Effects of endurance resistance exercise on knee joint cartilages in young male rats – a randomized controlled trial

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SUMMARY

To study the effects of endurance resistance exercise on the cellularity of knee joint hyaline cartilages and fibrocartilage in young male rats, twelve healthy young male Sprague Dawley rats were equally divided into Endurance resistance training (ERT) and Sedentary or Control (C). ERT group climbed a ladder carrying 5% of body weight with 3 sets for adaptation. From week 2, 10% and 3 sets then progressed to 20% and 4 sets, 30% and 5 sets, and 40% of the body weight and 6 sets every week till week 5 with 12-15 repetitions per set and 2-minute intervals and 5 days per week. Each training session's duration was 30 minutes. The effects on the number of chondrocytes per lacuna of fibrocartilage and tibial hyaline cartilage were found to be significantly better in ERT group. For the number of chondrocytes per lacuna of femoral hyaline cartilage, no significant difference was observed between the group analyses. These results collectively imply that endurance resistance training has potential for enhancing cartilage integrity and general health, specifically the fibrocartilage and tibial hyaline cartilage.

Key words: Endurance – Resistance – Exercise – Hyaline cartilage – Fibrocartilage

INTRODUCTION

Cartilage is a pliable, smooth, elastic tissue that is firm and less flexible than soft tissues like muscle, ligament, and tendon, but not as stiff and solid as bone (Zhang et al., 2018). Hyaline (e.g., articular cartilage), elastic (e.g., ear cartilage), and fibrous (e.g., intervertebral disc) cartilage are the types present in the human body (Lin and Klein, 2021). Hyaline cartilage, or articular cartilage (AC), is a highly specialized connective tissue that lines the articular surfaces of bones (Branly et al., 2017). AC lacks vascularity, lymphatics and nerve supply (Nie et al., 2020). AC enables bones to move over one another, reduces friction and protects from damage in lubricated joint movements (Costa et al., 2018). The fibrocartilage plates in the knee joint are known as the menisci. These circular wedge-shaped cartilages are located in pairs between the tibial plateaus and the femoral condyles, laterally and medially, respectively (Murphy et al., 2019). Due to meniscal

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injury, articular cartilage may be subjected to excessive biomechanical pressures (Favero et al., 2019). Deprivation of the meniscus puts the knee joint at risk of degeneration (Rhim et al., 2021). The mainstay of treatment in degenerative cartilage disorders is prevention via exercise and physiotherapy (Colella et al., 2020). Traditional exercise programs, including resistance, aerobic and flexibility training, and non-traditional exercises, including tai chi, yoga, and aquatic exercise programs, have successfully demonstrated relief (Wellsandt and Golightly, 2018). The morphological effects of therapeutic exercise on the individual components of articular cartilage of the knee joint need to be better recognized (Bricca et al., 2019). Although exercise is extensively suggested for preventing joint deterioration, the diversity of exercise choices evaluated is far broader. There is inadequate literature to advocate a particular exercise over another; thus, patients and clinicians are looking for guidelines on the "optimal" exercise and its frequency, intensity, time and type (FITT) (Kolasinski et al., 2019).

Zhou (2004) studied knee joint articular cartilages of male Sprague-Dawley (SD) rats using low, medium and high-intensity exercises for 8 weeks, 1 hour per day. The results showed that moderate-intensity active exercise can enhance cartilage repair and delay degeneration, while high intensity may increase damage (Zhou, 2004). In addition, Yao et al. (2019) investigated that running at high speed or intensity, rather than long-distance running, may cause cartilage degeneration in female mice (Yao et al., 2019). Furthermore, Moshtagh et al. (2018) added that intense running can result in mild cartilage degeneration and the study was done in male Wistar rats (Moshtagh et al., 2018). On the other side, Lee et al. (2011) studied the histology of medial knee joints of treadmill-running Wistar rats using a modified Mankin system (MMS) and Osteoarthritis Research Society International (OARSI) scores. MMS scores showed a greater prevalence of mild OA at 6 weeks and moderate OA at 10 weeks in the exercise group than in the control group. There were significantly higher OARSI scores in the exercise group than the control group at 10 weeks for both femoral and tibial cartilages. Therefore, this literature recognized that treadmill-running exercise can lead to the development of OA in rats (Lee et al., 2011). As the evidence regarding the effects of exercises on cartilage is enormous, literature on improving chondrocytes number is limited. Therefore, this study aims to determine the effects of endurance resistance exercise on the cellularity of knee joint hyaline cartilages and fibrocartilage in young male rats.

