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Exercise Order Differentially Affects Thymic Oxidative Stress, Inflammation, and SIRT1/FOXO3 Signaling in Aged Male Rats: Implications for Thymic Involution

Hamidreza Choobdari
Bu-Ali Sina University
Hesam Parsa
h.parsa@basu.ac.ir

Bu-Ali Sina University

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Abstract

Age-related thymic involution contributes to immunosenescence. While exercise mitigates thymic aging, the optimal order of combined exercise components remains poorly understood. This study investigated whether exercise order differentially affects thymic oxidative stress, inflammation, SIRT1/FOXO3 signaling, and histomorphometry in aged male rats. Aged male Wistar rats (n = 7 per group) were assigned to sedentary control (SED), aerobic-then-interval (AIT), or interval-then-aerobic (ITA) exercise. Swimming was performed 5 days/week for 10 weeks. Thymic MDA, SOD, GPX, TNF-α, SIRT1 and FOXO3 (by Western blot), and histomorphometry (cortical thickness, medullary thickness, cortex-to-medulla ratio) were measured. Data were analyzed by one-way ANOVA and Tukey's test. Both exercise orders reduced MDA and TNF-α and increased SOD versus SED, with no significant difference between AIT and ITA. GPX activity was significantly higher in both exercise groups versus SED, with the highest values in ITA, but no significant difference between ITA and AIT. SIRT1 expression was significantly higher in both exercise groups versus SED, with the highest expression in AIT; however, AIT and ITA did not differ significantly. FOXO3 did not differ among groups. AIT was the only group showing significantly greater cortical thickness and cortex-to-medulla ratio versus SED. Medullary thickness did not differ. Exercise order may influence some thymic markers, but no clear superiority of one order was observed across all outcomes. These findings suggest that exercise order should be considered in future studies on exercise interventions to combat age-related thymic involution.

Introduction

Aging is characterized by a progressive decline in immune function, termed immunosenescence, which contributes to increased susceptibility to infections, autoimmune diseases, and cancer in the elderly (1, 2). A central driver of immunosenescence is the age-related involution of the thymus, the primary lymphoid organ responsible for the maturation and selection of naive T lymphocytes (3). Thymic involution is marked by cortical atrophy, loss of thymic epithelial cells, and a significant reduction in the output of naive T cells, leading to a restricted T cell receptor repertoire and impaired adaptive immunity (3, 4). Therefore, preserving thymic structure and function has emerged as a key therapeutic target to delay or reverse immune aging.

Oxidative stress and inflammation are major contributors to thymic involution. With advancing age, the balance between reactive oxygen species (ROS) production and endogenous antioxidant defense shifts toward oxidative damage in thymic tissue (5). The activity of key antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPX), declines, while lipid peroxidation products, particularly malondialdehyde (MDA), accumulate (5, 6). In parallel, the thymic microenvironment exhibits a chronic low-grade inflammatory state, characterized by elevated levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) (7, 8). TNF-α not only induces apoptosis of thymocytes and thymic epithelial cells but also amplifies oxidative stress by stimulating ROS production through NF-κB signaling (7, 9). This creates a vicious cycle in which oxidative stress and inflammation mutually reinforce each other, accelerating thymic atrophy (6, 9). Thus, interventions that

simultaneously target oxidative stress and inflammation within the thymus may mitigate age-related thymic involution.

The SIRT1/FOXO3 signaling axis plays a pivotal role in cellular resistance to oxidative stress and in the regulation of longevity. SIRT1, an NAD+-dependent deacetylase, deacetylates FOXO3, thereby enhancing its transcriptional activity and promoting the expression of antioxidant genes such as SOD2 and catalase (10, 11). Importantly, SIRT1 also suppresses inflammation by deacetylating NF-κB, thereby reducing TNF-α production (12). In thymic tissue, both SIRT1 and FOXO3 levels decline with age, and this reduction is associated with increased oxidative damage, impaired thymic epithelial cell function, and accelerated involution (13, 14). Conversely, activation of the SIRT1/FOXO3 pathway has been shown to protect against oxidative stress-induced thymic atrophy in animal models (14). Therefore, strategies that upregulate this pathway may preserve thymic architecture during aging by simultaneously reducing oxidative stress and inflammation.

