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Original Article

Protective effects of swimming exercise on experimental rheumatoid arthritis in rats through modulation of leptin receptor expression and inflammation

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ABSTRACT

Objectives: This study aims to investigate the effects of swimming exercise on arthritis severity, radiological joint damage, and leptin-related molecular changes in experimental rheumatoid arthritis (RA) in rats.

Materials and methods: A total of 30 male Wistar rats were randomly assigned to three groups (n=10 per group): Control, Arthritis, and Exercise + Arthritis. Experimental arthritis was induced by a single intradermal injection of Complete Freund's Adjuvant (CFA). The Exercise + Arthritis group underwent a six-week swimming protocol (1 h/day, five days/week), beginning two weeks before arthritis induction and continuing thereafter. Arthritis severity was assessed by visual scoring, radiological analysis, and histopathological evaluation. Plasma concentrations of leptin and matrix metalloproteinases (MMP-1, MMP-3, MMP-8, MMP-13) were measured using enzyme-linked immunosorbent assay (ELISA), and leptin receptor expression in paw tissues was analyzed immunohistochemically.

Results: The Exercise + Arthritis group exhibited significantly lower visual arthritis scores ($p=0.002$), reduced ankle swelling ($p<0.001$), and decreased cartilage and bone damage radiologically ($p=0.045$) compared to the Arthritis group. Histopathological evaluation revealed that swimming exercise markedly attenuated inflammatory cell infiltration, pannus formation, and bone resorption. Although plasma leptin and MMP levels did not differ between the groups ($p>0.05$), leptin receptor expression, considered to be associated with joint damage, was found to be significantly lower in the Exercise + Arthritis group ($p=0.009$). Swimming exercise reduced arthritis severity and joint damage.

Conclusion: Moderate-intensity swimming exerts protective, anti-inflammatory effects in experimental RA by reducing inflammation and preserving joint structure in rats, possibly through modulating leptin receptor expression and inflammatory pathways. Although regular swimming may serve as a useful adjunct to slow disease progression and joint damage in RA in rats, the translational relevance of these results remains uncertain in humans.

Keywords: Inflammation, leptin, matrix metalloproteinase, rat model, rheumatoid arthritis, swimming exercise.

Rheumatoid arthritis (RA) is a common chronic inflammatory disease which affects approximately 0.5 to 1% of the global population.[1] Clinically, the symptoms of the disease vary significantly between early and late stages. While patients in the early stage often present with non-specific symptoms such as fatigue, tender and swollen joints, and morning stiffness, inadequately treated late-stage patients may experience joint erosion, deformities, and loss of range of motion (ROM).[2]

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To control disease activity and mitigate its harmful effects, disease-modifying antirheumatic drugs (DMARDs) are commonly used in RA management.[3] However, some studies suggest that pharmacological treatment should be combined with physical activity.[4,5] The relationship between RA and exercise has been widely studied, with research focusing on the types and intensities of exercise appropriate for RA patients. High-intensity dynamic exercises such as cycling, brisk walking, and resistance training are designed to stimulate muscle growth and enhance physical fitness, but they can also lead to joint damage. Compared to dynamic exercises, RA patients prefer ROM, stretching, and isometric exercises that place less stress on the joints.[6] Among these, swimming is particularly noteworthy for exerting minimal stress on joints and has been reported to improve aerobic capacity and muscle strength in RA patients, as well as to modulate immune cell activity.[7] Evidence from studies involving patients with osteoarthritis indicates that routine swimming exercise reduces joint pain and stiffness, accompanied by notable improvements in muscular strength and functional performance.[8] There are several studies showing the positive effects of exercise on RA.[9-11] However, to the best of our knowledge, no studies have examined the effects of swimming on arthritis severity or radiological damage yet.

A growing body of research has investigated the interaction between leptin and exercise. Most studies indicate that exercise, particularly aerobic modalities such as swimming, tends to reduce circulating leptin levels.[12-15] Nevertheless, conflicting evidence exists, with several studies reporting increases in leptin concentrations following exercise interventions.[16,17] Leptin, first identified in 1994 as the prototypical adipokine, has since been recognized as a key mediator in the pathogenesis of RA.[18] Although initially characterized for its regulatory function in appetite and energy homeostasis, leptin has emerged as a critical immunomodulatory molecule. Beyond its metabolic role, leptin augments immune activation by enhancing natural killer cell cytotoxicity and promoting the activation of granulocytes, macrophages, and dendritic cells, thereby amplifying inflammatory responses.[19] Moreover, leptin facilitates T-cell polarization toward the T-helper-1 (Th1) phenotype, resulting in elevated production of proinflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6 (IL-6), interferon-gamma (IFN-γ), and IL-17, while concurrently suppressing T-helper-2 (Th2) cell differentiation. This pronounced Th1-skewed immune response represents a hallmark immunological disturbance in RA, confirming leptin as a potent proinflammatory adipokine implicated in disease progression.[20]

A growing body of evidence highlights the role of physical activity in modulating inflammatory pathways and improving clinical outcomes in rheumatic diseases. However, the mechanisms through which exercise exerts its potential protective effects in RA still remain incompletely understood. In particular, the interaction between exercise and adipokines such as leptin, an established proinflammatory mediator implicated in RA pathogenesis, has attracted increasing scientific interest.