MATERIALS AND METHODS

Animal model selection and study setting

Young male Sprague Dawley rats were obtained from the Animal House of the International Centre for Chemical and Biological Sciences, University of Karachi. They were kept in cages at room temperature while maintaining a day and night cycle and were given ad libitum water and feed at Ziauddin College of Rehabilitation Sciences. The rats performed the exercise training at the same location. The histological procedures and microscopic examination of slides were performed at the Cell Biology and Histology Lab, MDRL-2, Ziauddin University, Clifton Campus. Sprague Dawley healthy young growing male rats and rats weighing 200 to 300 grams were included. Unhealthy rats having ambulation problems or any injury were excluded.

Randomization

A total of n=12 healthy young male Sprague Dawley rats weighing 200 to 300 grams were equally divided into two groups: Endurance resistance training (ERT) and Sedentary or Control (C) through simple random sampling technique (Charan and Biswas, 2013; Luciano et al., 2017).

Interventions Protocol

Generally, all the male rats enrolled in the ERT climbed the ladder carrying the load, which was tied to the proximal end of the tail. Height was 110 cm with 80° inclination and 2 cm distance between the steps (Neto et al., 2019). For ERT, initially, a one-week adaptation period was completed carrying 5% of individual body weight with 3 sets. The initial load to be carried and sets from week 2 were 10% and 3 sets, then progressed to 20% and

4 sets, 30% and 5 sets, and 40% of the body weight and 6 sets every week till week 5. There were 12-15 repetitions per set and 2-minute intervals in between. For Control, no training was done. At the end of the 5th week, all rats were euthanized within 48 hours. The total training protocol period was 5 weeks, including an initial one-week adaptation period for the exercise group (Mazor et al., 2019). The frequency of exercise training was 5 days per week, excluding weekends. Each training session's duration was 30 minutes.

Histological Evaluation

The knee joints were harvested en bloc by dissecting the shaft of lower limbs from the shaft of the femur to the shaft of the tibia/fibula. The harvested cartilage tissues were kept in a sealed plastic jar containing Bouin's fixative, and bone decalcification was done. The tissues were dehydrated with alcohol, cleared with xylene and fixed in paraffin. 0.4 um sections were taken on glass slides, and Haematoxylin and Eosin (H&E) staining was performed. After mounting the tissue, the slides were examined under a Nikon INTENSE LIGHT C-HGFI microscope. Microscopic images were analysed, and morphometry was performed by using NIS Elements software. The number of chondrocytes per lacuna was assessed in fibrocartilage and hyaline cartilages of the femur and tibia.

Data Analysis

The analysis was performed on SPSS (Statistical Package for Social Sciences) version 25. All numerical variables were expressed in mean and standard deviation. An independent sample t-test was applied to compare numerical variables. A p-value of <0.05 was considered significant in all statistical values.

Ethical Considerations

Ethical approval was obtained from the Animal Ethics Committee of Ziauddin University (Protocol No#2022-06/KY/ZCRS) before starting research. The protocols of animal ethics were followed throughout the intervention.

RESULTS

The effects of exercises on fibrocartilages were identified, and the findings were analysed on the number of chondrocytes per lacuna. The results provided evidence that the outcome measure was significantly $p<0.05$ better in the experimental group. (Table 1, Figs. 1a and 2).