Regular physical exercise is a potent non-pharmacological intervention that can counteract oxidative stress and inflammation. However, the optimal design of combined exercise protocols—particularly the order in which different exercise components are performed—remains poorly understood. In skeletal muscle, the sequence of concurrent training can differentially affect molecular signaling pathways, including AMPK and mTOR (15). For instance, performing endurance exercise after resistance training can blunt anabolic signaling, whereas the reverse order may enhance mitochondrial adaptations (15, 16). Emerging evidence suggests that exercise order also influences systemic immune responses (17). However, no study to date has examined whether the order of interval and aerobic exercise within a combined session differentially affects thymic oxidative stress, inflammation, and the SIRT1/FOXO3 pathway in aged animals. This gap is significant because the thymic microenvironment may respond differently to exercise order than skeletal muscle, given its distinct vascular, cellular, and hormonal milieu.

Therefore, the present study aimed to investigate the effects of exercise order—interval followed by aerobic (ITA) versus aerobic followed by interval (AIT)—on thymic oxidative stress markers (MDA, SOD, GPX), the inflammatory marker TNF-α, the SIRT1/FOXO3 signaling pathway, and histomorphometric indices of thymic involution in aged male Wistar rats. We hypothesized that (1) both exercise orders would reduce oxidative stress and inflammation and upregulate SIRT1/FOXO3 compared with sedentary controls; and (2) the two exercise orders would differentially modulate these markers, with the aerobic-then-interval (AIT) order potentially exerting superior antioxidant, anti-inflammatory, and thymoprotective effects.

Materials and Methods

Animals

Twenty-one male Wistar rats (21 months old, 400–450 g) were sourced from the Pasteur Institute (Tehran, Iran). Upon arrival, the animals were transferred to the animal facility at Bu-Ali Sina University (Hamedan, Iran) and allowed to acclimate for one week. The rats were housed five per cage in polycarbonate cages under a 12-h light/dark cycle at 22 ± 2°C and 55% humidity, with free access to standard chow and water. Using a computer-generated sequence, the animals were randomly allocated into three groups (n = 7 each): sedentary control (SED), aerobic-then-interval (AIT), and interval-then-aerobic (ITA) exercise. Sample size was determined a priori via G*Power (18) (effect size f = 0.40, α = 0.05, power = 0.80 for a one-way ANOVA). All experimental procedures were approved by the Ethics Committee of Bu-Ali Sina University (IR.BASU.REC.1404.025). All methods were performed in accordance with the relevant guidelines and regulations.The study complied with the NIH guidelines for animal care and was reported in accordance with the ARRIVE 2.0 standards (19).

Exercise training protocol

Exercise training protocol
The swimming protocol was adapted from da Rocha et al. (20) and specifically modified for aged rats. To establish the correct exercise intensities, a two-phased incremental swimming test was applied (21). The training program lasted for 10 weeks, with five sessions per week. Each session was conducted in a circular pool (120 cm diameter, 60 cm depth, 40 cm water depth) at 30–32°C to minimize cold stress (20), with a maximum of five rats per session.

Each session consisted of two sequential components: interval and aerobic swimming. For the ITA group, the protocol began with interval swimming (4–6 sets of 30–45 s at 4–12% body weight [BW], separated by 45–60 s rest periods), immediately followed by continuous aerobic swimming (25–45 min at 0–3% BW). The AIT group completed the identical workload but in the reverse order (aerobic prior to interval). Weights corresponding to the designated BW percentage were attached to the base of the tail. Weekly progression of loads and durations is detailed in Table 1. Rats exhibiting signs of distress or exhaustion (e.g., repeated submersion) were briefly removed to rest. Sedentary controls were handled under identical conditions but were not exposed to swimming. **Table 1.** Exercise training protocol for aged male Wistar rats.

Week	Aerobic duration (min)	Aerobic load (%BW)	Number of interval sets	Interval duration (s)	Rest (s)	Interval load (%BW)
1	25	0	4	45	60	4
2	30	0	4	45	60	5
3	30	1	4	45	60	6
4	35	1	5	40	50	7
5	35	2	6	35	45	8
6	40	2	6	35	45	9
7	40	2.5	6	30	45	10
8	40	3	6	30	45	10
9	42	3	6	30	45	11
10	45	3	6	30	45	12

Tissue collection

Forty-eight hours after the final training session, the animals were deeply anesthetized via intraperitoneal injection of ketamine (40 mg/kg) and xylazine (4 mg/kg). Euthanasia was performed by cervical dislocation, strictly adhering to the AVMA Guidelines for the Euthanasia of Animals (22). Subsequently, the thymus gland was carefully excised. For histological evaluations, a tissue fragment was immediately fixed in 10% neutral-buffered formalin. The remaining thymic tissue was snap-frozen in liquid nitrogen and subsequently stored at − 80°C until further Western blot and oxidative stress analyses.