In the light of literature data, in the present study, we hypothesized that exercise could attenuate both the development and severity of arthritis and swimming exercise preconditioning could attenuate arthritis severity and joint destruction in rats by downregulating leptin receptor expression and associated inflammatory responses. We, therefore, aimed to investigate the effects of swimming exercise on the onset and clinical progression of arthritis in experimental RA in rats and to evaluate whether the potential protective effects of exercise on arthritis were mediated through leptin.

MATERIALS AND METHODS

This experimental study was conducted at Çanakkale Onsekiz Mart University (ÇOMU), Department of Physiology, Experimental Research and Application Center between October 2021 and October 2022. The study protocol was approved by the ÇOMU Local Ethics Committee for Animal Experiments (Date: 30/07/2021, No: 2021/06-05). All procedures were carried out in accordance with institutional guidelines for animal research and complied with relevant national and international ethical standards.

Animals and study design

A total of 30 male Wistar rats (seven to 10 weeks old) were obtained from the Experimental Research and Application Center of ÇOMU. The animal sample size in this study was determined based on prior studies in the literature.[21,22] All animals were clinically healthy at the beginning of the

study. A randomized parallel-group experimental design was used. Animals were housed in standard laboratory cages under controlled environmental conditions (22±2°C; 50 to 60% humidity; 12-h light/dark cycle). Standard commercial pellet chow and tap water were provided *ad libitum*. Environmental enrichment was provided in the form of nesting material and shelter. Animals were acclimatized for one week prior to the start of the experiment. Animals were randomly assigned to one of three groups using a computer-based random-number generator (available at https://www.random.org/) with simple randomization:

1. Control group (n=10): Received no intervention and served as the baseline control.
2. Arthritis group (n=10): Experimental arthritis was induced following the standard induction protocol, as described below. No exercise regimen was applied.
3. Exercise + Arthritis group (n=10): The rats underwent a structured swimming exercise protocol prior to arthritis induction. The same arthritis protocol used in the Arthritis group was subsequently applied.

Cage distribution was adjusted to avoid group clustering. Animals were monitored daily for general health status, mobility, appetite, and clinical signs of arthritis or distress. Veterinary care was available, if required. The study flowchart is shown in Figure 1.

Arthritis induction protocol

Experimental arthritis was induced using Complete Freund's Adjuvant (CFA) containing *Mycobacterium tuberculosis* H-37 RA (10 mg/mL). Animals in the arthritis-induced groups received a single intradermal injection of 100 µL of CFA at the base of the tail. This protocol was adapted from previously established methods.[23] The CFA administration was performed under brief manual restraint, and injection sites were inspected daily for local reactions. No additional analgesia was administered to avoid interference with inflammatory outcomes.

Exercise protocol

The swimming exercises were performed in a polypropylene rectangular tank measuring 67.5 cm wide, 94 cm long, and 64 cm high. Before the experimental procedures, the rats allocated to the Exercise + Arthritis group underwent a five-day water acclimatization protocol. During this period, animals performed daily swimming sessions lasting 5 to 10 min without additional load to reduce stress and facilitate adaptation to the aquatic environment.[7] Following acclimatization, rats in this group completed a six-week swimming training program consisting of 1-h sessions, five days per week, for a total of 30 sessions.[24] On Day 14 of the exercise period, arthritis was induced via a CFA injection. Throughout all swimming sessions, the water temperature was maintained at 30 to 32°C to ensure thermal comfort and prevent cold-induced stress.[25] During the investigation of the protective effects of exercise in the arthritis model, no additional measurements were performed to assess the rats' fatigue levels.

Clinical evaluation and arthritis scoring

The body weights and paw diameters of all animals were measured and recorded weekly throughout the experimental period. For weight

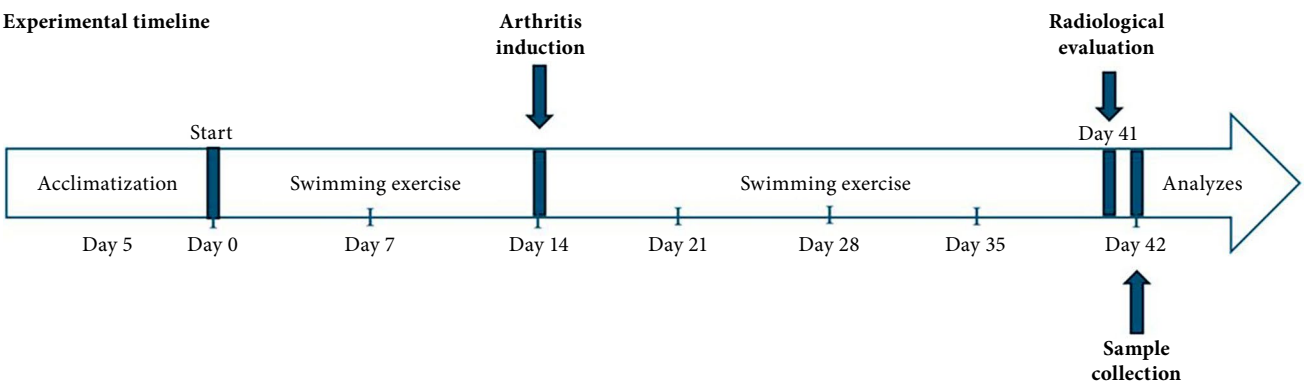

Figure 1. Experimental timeline.

measurement, an empty cage was first placed on a digital scale to obtain the tare value. Afterward, each rat was placed in the cage, and its body weight was recorded.

