Unlocking Resilience: How Endurance Resistance Training Transforms Knee Cartilage in Young Male Rats

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Abstract

Objective: This study examines how endurance resistance training affects the thickness of young male rats' knee joint hyaline cartilage. These results can later be replicated for the benefit of humans, and an appropriate exercise-based plan can be designed accordingly.

Methods: Twelve healthy young male rats were divided into an endurance exercise group (EG) and a sedentary control group (CG). The EG group climbed a ladder with weights, starting at 5% of their body weight and increasing to 40% over five weeks. Each session included 12-15 repetitions, 2-minute breaks, and lasted 30 minutes, five days a week.

Results: It was discovered that the EG had considerably thicker femoral hyaline cartilage than the CG ($p=0.0048$, Rt side; $p=0.0049$, Lt Side). The effects on tibial hyaline cartilage thickness were also significantly favoring EG over CG ($p<0.001$ on both sides).

Conclusion: This study indicates that endurance resistance exercise significantly improved femoral and tibial hyaline cartilage thickness parameters. These results imply endurance resistance training can enhance cartilage integrity and general health. These results support our hypotheses and point to promising directions for future research in exercise-based interventions for cartilage health. In particular, our findings imply that resistance training with endurance may benefit both hyaline cartilages in the knee joint of young male rats.

Key Words: Cartilage, Endurance training, Exercise, Hyaline Cartilage, Knee Joint, Rats, Resistance training.

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Introduction

Rat knee joints are smaller and have quadrupedal movement, however they are anatomically similar to human knee joints. It is composed of the patella, femur, tibia, and fibula, with articular cartilage covering the ends of the bones. Stability is ensured by ligaments, including the medial and lateral collateral ligaments and the anterior and posterior cruciate ligaments. The medial and lateral menisci act as shock absorbers[1]. Muscles such as the gastrocnemius, hamstrings, and quadriceps promote movement. The synovial membrane and fluid nourish and lubricate the joint. Although the basic characteristics are similar to those of humans, the rat's physiology may require specific changes in size and form, which emphasizes the relevance of recognizing these details for efficient research and veterinary care[2].

As the knee joint is structured to support the weight of the body, soft tissue structures serve a critical function in maintaining the joint's range of

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motion[3]. Cartilages are avascular, aneural, and alymphatic connective tissues that are located in the ribs, spine, and synovial joints. There are three main types of cartilage: hyaline, fibrocartilage, and elastic cartilage[4]. Hyaline cartilage is the most common type found in the human body and has a glassy appearance. Considering water makes up between 60 and 85% of its weight, it is a key lubricant that prevents friction between the articulating surfaces[5].

Various types of collagen molecules are linked together to form an inner filamentous template of hyaline cartilage that combines to form a network and accounts for the dry weight of the tissue. The filamentous structure of hyaline cartilage as described in several studies is formed by the covalent linkage of type II collagen fiber with the surface type IX and XII collagen, providing the network heteropolymeric structure thereby enabling tissue to bear tensile and shear strength[6]. Besides this, aggrecan which is the dominant form of proteoglycan that comprises more than 100 chondroitin sulfate glycosaminoglycan (GAG) chains and a keratan sulfate chain provides a bottle brush structure to cartilage. The formation of a GAG chain provides a negative charge to the structure that creates repulsive interaction between two GAG chains at the molecular level. This repulsive interaction at the molecular level provides compressive strength to the structure thereby enabling the cartilage to act as a load-bearing tissue in the joints[4].

Growth plates and articulating surfaces of synovial joints both are made of hyaline cartilage[7]. It also serves as a basis of support for several structures, including the larynx, trachea, bronchi, and nose. Joint mobility is achieved by articulating hyaline cartilage, which offers lubricating surfaces with a comparatively low coefficient of friction of 0.001 to 0.01[4, 8]. Hyaline cartilages have various functions ranging from weight bearing to lubrication and structural support. These functions necessitate a well-organized structure of the extracellular matrix (ECM), which plays a key role in determining its overall functionality[9,10]. Therefore, the disease progresses quickly in any cartilage-affecting disorder that modifies the ECM. This is observed in diseases like osteoarthritis (OA), where changes to the ECM increase the primary markers of the condition. In addition, hereditary or genetic mutations may result in disorders like dwarfism. Moreover, pathogenic processes can cause secondary effects, which are evident in inflammatory arthropathies and osteochondritis dissecans[4]. Various factors like delay in the diagnosis of cartilaginous disease, poor understanding of the etiology and causative agents, and aneural nature of cartilaginous tissue along with its avascular form; limit appropriate drug delivery thereby creating severe challenges in its management[11,12].-