Western blot analysis

Thymic protein expression of SIRT1 (~ 110 kDa) and FOXO3 (~ 71 kDa) was assessed by Western blotting, following a previously established protocol (23, 24). Briefly, thymus samples were lysed in RIPA buffer containing protease and phosphatase inhibitors. Protein concentration was determined using a BCA assay. Equal amounts of protein (20–40 µg) were separated by SDS-PAGE and transferred onto PVDF membranes. The membranes were blocked for 1 hour at room temperature in 5% non-fat dry milk/TBST, followed by overnight incubation at 4°C with primary antibodies against SIRT1 (ab189494; 1:1000), FOXO3 (ab47285; 1:1000), and β-actin (ab8227; 1:5000) (all from Abcam). All antibodies were validated according to standard guidelines (26). After washing with TBST, an HRP-conjugated goat anti-rabbit secondary antibody (ab6721; 1:2000, Abcam) was applied for 1 hour. Protein bands were visualized using ECL and quantified with ImageJ (25). SIRT1 and FOXO3 band intensities were normalized to β-actin. Densitometric analysis was performed by an investigator blinded to the group allocations.

Measurement of oxidative stress and inflammatory markers

Thymic levels of TNF-α, MDA, SOD, and GPX were measured using commercial ELISA kits according to the manufacturers' instructions. Tissues were homogenized in cold buffer, centrifuged (10,000 × g, 10–15 min, 4°C), and the supernatants were collected. TNF-α content was quantified with a rat-specific kit (KPG-TNF-α-48, Karmania Pars Gene, Iran) at 450 nm. MDA levels were determined via a thiobarbituric acid assay (27) (Teb Pazhouhan Razi, Iran) at 530–540 nm. SOD and GPX activities were assessed using commercial kits (Navand Lab Kit, Iran) based on pyrogallol autoxidation inhibition (28) (400 nm) and NADPH oxidation (29) (340 nm), respectively. Total protein was measured by the Bradford method (30) (Bio-Rad, USA) to normalize the data. All samples were processed in a single batch to minimize inter-assay variation. ELISA readings were performed by an investigator blinded to the group assignments.

Histology and histomorphometry

Thymus tissue was processed for histological examination. Following dissection, the thymus was fixed in 10% neutral-buffered formalin, embedded in paraffin, and serial sections of 4–6 μm thickness were obtained using a microtome. For histomorphometric analysis, the sections were stained with hematoxylin and eosin (H&E) only. Digital images were captured and analyzed using ImageJ software (25). Random fields per section were evaluated at 40× magnification. Cortical thickness, medullary thickness, and the cortex-to-medulla ratio were measured according to standard criteria (31). All evaluations were performed by a blinded investigator.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc test for pairwise comparisons. Statistical significance was set at $p < 0.05$. All analyses were performed using GraphPad Prism (version 9.4.0). No animals were lost during the study, and no data points were excluded from the analyses.

Results

Oxidative stress markers

The effects of exercise order on thymic oxidative stress markers are presented in Fig. 1. Thymic MDA concentration was significantly higher in the SED group (4.04 ± 0.72 μmol/mg protein) compared with both AIT (1.89 ± 0.61 μmol/mg protein; $p < 0.0001$) and ITA (2.36 ± 0.68 μmol/mg protein; $p < 0.001$) groups, with the AIT group showing the lowest values (Fig. 1A). In contrast, SOD activity was significantly lower in SED (228.12 ± 30.15 U/mg protein) compared with AIT (353.52 ± 45.28 U/mg protein; $p < 0.0001$) and ITA (325.58 ± 38.76 U/mg protein; $p < 0.001$), and the AIT group exhibited the highest activity (Fig. 1B). GPX activity was also significantly lower in SED (28.12 ± 5.34 mU/mg protein) compared with ITA (49.92 ± 8.67 mU/mg protein; $p < 0.0001$) and AIT (43.47 ± 7.12 mU/mg protein; $p < 0.01$), with ITA

showing the highest activity (Fig. 1C). No significant differences were observed between AIT and ITA for MDA, SOD, or GPX ($p > 0.05$).