Paw diameter measurement: The mediolateral diameters of both the right and left hind paws were measured weekly using a digital caliper. The same investigator performed all measurements to minimize inter-observer variability.

Clinical arthritis scoring: Scoring was performed daily by two independent observers, blinded to group allocation, until the end of the experiment. Each paw was scored on a 0–4 scale based on the degree of swelling and erythema, yielding a maximum possible score of 16 per animal. The rats were considered arthritic, when their clinical arthritis score reached 1 or higher.[26] The scoring criteria were as follows:

- 0= no visible swelling or erythema
- 1= mild swelling and erythema limited to the ankle or tarsal joints
- 2= mild swelling and erythema extending from the ankle to the tarsal region
- 3= moderate swelling and erythema extending to the metatarsal joints
- 4= severe swelling and erythema involving the ankle, foot, and toes, or presence of ankylosis

Radiological evaluation

One day prior to euthanasia, radiographic imaging of all limbs was performed and evaluated blindly by two independent observers experienced in radiological scoring. Imaging was performed using a mammography system (Senographe Essential ADS 56.21.3; GE Healthcare, Chalfont St Giles, UK) with exposure parameters of 52 mA and 32 kV in the anteroposterior plane. Anesthesia was induced with ketamine (100 mg/kg) and xylazine (10 mg/kg) prior to imaging. Radiographs were acquired at a source-to-object distance of 32 cm, leaving an 18.5-cm gap between the animal and the detector using a magnification apparatus. Radiographic severity was scored using a 0-3 semi-quantitative scale, based on swelling, osteoporosis, cartilage loss, bone erosion, heterotopic ossification, and periosteal new bone formation. Each variable was scored as follows: 0= normal; 1= mild; 2= moderate; 3= severe. The total radiological score was calculated by averaging all scores.[27]

Termination of the experiment and sample collection

At the end of the experiment, the rats were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg). While under anesthesia, animals were euthanized by blood collection from the abdominal aorta using a sterile disposable syringe. Plasma was obtained immediately by rapid centrifugation of the collected blood and stored at −40°C for subsequent biochemical analyses. All procedures were performed to minimize pain and distress, and the absence of vital reflexes confirmed euthanasia. All animals were euthanized on Day 42 following the completion of all experimental procedures.

Biochemical analyses

Plasma concentrations of leptin and matrix metalloproteinases (MMP-1, MMP-3, MMP-8, and MMP-13) were quantified using enzyme-linked immunosorbent assay (ELISA) kits (Bioassay Technology Laboratory, China; Leptin: ELK1244, MMP-1: ELK2542, MMP-3: ELK2536, MMP-8: ELK2532, MMP-13: ELK2538).

Histopathological evaluation

To examine the impact of swimming exercise on cellular infiltration, pannus formation, and bone resorption, the hind paws (including the ankle joints) were harvested from all groups and fixed in 10% neutral-buffered formalin for seven days prior to histological processing. The samples were decalcified in 15% ethylenediaminetetraacetic acid (EDTA) for three weeks, dehydrated, embedded in paraffin, and sectioned at 5 µm. Tissue sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope. Each section was individually evaluated for cellular infiltration, pannus formation, and bone erosion using a semi-quantitative scoring system described by Banda et al.[28] and Scotece and Mobasheri,[29] with scores ranging from 0 to 5. The cumulative score for each animal was used to determine the histopathological severity of arthritis. All histopathological assessments were carried out by a blinded histopathologist.

Immunohistochemical evaluation

To evaluate leptin receptor expression, tissue sections were mounted on poly-L-lysine–coated slides. After drying, sections were deparaffinized in xylene at 60°C for 15 min, rehydrated through

a graded ethanol series, and rinsed in distilled water. Antigen retrieval was performed in citrate buffer (pH 6.0) at 95°C for 30 min. The sections were allowed to cool for 45 min, washed, and incubated with 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity. A blocking solution (ab93705, Abcam, MA, USA) was applied for 15 min, followed by overnight incubation at 4°C with a 1:200-diluted anti-leptin receptor primary antibody (BS0961R, Bioss Inc., MA, USA). For negative controls, the dilution buffer was used instead of the primary antibody. Following washing, the sections were incubated with biotin (ab93705, Abcam, MA, USA) for 15 min and avidin for 20 min. Aminoethylcarbazole (AEC; ab93705, Abcam, MA, USA) was applied as the chromogen for 10 min, and slides were counterstained with H&E for 30 seconds. The stained sections were examined and imaged under an Olympus BX53 light microscope.

The H-score analysis was used to quantify leptin receptor expression in five randomly selected fields at 40× magnification. The H-score was calculated as the sum of the product of the percentage of positively stained cells and the corresponding staining intensity at each level. Staining intensity was graded as follows: 0= no staining; 1= weak staining; 2= moderate staining; 3= strong staining.

The H-score is calculated using the formula: H-score = intensity level x %positive cells.