A comprehensive evaluation of various studies has demonstrated the importance of exercise in improving muscular strength and reducing the range of clinical symptoms associated with different cartilaginous diseases[13,14]. Additionally, it has been shown that exercise-based therapeutic strategies improve muscular strength, and aerobic capacity, and reduce discomfort during functional movement in a variety of inflammatory conditions affecting joints and cartilages[15]. Studies have suggested various forms of intermittent load-bearing and dynamic exercises have chondroprotective effects, preventing structural deregulation of joints, and multiple clinical guidelines advocate for exercises as an integral component of conservative management for complications affecting the cartilage and articular components of weight-bearing joints[14,16]. The purpose of this research is to examine how young male rats' knee joint hyaline cartilage thickness is affected by endurance resistance training.

Methodology

Young male Sprague Dawley rats were sourced from the International Center for Chemical and Biological Sciences at the University of Karachi. They were housed in temperature-controlled cages at Ziauddin College of Physical Therapy, with a regular day/night cycle and free access to food and water. The exercise training for the rats was conducted at the same facility. Histological procedures and slide analysis were conducted in the Cell Biology and Histology Lab at Ziauddin University, Clifton Campus. The rats weighed between 200 and 300 grams and were healthy young males; those with mobility issues or injuries were excluded from the study.

Twelve young male Sprague Dawley rats weighing between 200 and 300 grams were divided equally into two groups using a simple random sampling procedure: Endurance resistance training (EG) and Sedentary or Control (CG)[17]. All of the male rats enrolled in the EG carried the weight as they climbed the ladder; it was attached to the proximal end of the tail. The stairs were 2 cm apart and had an 80° inclination at a height of 110 cm[18]. For EG, an initial adaption phase of one week was completed with three sets of 5% of each rat's body weight. 10% of the body weight was the starting load to be carried and 3 sets in week two. From that point until week 5, the exercise regimen included 20% of body weight for four sets, 30% for five sets, and 40% for six sets each week. 12–15 repetitions were performed in each set, with a 2-minute break in between. No training was given to CG. All rats were subjected to euthanasia within 48 hours of the fifth week's completion. The exercise group had a one-week adaptation period before the whole five-week training protocol started[19]. Exercise training was performed five days a week, excluding weekends. The total duration of each training session was thirty minutes.

Knee joints were obtained by dissecting the lower limbs from the femur to the tibia and fibula. After removing the calcium from the bones, the cartilage was placed in a sealed jar with Bouin's fixative. The samples then underwent xylene clearing, alcohol dehydration, and paraffin embedding. Thin sections (5 mm) were cut and stained with Hematoxylin and Eosin (H&E) on glass slides. After mounting, they were examined under a Nikon INTENSE LIGHT C-HGFI microscope. Morphometric analysis of hyaline cartilage thickness in the femur and tibia was done using NIS Elements software.

Version 25 of the SPSS (Statistical Package for Social Sciences) was used to conduct the analysis. The mean and standard deviation were used to express all numerical variables. To compare numerical variables, an independent sample t-test was performed. All statistical values were considered significant at a p-value of 0.05. The University's Animal Ethics Committee provided ethical permission (Protocol No. 2022-06/KY/ZCRS) before the study's commencement. Throughout the intervention, animal ethics procedures were followed properly.