Inflammatory marker

The effect of exercise order on thymic TNF-α concentration is shown in Fig. 2. TNF-α concentration was significantly higher in the SED group (43.78 ± 4.67 pg/mg protein) compared with both AIT (29.78 ± 7.36 pg/mg protein; $p < 0.001$) and ITA (31.87 ± 4.97 pg/mg protein; $p < 0.01$), with the AIT group showing the lowest values (Fig. 2). No significant difference was observed between AIT and ITA ($p > 0.05$).

SIRT1/FOXO3

Representative Western blot images for SIRT1, FOXO3, and β-actin (loading control) and quantification of SIRT1 and FOXO3 are shown in Fig. 3. SIRT1 intensity was significantly lower in the SED group (0.51 ± 0.12 a.u.) compared with both AIT (1.18 ± 0.20 a.u.; $p < 0.01$) and ITA (1.06 ± 0.19 a.u.; $p < 0.05$) groups, with the AIT group exhibiting the highest expression (Fig. 3B). Although FOXO3 intensity appeared higher in the exercise groups (ITA: 0.95 ± 0.22 a.u.; AIT: 1.10 ± 0.28 a.u.) compared with SED (0.59 ± 0.10 a.u.), the differences did not reach statistical significance ($p > 0.05$) (Fig. 3C).

Histomorphometry

Representative H&E-stained thymic sections and histomorphometric analysis are shown in Fig. 4. In panel A, white arrows indicate the cortex, and orange arrows indicate the medulla. Cortical thickness was significantly lower in the SED group (156.50 ± 5.28 μm) compared with the AIT group (187.17 ± 6.24 μm; $p < 0.05$). However, no significant differences were observed between the SED and ITA groups (171.67 ± 5.32 μm) or between the AIT and ITA groups (Fig. 4B). Medullary thickness showed an opposite trend, being highest in the SED group (98.17 ± 2.32 μm) and lowest in the AIT group (85.00 ± 3.74 μm), but these differences were not statistically significant (Fig. 4C). The cortex-to-medulla ratio was significantly lower in the SED group (1.59 ± 0.06) compared with the AIT group (2.20 ± 0.11; $p < 0.05$), with the ITA group showing intermediate values (1.89 ± 0.07) (Fig. 4D). No animals were lost, and no data points were excluded from the analyses.

Discussion

The present study examined whether the sequencing of interval and aerobic exercise within a single swimming session could differentially modulate thymic oxidative stress, SIRT1/FOXO3 signaling, and histomorphometric parameters in aged male rats. Both exercise sequences offered protective effects compared to a sedentary lifestyle. However, the AIT (aerobic-then-interval) order yielded more favorable outcomes. Specifically, the AIT regimen resulted in lower MDA levels, elevated SOD activity, and enhanced SIRT1 expression. It also led to better preservation of cortical thickness and the cortex-to-medulla ratio. However, the statistical significance between AIT and ITA was restricted to SOD and

SIRT1. GPX activity peaked in the ITA group, while FOXO3 expression remained statistically unchanged across all groups. These findings suggest that the sequence of exercise modalities is a determining factor in thymic protection during aging, though the benefits are marker-specific.

Aging is intrinsically linked to elevated oxidative stress within the thymus, which subsequently drives thymic involution by damaging thymic epithelial cells and thymocytes (5, 6). In the current study, both exercise paradigms successfully decreased MDA concentrations and enhanced SOD activity relative to the SED group. This corroborates the established antioxidant properties of physical training (32, 33). The more pronounced improvements observed in the AIT group could be attributed to the physiological sequence of stimuli. Initiating the session with aerobic exercise may promote a gradual upregulation of mitochondrial biogenesis and antioxidant enzymes, effectively preconditioning the thymus for the subsequent high-intensity interval phase (12, 34). Conversely, starting with interval exercise might cause an abrupt surge in reactive oxygen species. This could overwhelm the adapting antioxidant defense system. Similar order-dependent molecular responses have been documented in skeletal muscle, where the final exercise bout dictates the acute signaling cascade (12, 15). The present data extend this concept to the thymus, implying that exercise sequencing warrants careful consideration in protocols designed for aging populations.

Interestingly, GPX activity was an exception, reaching its highest level in the ITA group. This discrepancy might indicate a distinct regulatory mechanism for different antioxidant enzymes based on exercise order. GPX is crucial for neutralizing hydrogen peroxide and lipid peroxides, and its expression might be more responsive to the metabolic stress induced by an initial interval bout (29). Nevertheless, the physiological relevance of this isolated GPX elevation is uncertain, particularly since MDA levels remained higher in ITA compared to AIT. Therefore, the overall redox balance appeared more advantageous in the AIT group, though the specific GPX response in ITA requires further exploration.