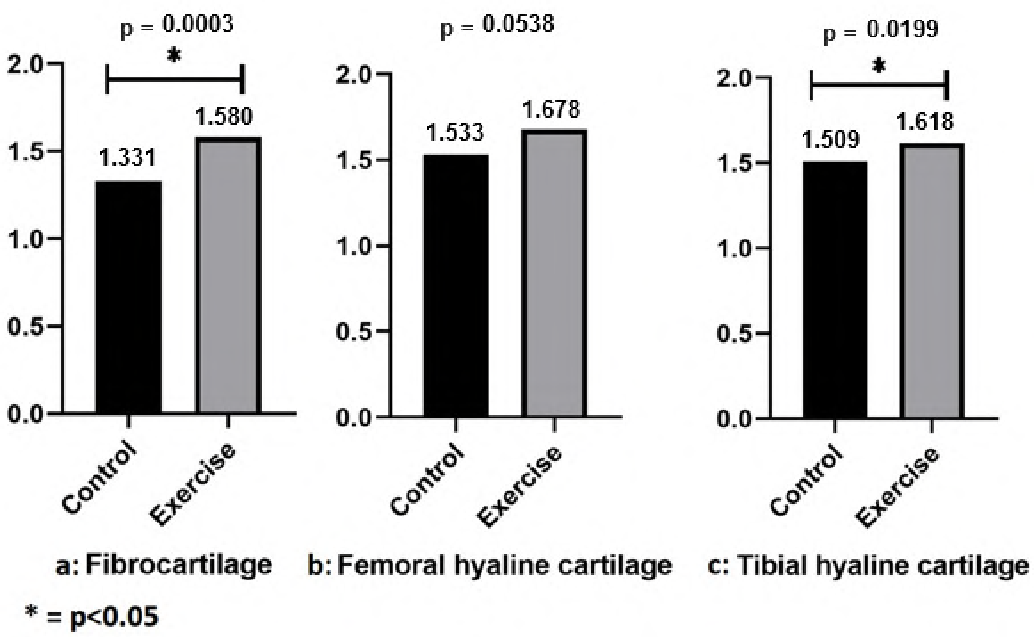

Fig. 1.- Graphically depicts the significant difference between the number of chondrocytes in fibrocartilage and tibial hyaline cartilage between the two groups (**a, c**). It also shows the no significant difference in number of chondrocytes per lacuna in femoral hyaline cartilage between the two groups (**b**).

Table 1. Represents the number of chondrocytes per lacuna in fibrocartilage, femoral hyaline cartilage and tibial hyaline cartilage. It was found to be significantly greater in the fibrocartilages and tibial hyaline cartilages of the exercise group. No significant differences were noted in the number of chondrocytes of femoral hyaline cartilages between the two groups.

Effects of Endurance Resistance Exercises on Knee Cartilage				
Variables	**Sample Size (n)**	**No. of Chondrocytes**		**p-value**
		Exercise Group (Mean±S.D)	**Control Group** (Mean±S.D)	
Fibrocartilage	12	1.580 ± 0.19	1.331 ± 0.04	0.0003*
Femoral Hyaline Cartilage	12	1.678 ± 0.18	1.533 ± 0.17	0.0538
Tibial Hyaline Cartilage	12	1.618 ± 0.09	1.509 ± 0.1	0.0199*