Results

Significant improvement in femoral hyaline cartilage thickness was observed in the EG with a mean and SD of 43.95 ± 13.44 (Rt side) and 48.93 ± 15.88 (Lt Side) as compared to CG with 23.29 ± 4.01 (Rt side) and 25.15 ± 3.22 (Lt Side) (p=0.0048, Rt side; p=0.0049, Lt Side). (Table-1; Figure-1 & 2)

The effects on tibial hyaline cartilage thickness were also significant favoring EG (Mean and SD 75.56 ± 8.36 Rt Side; 77.40 ± 8.54 Lt Side) over CG (41.66 ± 5.24 Rt Side; 43.49 ± 6.50 Lt Side) (p<0.0001 both sides). (Table-1; Figure-1 & 2)

Table 1. Impact of Endurance Resistance Training on Hyaline Cartilage in the Knee Joint

Variable	Sample Size(n)	Control Group (Mean±S.D)	Exercise Group (Mean±S.D)	p-value
Femoral Hyaline Cartilage				
Average Cartilage Thickness (Right Knee)	6	23.29 ± 4.01	43.95 ± 13.44	p = 0.0048*
Average Cartilage Thickness (Left Knee)	6	25.15 ± 3.22	48.93 ± 15.88	p = 0.0049*
Tibial Hyaline Cartilage				
Average Cartilage Thickness (Right Knee)	6	41.66 ± 5.24	75.56 ± 8.36	p<0.0001*
Average Cartilage Thickness (Left Knee)	6	43.49 ± 6.50	77.40 ± 8.54	p<0.0001*

*p<0.05

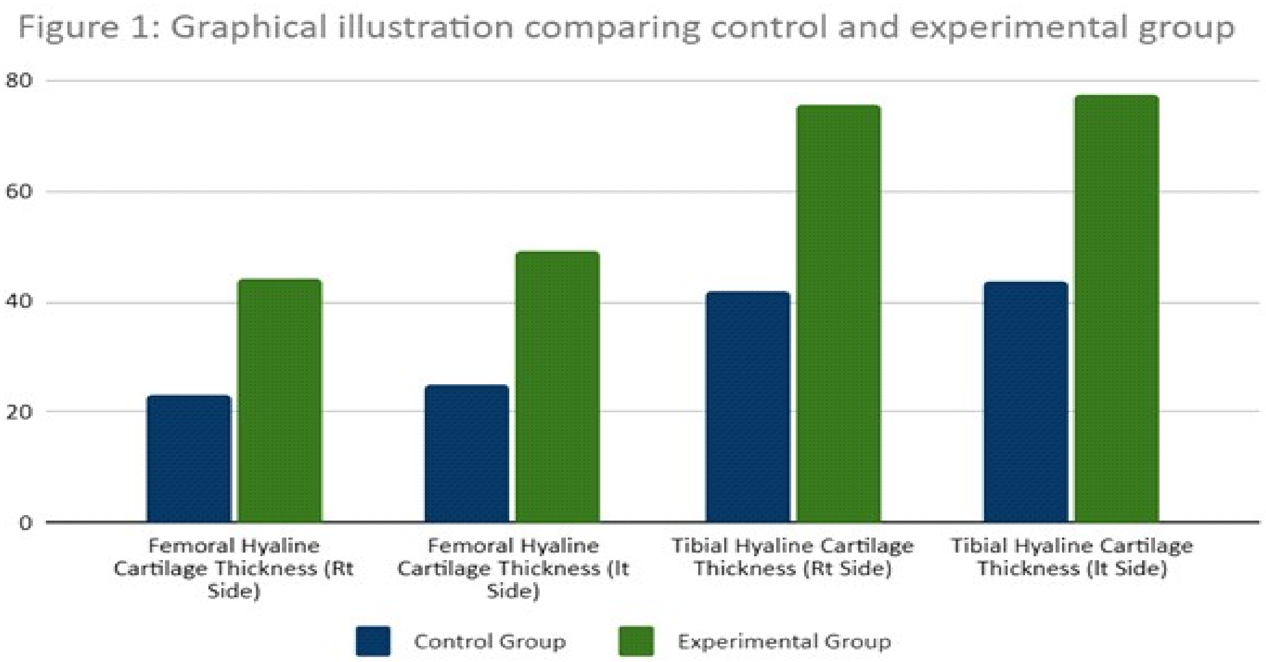
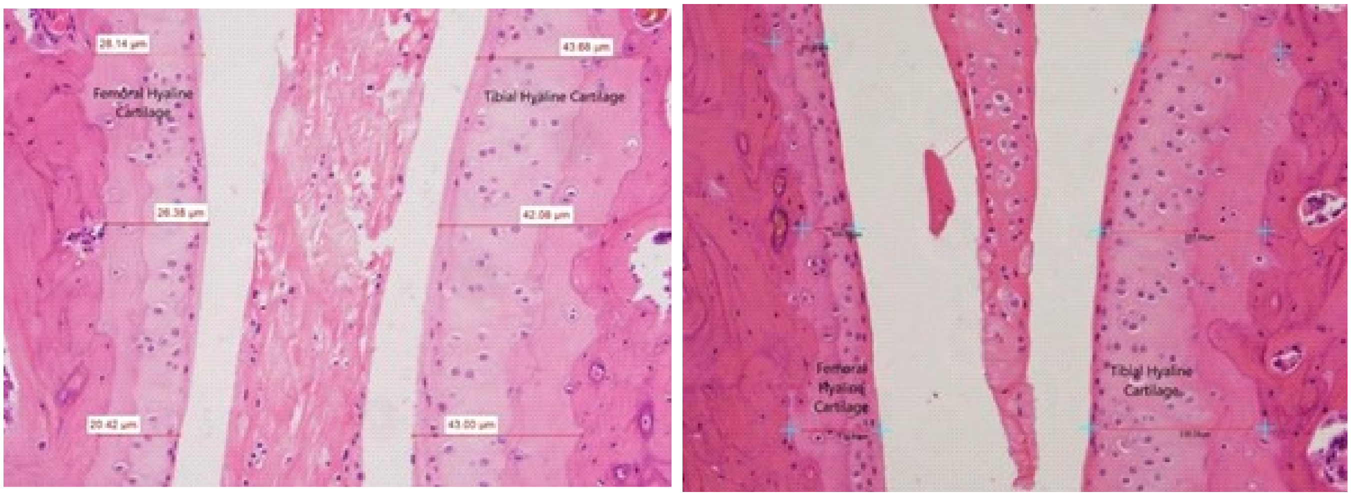