Statistical analysis

Statistical analysis was performed using the SPSS for Windows version 16.0 software (SPSS Inc., Chicago, IL, USA). Descriptive data were presented in median with interquartile range (IQR) or number and frequency, where applicable. Normality of distribution was assessed using the Kolmogorov-Smirnov test. Differences between two groups were analyzed using the Mann-Whitney U test, while comparisons among multiple groups were evaluated using the Kruskal-Wallis test. Following a significant Kruskal-Wallis test, pairwise *post-hoc* comparisons were performed using the Mann-Whitney U test with Bonferroni-adjusted p values to control for multiple comparisons. A p value of <0.05 was considered statistically significant.

RESULTS

A total of 30 Wistar rats were enrolled in the study and randomly assigned to three groups. No mortality was observed in any group, and all animals completed the study. Body weight was monitored throughout the study. As of Day 14 and continuing through the end of the experimental period, both the Arthritis and Exercise + Arthritis groups exhibited significantly lower body weight gain than the Control group (p=0.005). In addition, the circumferences of the left and right ankle joints were measured weekly throughout the experiment to assess joint swelling and inflammation. Ankle circumferences of both the Arthritis and Exercise + Arthritis groups increased significantly compared to the Control group starting on Day 28 (p=0.043 and p=0.008, respectively). In the Arthritis group, it sustained until the end of the study. However, ankle circumference measurements in the Exercise + Arthritis group were similar to the Control group after Day 35 until the end of the study (p=0.065).

Clinical and biochemical findings

An image of paw demonstrating the maximum arthritis score is shown in Figure 2. Clinical signs of arthritis became observable and quantifiable in both the arthritis and Exercise + Arthritis groups 14 days after arthritis induction. The rats in the Exercise + Arthritis group exhibited significantly lower visual arthritis scores compared to the Arthritis group on Day 14 (p=0.002) and from Day 31 to the end of the study (p=0.001) (Figure 3).

Plasma leptin and MMP-1, MMP-3, MMP-8, and MMP-13 levels were compared across all experimental groups. No statistically significant differences were detected (leptin: p=0.105; MMP-1: p=0.674; MMP-3: p=0.207; MMP-8: p=0.280; MMP-13: p=0.844).

Leptin receptor expression

Leptin receptor expression in paw tissues of all groups was assessed using immunohistochemistry (Figure 4). In the Control group, weak leptin receptor expression was detected in articular cartilage cells and bone cells. Additionally, low-level expression was observed in endothelial cells lining the bone marrow cavities and in adipocytes within the bone marrow. In the Arthritis group, leptin receptor expression was markedly increased in articular cartilage cells and bone cells. Strong immunoreactivity was particularly evident in adipocytes within the bone marrow cavities and in inflammatory cell populations. In contrast, the Exercise + Arthritis group exhibited weak leptin receptor expression compared to the Arthritis

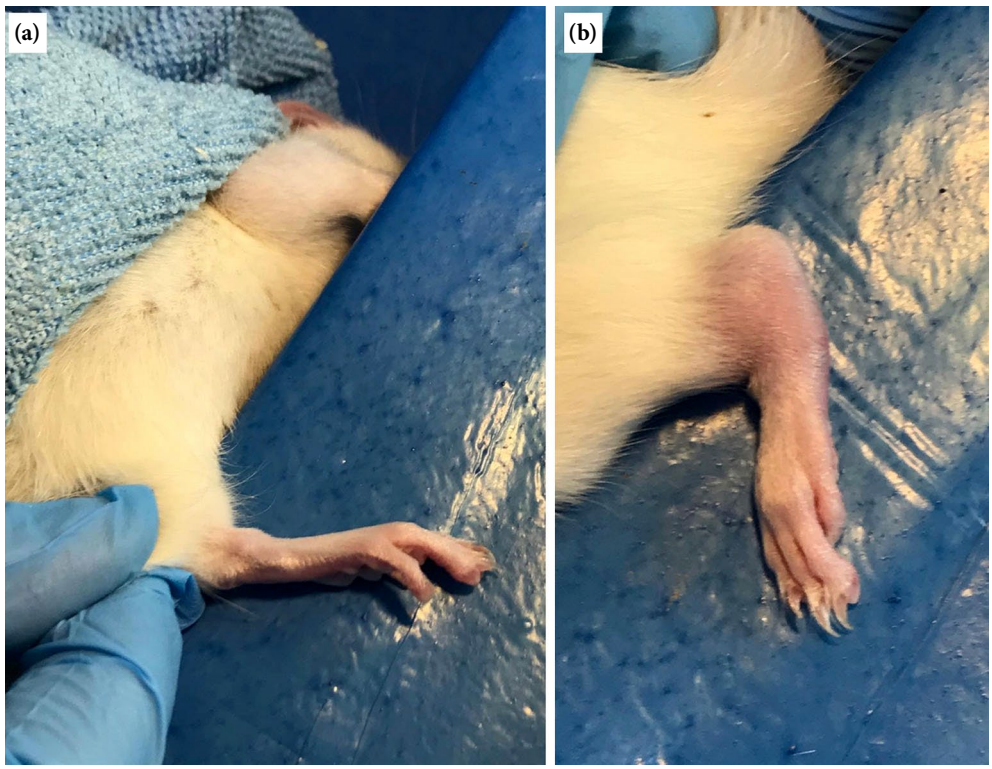

Figure 2. Paw images of control and arthritis groups. **(a)** Paw image from the control group. **(b)** Paw image from the arthritis group.

group (p=0.009) (Table I). Minimal expression was observed in articular cartilage cells and bone marrow adipocytes. These findings indicated that swimming exercise attenuated the arthritis-associated upregulation of leptin receptors.