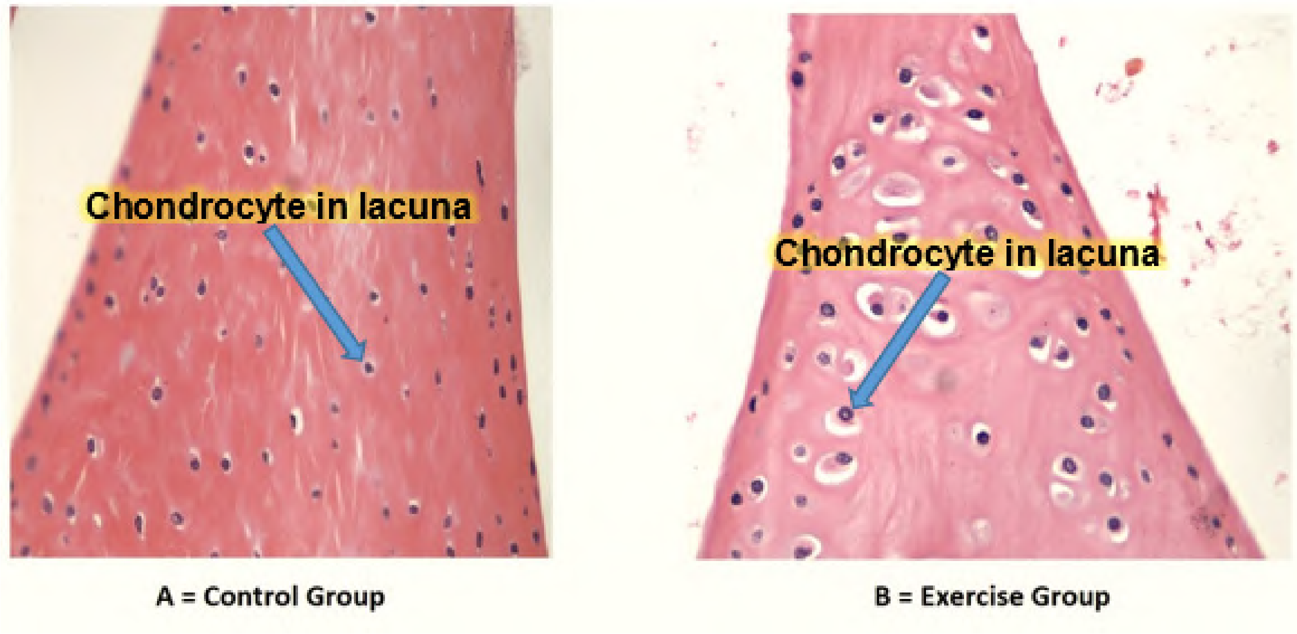

Fig. 2.- Shows difference between the number of chondrocytes per lacuna in knee joint fibrocartilage between the two groups (**A, B**).