The SIRT1/FOXO3 pathway is a well-established regulator of cellular resilience to oxidative stress and longevity (10, 11). In this study, SIRT1 protein expression was significantly elevated in both exercise groups compared to the SED group, with the AIT group showing the most substantial increase. This aligns with previous literature indicating that exercise upregulates SIRT1 across various tissues, including skeletal muscle and the thymus (14, 35). Because SIRT1 deacetylates FOXO3 to enhance its transcriptional activity and antioxidant gene expression (10), the higher SIRT1 levels in the AIT group may explain the superior SOD activity and lower MDA levels observed.

Contrary to expectations, FOXO3 protein levels did not show significant differences among the groups. Several factors could account for this. First, FOXO3 is primarily regulated via post-translational modifications rather than changes in total protein abundance (36). It is plausible that SIRT1-mediated deacetylation of FOXO3 was enhanced in the AIT group without altering its total protein expression. Second, the 10-week intervention period might not have been sufficient to elicit detectable changes in FOXO3 protein levels. Third, the limited thymic tissue available for Western blotting may have restricted

the statistical power to identify subtle differences. Future investigations should evaluate FOXO3 acetylation status and downstream target genes to clarify the functional activation of this axis.

Thymic involution is characterized by the progressive depletion of cortical thymocytes and a reduction in cortical thickness, which ultimately decreases the cortex-to-medulla ratio (31). In the current study, the AIT group was the only cohort demonstrating significant preservation of cortical thickness and a significant increase in the cortex-to-medulla ratio compared to the SED group. These histological observations align with the biochemical data. It suggests that reduced oxidative stress and elevated SIRT1 expression in the AIT group may have shielded thymic epithelial cells from oxidative damage, thereby decelerating cortical atrophy. Conversely, the ITA group displayed intermediate values, while the SED group exhibited the most severe atrophy. Medullary thickness remained statistically unchanged across groups, which is consistent with the premise that the medulla is less susceptible to age-related involution than the cortex (3).

The superior structural preservation in the AIT group might also be associated with its influence on systemic inflammation. Although systemic inflammatory cytokines were not directly measured in this study, prior research indicates that exercise order can modulate acute immune responses (17). For instance, Markov et al. reported that performing strength exercise prior to endurance exercise induced greater acute immunological stress in young athletes (17). It is conceivable that a comparable mechanism exists in the aged thymus: commencing with aerobic exercise may establish a more favorable systemic environment for the subsequent interval bout. This could mitigate the overall stress response and preserve thymic architecture. Nonetheless, this remains a hypothesis that requires direct empirical validation.

Jahan-Mahin et al. previously demonstrated that combined water-based training protocols could alleviate thymus atrophy and improve markers of cellular immune senescence in aged male rats (37). The present study expands upon these findings by demonstrating that the specific order of exercise components within a combined session is a critical determinant of thymic outcomes. To the best of our knowledge, this is the first study to evaluate the differential effects of exercise order on thymic oxidative stress and SIRT1/FOXO3 signaling in aged animals. The results also corroborate skeletal muscle studies showing that exercise order influences molecular adaptations, such as AMPK and mTOR signaling (12, 15). However, the optimal sequence appears to be tissue-specific. While resistance exercise before endurance exercise is often recommended for maximizing muscle anabolic adaptations (16), the present data suggest that for the thymus, aerobic exercise preceding interval exercise may be more advantageous for antioxidant defense and structural preservation.

Several limitations must be acknowledged in this study. First, the investigation was restricted to male rats, limiting generalizability to females. Second, due to the severe age-related involution of the thymus and the resulting scarcity of viable tissue, Western blot analysis and histomorphometry were conducted on fewer independent biological replicates (n = 3 and n = 2 per group, respectively) compared to the ELISA data (n = 7). Consequently, the statistical power for these specific assays was reduced, and the

negative findings for certain markers (e.g., FOXO3) should be interpreted with caution rather than as definitive evidence of no effect. Third, the 10-week intervention period may have been insufficient to observe more pronounced alterations in FOXO3 expression and medullary thickness. Fourth, FOXO3 acetylation and downstream target genes were not directly measured, which would have provided a more comprehensive assessment of SIRT1/FOXO3 pathway activity. Fifth, functional immune outcomes were not evaluated, leaving the physiological implications of the histological changes unconfirmed. Finally, despite utilizing a well-established swimming protocol, extrapolating these findings to human exercise prescriptions requires caution.