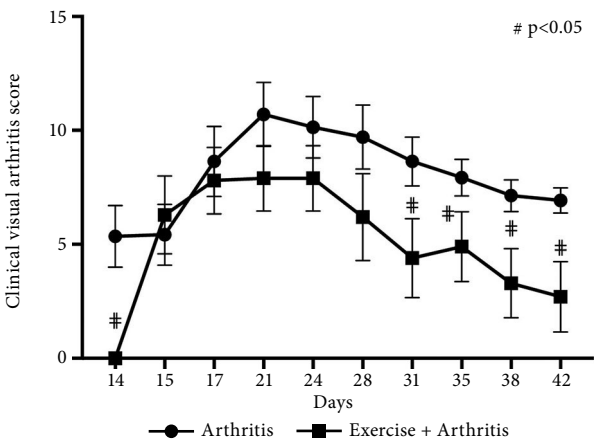

Figure 3. Time-course comparison of visual arthritis scores between the Arthritis and Exercise + Arthritis groups. Clinical signs of arthritis emerged in both groups on Day 14 and remained present until study completion. Rats in the Exercise + Arthritis group exhibited significantly lower visual arthritis scores compared to the Arthritis group on Day 14 and from Day 31 to the end of the study.

# p<0.05 compared to the arthritis group. Analyzed with the Mann-Whitney U test.

Radiological findings

Radiographs of animals from all experimental groups were obtained and scored one day before euthanasia (Figure 5). Radiological evaluation included variables associated with inflammation and arthritis, including soft-tissue swelling, cartilage loss, osteoporosis, erosion, heterotopic ossification, and periosteal new bone formation. The Exercise + Arthritis group demonstrated lower levels of soft-tissue swelling, erosion, heterotopic ossification, and periosteal new bone formation than the Arthritis group (p=0.006, p=0.048, p=0.004, and p=0.031, respectively) (Table 1).

Histopathological findings

Light microscopic examination of paw tissue samples from the Control group showed a normal histological appearance of the articular cartilage surrounding the distal bone. In the Arthritis group, marked cellular infiltration, pannus formation, and bone resorption were observed. Pannus formation