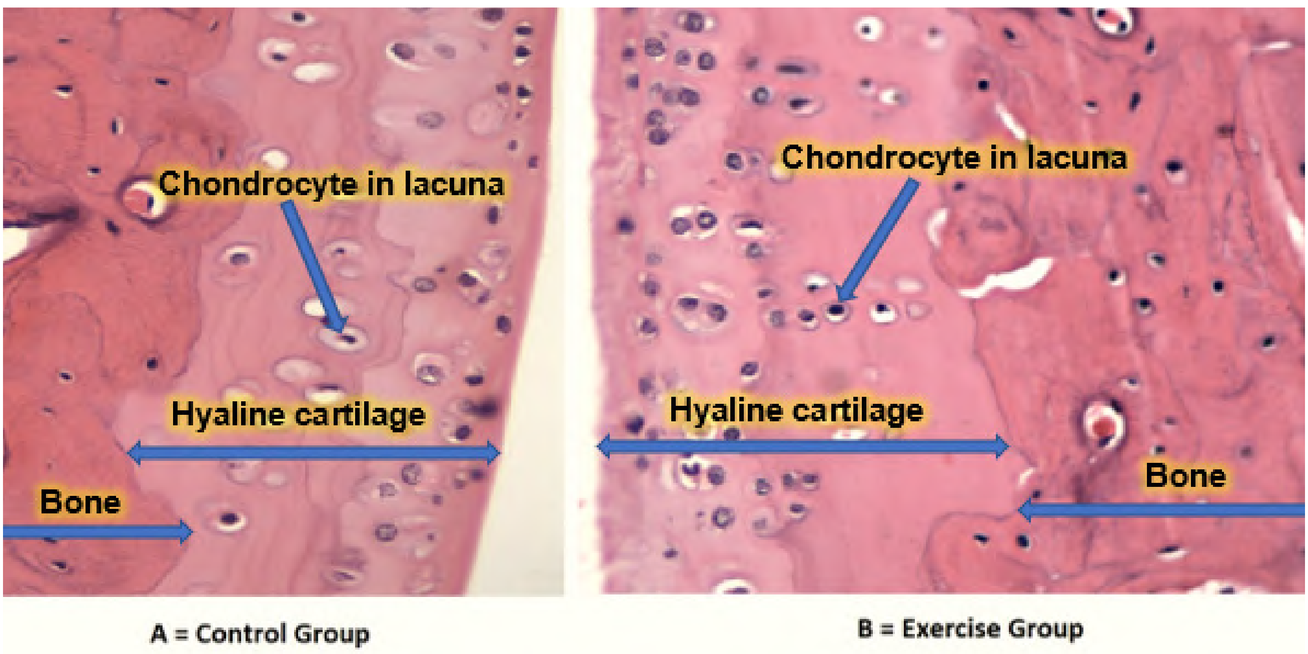

Fig. 3.- Shows difference between the number of chondrocytes per lacuna in knee joint femoral hyaline cartilage between the two groups (**A, B**).

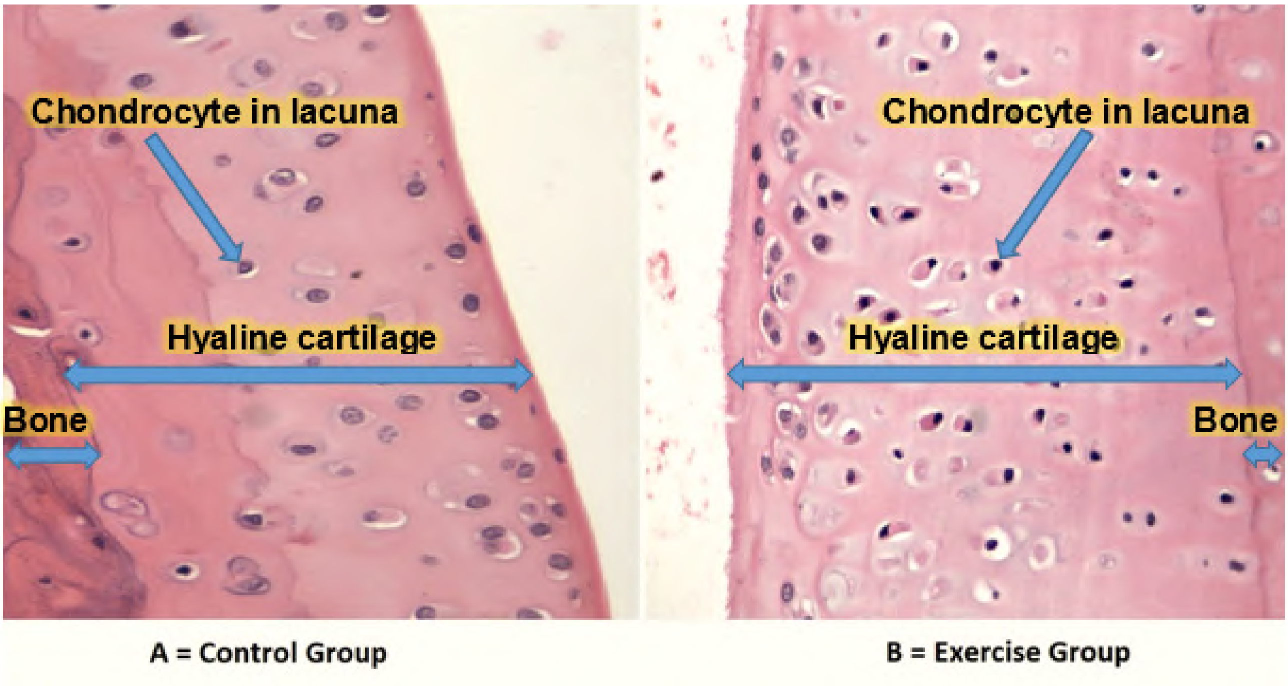

Fig. 4.- Shows difference between the number of chondrocytes per lacuna in knee joint tibial hyaline cartilage between the two groups (**A, B**).