Conclusion

The present study demonstrates that the order of interval and aerobic exercise within a combined swimming session differentially affects thymic oxidative stress, SIRT1 expression, and histomorphometric indices of thymic involution in aged male rats. Both exercise orders reduced oxidative stress and improved antioxidant defense compared with sedentary controls, but the aerobic-then-interval (AIT) order showed some advantages over the interval-then-aerobic (ITA) order, particularly for SOD activity, SIRT1 expression, cortical thickness, and the cortex-to-medulla ratio. However, these differences were not uniform across all markers, and FOXO3 expression did not differ significantly among groups. Overall, the findings suggest that exercise order should be carefully considered when designing combined training protocols aimed at preserving thymic structure and function during aging.

Declarations

Competing interests

The authors declare no competing interests.

Author Contribution

H.C. conducted the experiments, analyzed the data, and drafted the manuscript. H.P. conceptualized and designed the study, supervised the project, and critically revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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Figures

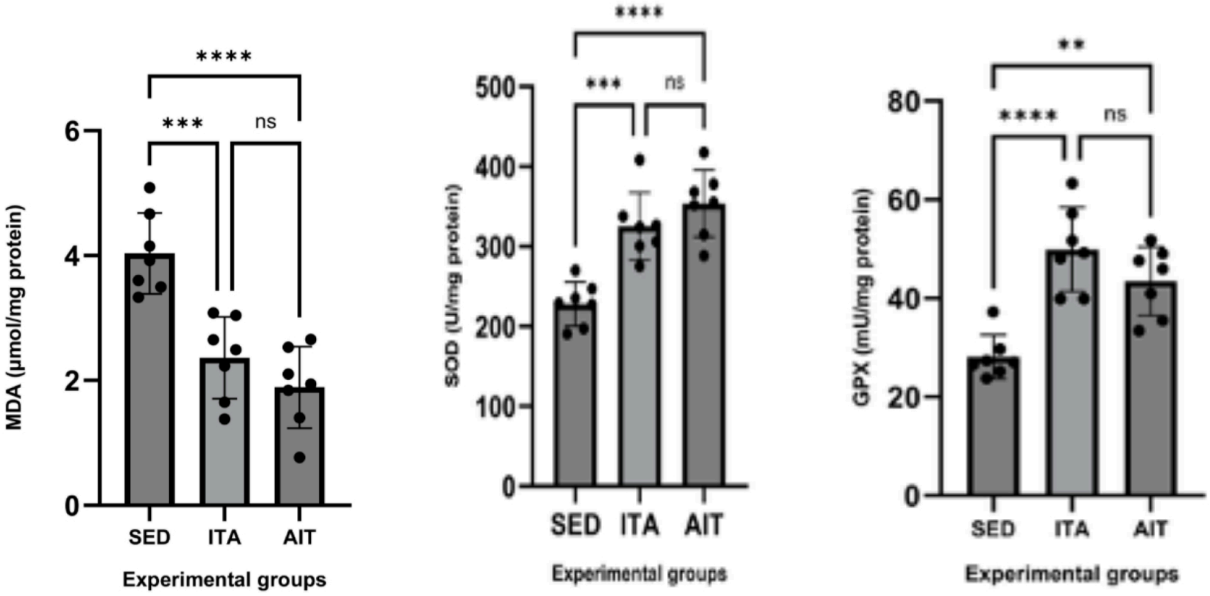


Figure 1

Effect of exercise order on thymic oxidative stress markers in aged male rats: MDA (A), SOD (B), and GPX (C). Data are presented as mean ± SD (n = 7 per group). Statistical significance is indicated as follows: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; ns, not significant. Abbreviations: SED, sedentary control; AIT, aerobic-then-interval exercise; ITA, interval-then-aerobic exercise; MDA, malondialdehyde; SOD, superoxide dismutase; GPX, glutathione peroxidase.