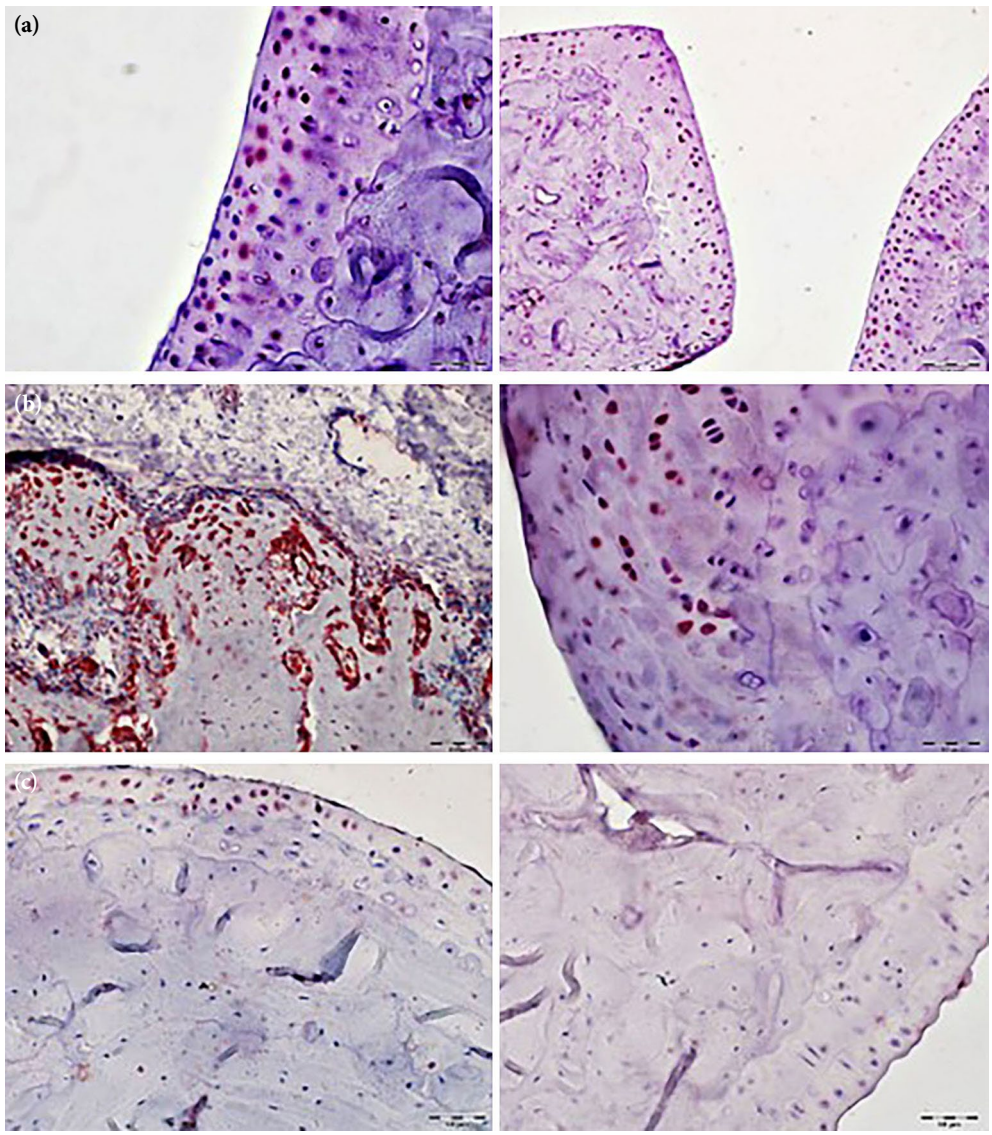

Figure 4. Immunohistochemical evaluation of leptin receptor expression in paw tissues. **(a)** Control group: weak expression in articular cartilage cells, bone cells, bone marrow endothelial cells, and adipocytes. **(b)** Arthritis group: markedly increased expression in articular cartilage and bone cells, with strong positivity in bone marrow adipocytes and inflammatory cells. **(c)** Exercise + Arthritis group: weak leptin receptor expression, with minimal staining in articular cartilage cells and bone marrow adipocytes.

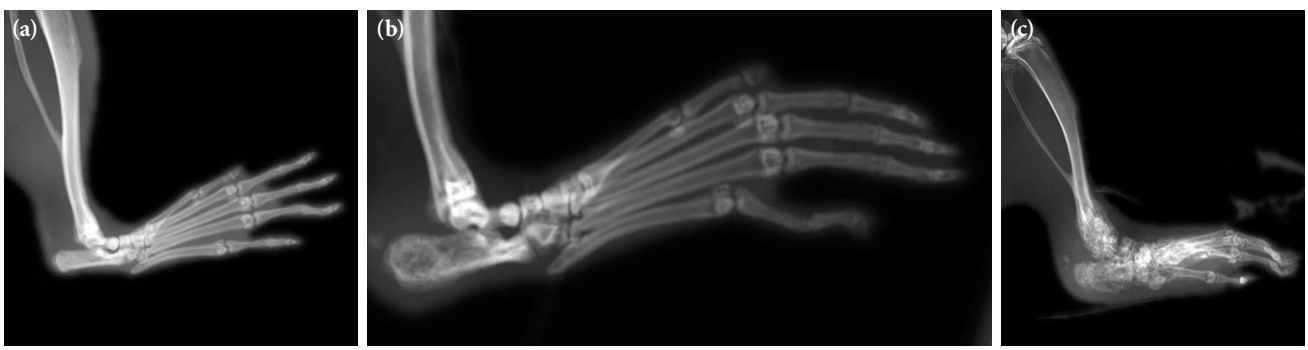

Figure 5. Radiological images of animal paws. **(a)** Control group; **(b)** Exercise + Arthritis group; **(c)** Arthritis group.

TABLE 1
Comparison of radiological, histopathological, and immunohistochemical scores between the Arthritis and Exercise + Arthritis groups

	Arthritis group		Exercise + Arthritis group		
	Median	IQR	Median	IQR	*p*
Radiological scores (according to visual scoring, scores ranging from 0 to 3)					
Soft-tissue swelling	3.00	1.00	0.00	2.00	0.006*
Cartilage loss	2.00	2.00	0.00	2.00	0.108
Osteoporosis	2.00	1.00	1.00	2.00	0.064
Bone erosion	2.00	2.00	0.00	3.00	0.048*
Heterotopic ossification	2.50	2.00	0.00	0.00	0.004*
Periosteal new bone formation	3.00	1.00	1.00	3.00	0.031*
Histopathological scores (scores ranging from 0 to 5)					
Cellular infiltration	4.00	1.00	2.00	1.00	0.009*
Pannus formation	4.00	0.00	1.50	3.00	0.041*
Bone resorption	4.00	1.00	1.50	3.00	0.041*
Total histopathological score	12.00	2.00	5.00	7.00	0.015*
Immunohistochemical score					
H-score	2.36	1.10	1.40	0.35	0.009*

IQR: interquartile range; * p<0.05, Mann-Whitney U test.

was accompanied by complete loss of the underlying cartilage and bone layers, together with intense inflammatory infiltrates. In the Exercise + Arthritis group, mild inflammatory cell infiltration was observed, with pannus formation in some specimens and mild edema (Figure 6). A statistically significant difference was observed compared to the Arthritis group (p=0.015) (Table 1).