Fig 1. Femoral and Tibial Hyaline Cartilages Thickness

Figure 1 graphically depicts the significant difference in the femoral and tibial hyaline cartilage thickness between the two groups.



Control

Exercise

Fig 2. Femoral and Tibial Hyaline Cartilages Thickness

Figure 2 shows the difference in the femoral and tibial hyaline cartilage thickness between the two groups.

Discussion

This study aimed to measure how exercise affects the thickness of hyaline cartilage in the knee joint. The exercise group showed a significant improvement in cartilage thickness compared to the control group. Our results suggest that endurance resistance exercise positively impacts the hyaline cartilage in the femur and tibia, indicating potential benefits for cartilage health and integrity. These findings highlight the need for more research on osteoarthritis and joint health in both animal models and humans.

A mechanical model of osteoarthritis (OA) was employed to investigate the impact of two common physical activities—swimming and treadmill walking—on the morphological features associated with OA. The study involved 48 male Wistar rats, which were divided into four groups: OA + Swimming (S), OA + Treadmill (T), Osteoarthritis (OA), and a Sham group (no OA induction). The results showed that the cartilage thickness didn't vary significantly across the groups[20]. A study conducted by Naeini et al. explored the impact of running on an inclined treadmill on osteoarthritis (OA) and investigated how training influenced cartilage thickness over six weeks. The results showed that the exercise group had lesser femoral thicknesses than the control group. This contributed to the conclusion that exercising beyond the adaptive threshold limit had an adverse effect rather than beneficial and that exercise must be done within the adaptive threshold limits. In comparison, our study revealed that exercise had a significant effect on the thickness of femoral hyaline cartilage. The exercise intervention in this study appeared to benefit the cartilage[21].