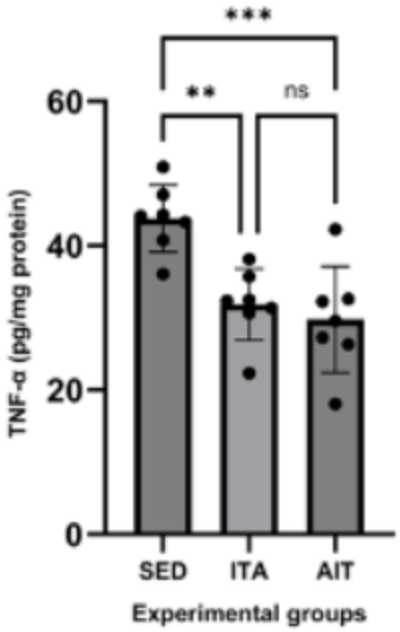


Figure 2

Thymic TNF-α concentration in aged male rats across experimental groups. Data are presented as mean ± SD (n = 7 per group). **p < 0.01, ***p < 0.001 versus SED; ns, not significant. Abbreviations: SED, sedentary control; AIT, aerobic-then-interval exercise; ITA, interval-then-aerobic exercise; TNF-α, tumor necrosis factor-alpha.

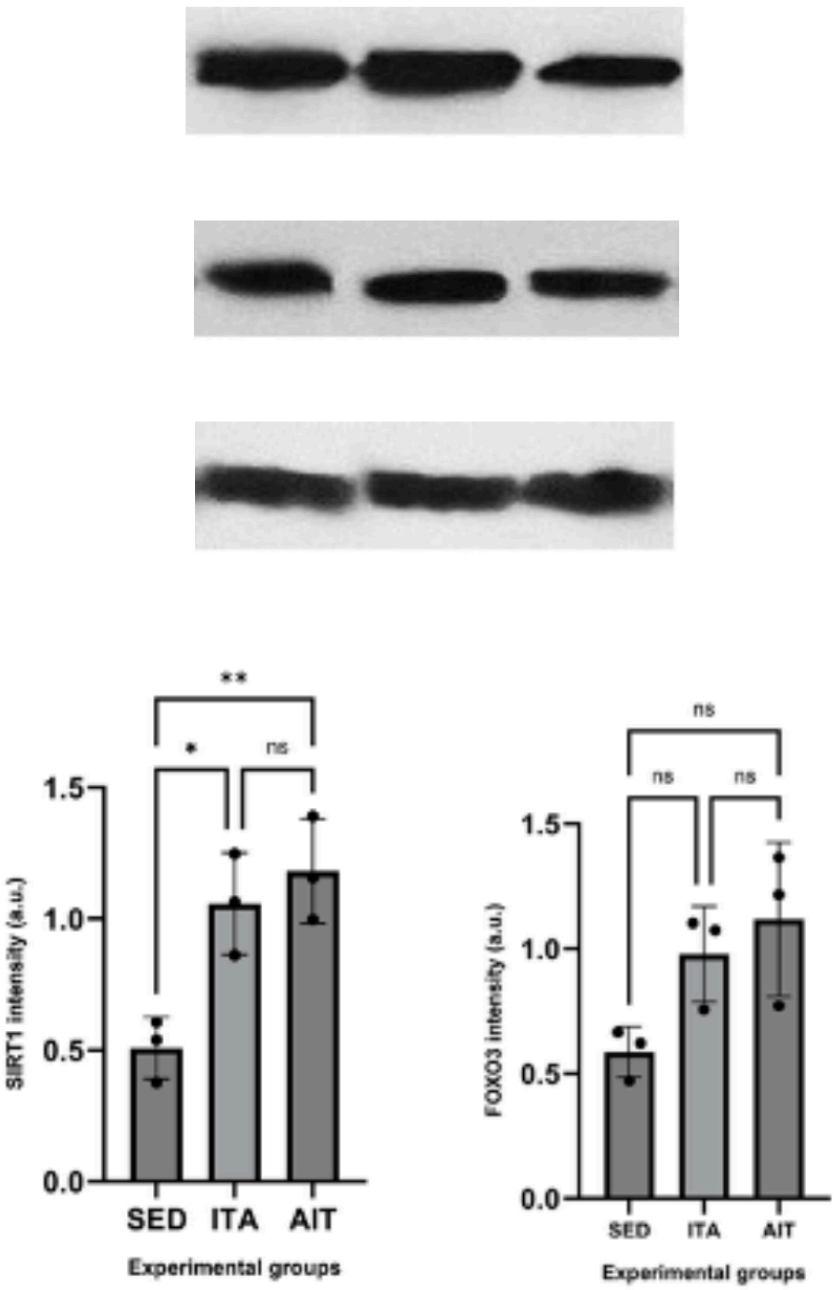


Figure 3

Thymic SIRT1 and FOXO3 protein expression in aged male rats: representative Western blot images for SIRT1, FOXO3, and β-actin (loading control) (A), SIRT1 quantification (B), and FOXO3 quantification (C). Data are presented as mean ± SD (n = 3 per group). Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$; ns, not significant. Abbreviations: SED, sedentary control; AIT, aerobic-then-interval exercise; ITA, interval-then-aerobic exercise; a.u., arbitrary units.