DISCUSSION

In this experimental study, we investigated the effects of swimming exercise on RA severity, radiological joint damage, and leptin-related molecular changes in a rat model. The main finding of this study was that the severity of arthritis induced in trained rats using the adjuvant-induced arthritis (AIA) model was lower, showing significant

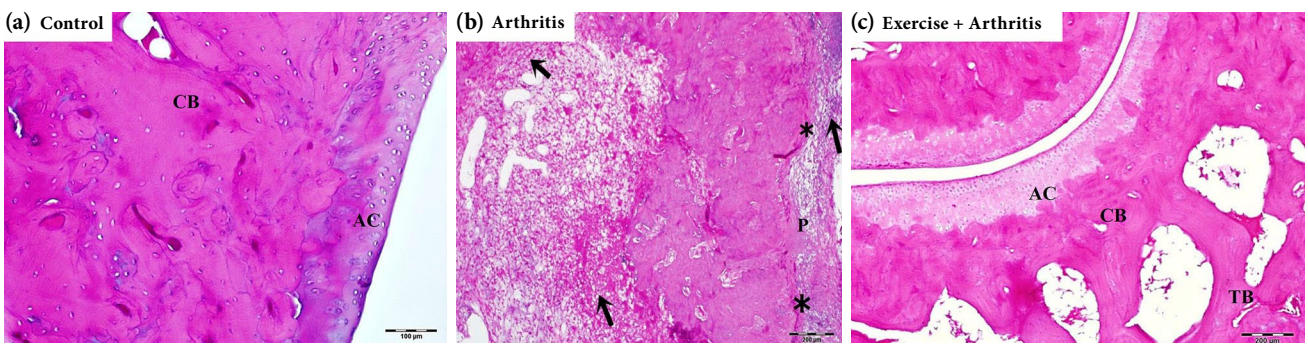

Figure 6. Histopathological images of the paw tissue. In images a, b, and c, histopathological images demonstrate differences in inflammatory infiltration, pannus formation, and bone destruction among the groups. **(a)** Histopathological section from a rat in the Control group, showing normal joint architecture without inflammatory infiltration or tissue destruction. **(b)**Histopathological section from a rat in the Arthritis group. Severe areas of infiltration (arrow), disruption in joint structure, and loss of cartilage and compact bone were observed. **(c)** Histopathological section from a rat in the Exercise + Arthritis group. Mild areas of infiltration (arrow) and moderate tissue damage in the joint and compact bone were observed.
P: Pannus; AC: Articular cartilage; CB: Compact bone; TB: Trabecular bone.

long-term improvement. This finding was clearly supported by ankle joint diameter measurements and visual arthritis scores, as well as by radiological imaging scores, all of which demonstrated a reduction in arthritis severity in the Exercise + Arthritis group compared to the Arthritis group. In addition, histopathological evaluations confirmed that regular swimming exercise alleviated inflammation and tissue damage associated with arthritis. Taken together, these results suggest that moderate-intensity swimming exercise has protective and therapeutic effects in the progression of experimental RA in rats.

Exercise has been widely recognized to modulate the immune system. Dorneles et al.[30] investigated systemic cytokine levels, $CD4^+$ T-cell subtypes, and monocyte frequencies in obese and lean individuals with varying levels of exercise-induced cardiorespiratory fitness. The study also evaluated serum concentrations of IL-6, IL-10, IL-17A, IL-33, leptin, and TNF-α. More importantly, the findings demonstrated that higher cardiorespiratory fitness was a key determinant of maintaining regulatory T cells (Tregs) and memory regulatory T cells (mTregs), as well as of preserving higher proportions of intermediate and non-classical monocytes and effector memory T cells (mTeffs).[30] These results highlight the immunoregulatory benefits of exercise in promoting a balanced inflammatory response. Similarly, Fang and Tang[31] reported that swimming exercise could prevent aortic dysfunction induced by a high-fat diet, and this protective effect was associated with reductions in oxidative stress and proinflammatory cytokines/adipokines. A high-fat diet increased circulating levels of proinflammatory adipokines such as leptin and resistin, whereas 16 weeks of swimming exercise markedly attenuated these effects. Moreover, exercise reduced the expression of proinflammatory cytokines, including IL-6 and IL-8.[31] These findings further support the notion that regular physical activity exerts systemic anti-inflammatory and antioxidant effects, which may contribute to improved vascular and metabolic health.

In our study, a moderate-intensity swimming exercise program was implemented for six weeks. In previous studies, moderate-intensity swimming exercise has been reported to be safe for patients with RA.[6,32] In addition to improving aerobic capacity and muscle strength, swimming exercise has been shown to modulate the immune system in individuals with RA. Several studies have also demonstrated that regular swimming exercise may regulate immune function by decreasing proinflammatory cytokines such as TNF-α and IL-6, while increasing anti-inflammatory cytokines such as IL-4 and IL-5.[21,33] Moreover, swimming exercise has been found to alleviate many of the symptoms of the disease (such as pain, joint stiffness, and muscle weakness) and, consequently, improve overall quality of life.[7,8]

On the other hand, due to financial constraints, we were unable to analyze serum cytokine levels in our study. Therefore, the assessments were limited to leptin, MMPs, and leptin receptor expression. The reduced severity of arthritis signs observed in our study may be associated with decreased proinflammatory cytokine activity, as reported previously. The experiment was primarily designed to examine the effects of swimming exercise on the onset and progression of arthritis. The finding that arthritis became less severe over time is noteworthy and may indicate a delayed protective effect of prior exercise. This observation warrants further investigation to clarify the underlying mechanisms and to determine whether similar outcomes can be reproduced in other experimental models of RA.