Zhou conducted a study to find out how exercise affected the metabolism of cartilage cells. It was determined that after eight weeks of training, the cartilage thickness of the exercise group had significantly improved in comparison to the control group. It further identified that activities improve cartilage thickness but should be performed within the adaptation limit as disregarding the barrier of adaptation limit during exercises may directly aggravate cartilage deterioration[22]. Qian et al. investigated the effects of passive motions on articular cartilage in osteoarthritic rats, examining changes in cartilage thickness at different time intervals. The results showed that in the sixth week, the group that did passive exercises had significantly increased their cartilage thickness in comparison to the control group. As a result, in addition to having to repair the impacts on degenerative cartilage, passive motion exercises also had to enhance the morphology of degenerating structures and, over time, lower the incidence of OA[23].

In another study, Ni et al. examined how different intensities of treadmill running affected knee articular cartilage in a rat model over eight weeks of training. They found that the moderate-intensity running group showed a significant increase in cartilage thickness compared to the control group[24]. Similarly, Neves et al. found that following a 14-day intervention to increase cartilage thicknesses, animals in the exercise group responded better. Consistent with this, our study suggests that exercise can enhance the integrity and health of the hyaline cartilage in the knee joints by increasing the thickness of the femoral and tibial hyaline cartilage in both knees[25]. These findings align with a study that investigated the impact of various mechanical loading regimes on an in vitro model of microfracture healing employing fibrin gel scaffolds containing mesenchymal stem cells (MSCs). This study revealed that dynamic compressive loading significantly enhanced the integration of repair tissue, strengthening the bond between newly generated cartilage tissue and the original tissue around it. Additionally, compression loading increased the expression of chondrogenic genes and favored a more hyaline-like cartilage phenotype, pointing to an environment that is conducive to cartilage development and repair. Conversely, shear loading had a less pronounced impact on the expression of chondrogenic genes and even resulted in the overexpression of catabolic markers, perhaps impeding the repair procedure. Drawing connections between these findings and our study, it can be inferred that early weight-bearing activities following microfracture surgery may help to promote the formation of higher quality cartilage repair tissue, whereas passive motion exercises without weight-bearing may not be as beneficial and may even negatively affect the formation of repair tissue during postoperative rehabilitation[26]. Therefore, incorporating dynamic compressive loading in rehabilitation protocols could be beneficial for optimizing cartilage repair outcomes.

Our research, along with previous studies, shows that exercise affects cartilage thickness based on factors like the type of exercise, the animal model (such as OA, RA, or kOA-like), and the specific cartilage being studied. While femoral and tibial hyaline cartilages in the knee were positively affected by our investigation, other studies on OA and RA also showed positive results under various conditions. These variations emphasize the importance of personalized exercise recommendations that take into account the specific characteristics and type of cartilage. They highlight that exercise may help maintain cartilage health in various arthritis conditions, despite different underlying mechanisms. To better understand these mechanisms and determine their practical effectiveness for human patients, further research is necessary.

A key strength of the study is using young male rats as a controlled model, which helps avoid challenges with human subjects. This method allowed researchers to assess how exercise influences the histology of knee joint cartilage. Additionally, there was a greater likelihood of increased physical activity among the young rats. To better understand exercise effects on cartilage health, researchers conducted a detailed analysis of knee joint cartilage. However, the use of only young male rats limits how the findings can apply to broader human populations. Moreover, since all the young male rats were identical in age, gender, and species, it complicates the generalization of the results to a wider demographic.

Moreover, the brief duration of the study might not accurately represent the enduring consequences of resistance training on the long-term effects of the reported modifications. Future studies should include larger sample sizes and evidence-based trials with functional testing to gain a thorough understanding of how exercise affects cartilage. These findings could potentially inform clinical recommendations for human exercise regimens.

Conclusion

This study shows that in young male Sprague Dawley rats, endurance resistance training significantly increases the thickness of hyaline cartilage in the knee joints. The findings show an advantageous association between cartilage health and exercise, indicating that regular physical activity may be essential for preserving and enhancing the integrity of knee joint cartilage. Although the results are consistent with previous research on the advantages of exercise for cartilage health, the sample size and duration constraints highlight the need for more studies. Following that, studies needed to explore the enduring impact of resistance training and its underlying mechanisms, in addition to examining diverse exercise techniques and their influence on various types of cartilage. These findings could affect clinical guidelines for exercise regimens that protect cartilage and decrease joint-related disorders.

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Explanations: None.

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