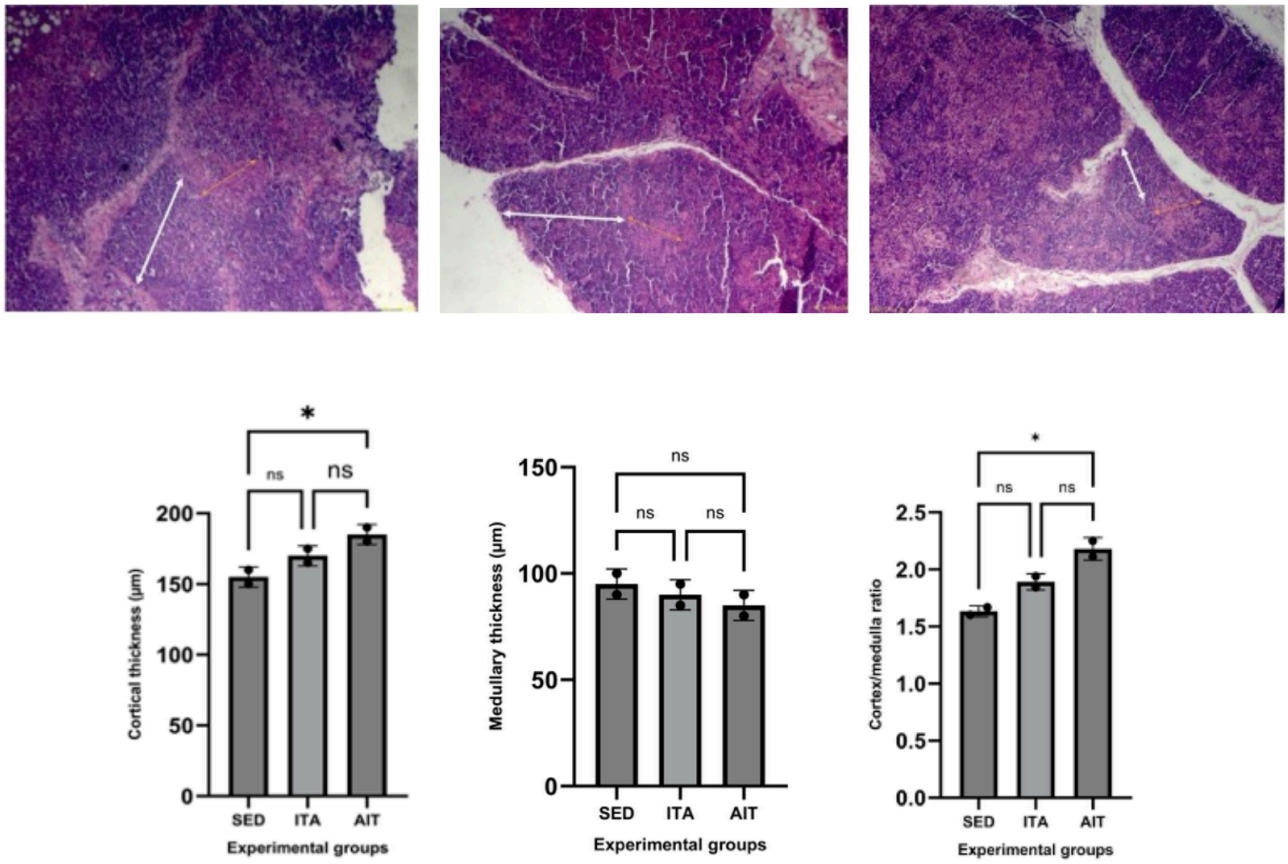


Figure 4

Thymic histology and histomorphometry in aged male rats: representative H&E-stained sections from AIT (A), ITA (B), and SED (C) groups. White arrows indicate the cortex, and orange arrows indicate the medulla. Scale bar = 50 µm; magnification ×40. Data are presented as mean ± SD (n = 2 per group). Statistical significance is indicated as follows: * $p < 0.05$; ns, not significant. Abbreviations: SED, sedentary control; AIT, aerobic-then-interval exercise; ITA, interval-then-aerobic exercise; H&E, hematoxylin and eosin.

Supplementary Files

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- SupplementaryInformation.pdf