Synovial inflammation plays a pivotal role in the pathogenesis of RA. Along with dense cellular infiltration in the synovial layer, this tissue gradually becomes hypertrophic. The synovial membrane forms the characteristic pannus tissue, which invades the underlying bone and cartilage. Within the pannus, mononuclear cells and fibroblast-like cells producing MMPs contribute to the degradation of articular cartilage and subchondral bone structure.[34] In our study, histopathological changes typically observed in RA, including cellular infiltration, pannus formation, and bone resorption, were evaluated. Histological examination of the paw tissues in the Arthritis group revealed intense inflammation. The inflammatory cell infiltration led to full-thickness loss of cortical bone and marked trabecular bone resorption. In contrast, the paws of rats in the Exercise + Arthritis group showed only mild edema with minimal destruction in both cortical and trabecular bone. Compared to the Arthritis group, arthritic rats subjected to exercise demonstrated a significant reduction in cellular infiltration, pannus formation, and bone resorption. Similarly, Fujii et al.[21] reported that exercise attenuated the destructive joint changes associated with arthritis, as demonstrated by histopathological analyses. Cifuentes et al.[35] also showed that exercise helps preserve articular cartilage. Consistent with these findings, the present

study demonstrated that regular swimming exercise reduced joint inflammation and joint damage in an experimental arthritis model.

Matrix metalloproteinases are synthesized by a wide range of cells, including fibroblasts, osteoblasts, endothelial and vascular smooth muscle cells, and immune cells such as macrophages, neutrophils, and lymphocytes. By degrading extracellular matrix components, MMPs significantly contribute to cartilage and bone destruction in the pathogenesis of arthritis.[36] In the Exercise + Arthritis group, the reduction in visual arthritis scores, as well as radiological and histopathological findings, may be associated with decreased MMP activity. However, no significant differences in plasma MMP levels were observed in our study. Two possible explanations can account for this finding. First, the presence of natural tissue inhibitors of metalloproteinases (TIMPs) plays a critical role in regulating MMP activity locally. The TIMPs suppress MMP-mediated extracellular matrix degradation by directly binding to active MMPs.[36] Second, exercise may have specifically reduced MMP activity within joint tissues rather than in circulation. Since our study did not assess TIMP expression or tissue-specific MMP activity, further investigations are warranted to clarify the regulatory relationship between exercise, TIMPs, and MMP-mediated tissue remodeling in arthritis.

In the present study, plasma leptin levels, considered a proinflammatory adipokine, were evaluated. However, no significant differences were observed between the groups. Several studies in the literature have reported that exercise reduces leptin levels.[12-14] Conversely, other studies have demonstrated an increase in leptin concentrations following exercise.[16] In our study, exercise did not affect leptin levels; however, the Exercise + Arthritis group exhibited lower leptin receptor expression compared to the Arthritis group. These findings indicate that swimming exercise may attenuate the arthritis-related upregulation of leptin receptors and potentially modulate intracellular signaling molecules involved in leptin receptor pathways. Nevertheless, the effects of exercise on leptin regulation still remain unclear, and further comprehensive studies are required to clarify the underlying mechanisms.

In the current study, radiological evaluations revealed that soft-tissue swelling, erosion, heterotopic ossification, and periosteal new bone formation were significantly lower in the Exercise + Arthritis group than in the Arthritis group. These findings suggest that regular swimming exercise may help reduce cartilage and bone damage associated with arthritis. The beneficial effects of swimming were further confirmed by histopathological analysis, which demonstrated a significant reduction in cellular infiltration, pannus formation, and bone resorption. The decreased infiltration of inflammatory cells consequently led to reduced inflammatory joint destruction.

The present study has several limitations. First, as cytokine levels which play a role in RA pathogenesis could not be measured, the mechanisms by which swimming exercise influences arthritis severity and radiological damage could not be fully elucidated. Another limitation is that the molecular effects of leptin on intracellular signaling pathways were not investigated. Previous studies have reported that leptin promotes IL-8 production in synoviocytes via the janus kinase/signal transducer and the activator of the transcription (JAK/STAT) pathway, thereby stimulating angiogenesis and chemotaxis, contributing to immune regulation, and ultimately enhancing joint destruction in RA.[37] A third limitation is that, by its experimental nature, this study differs from human research. Therefore, the findings should be validated in clinical studies involving patients with RA.

On the other hand, our study also has certain strengths. To the best of our knowledge, no previous study has investigated the effects of a swimming exercise program initiated before arthritis induction and continued for four weeks thereafter on arthritis severity and radiological damage in RA and this is the first study in the literature.

In conclusion, our study results indicate that moderate-intensity, regular swimming exercise provides both protective and therapeutic benefits in an experimental arthritis model in rats. As a low–mechanical load activity, swimming may offer a meaningful potential as a complementary strategy for managing RA in humans, as well. Notably, initiating exercise prior to disease onset was associated with reduced disease severity and delayed progression, suggesting its value as a preventive approach for individuals at high risk. Overall, moderate-intensity swimming attenuated inflammation, radiological damage, and histopathological deterioration, while the rats subjected to pre-induction exercise displayed markedly lower arthritis severity. These

effects may be mediated, at least in part, through modulation of leptin receptor activity and related signaling pathways. Although the precise molecular mechanisms still remain to be clarified, regular swimming exercise appears to be a promising non-pharmacological option for limiting joint damage and slowing disease progression in RA.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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