Further femoral hyaline cartilage analyses were performed, and the findings revealed that for the number of chondrocytes per lacuna, an insignificant p-value was observed. (Table 1, Figs. 1b and 3).

Analyses of the effects of exercises on tibial hyaline cartilage were performed, and the findings revealed that a significant $p<0.05$ was observed in the number of chondrocytes per lacuna. (Table 1, Figs. 1c and 4).

DISCUSSION

We carried out this study to quantitatively analyse the effects of exercise on knee joint cartilage cellularity. The exercise group significantly outperformed the control group regarding the number of chondrocytes per lacuna in fibrocartilage. However, in the study of the femoral hyaline cartilage, the number of chondrocytes per lacuna did not differ between the groups. On the contrary, tibial hyaline cartilage examination showed significant changes in the number of chondrocytes per lacuna. Our results show potential advantages for cartilage health and integrity by showing that the endurance resistance exercise intervention favoured both fibrocartilage and hyaline cartilage in the tibia bones. The more enormous ramifications of these findings require more investigation, particularly about osteoarthritis and joint health, using both animal models and maybe human experiments.

A study examined the effects of two prevalent physical activities—walking on a treadmill and swimming—on a mechanical model of osteoarthritis (OA), and observed how both affected morphological factors associated with OA. Forty-eight male Wistar rats were used in the experiment, and they were divided into four groups for the study: Sham (no OA induction), Osteoarthritis (OA), OA + Treadmill (T), and OA + Swimming (S). The findings revealed that the number of chondrocytes per lacuna was significantly reduced in the OA and OA + S groups than in the Sham group ($p < 0.05$), pointing to a detrimental influence on the health of the cartilage. However, compared to the OA group, the OA + T group showed a significant increase in the number of chondrocytes per lacuna ($p<0.001$), indicating that treadmill exercise may have a positive effect on chondrocyte count in this OA model (da Silva et al., 2023). Similarly in our study, the number of chondrocytes within the fibrocartilages of both knees appears to have increased as a result of the exercise intervention, which suggests that physical activity may enhance the integrity and health of knee fibrocartilage.

Another study found how Wistar rats with experimental rheumatoid arthritis (RA) responded to low-level laser treatment (LLLT), stair climbing exercise, and their combination. Eight groups of male Wistar rats were created: controls with LLLT, controls with exercise, controls with both LLLT and exercise, arthritis only, arthritis with LLLT, arthritis with exercise, and both LLLT and exercise for arthritis. There were five rats per group. According to the findings, the arthritis exercise group had more chondrocytes in its tibia and talus than the other groups (Retameiro et al., 2022). Comparatively, the number of chondrocytes in the femoral hyaline cartilage was not statistically significantly affected by exercise. Because of this, it does not seem that the exercise intervention in this study had the same effect on this specific type of cartilage as it did on the knee fibrocartilage.

Furthermore, researchers investigated how an 8-week, moderate-intensity treadmill aerobic training program affected rats with knee osteoarthritis (kOA)-like alterations caused by monosodium iodoacetate (MIA) in a study involving 27 rats. Three groups of rats were generated: SHAM (Control), kOA (induced), and kOA with aerobic exercise (OAE). According to the study, the number of chondrocytes in the rats in the OAE group decreased by 35%, showing that these crucial cells were preserved. Additionally, in rats with generated kOA-like alterations, aerobic training controlled inflammatory biomarkers and enhanced motor function. Overall, the results indicate a potential role for moderate-intensity aerobic exercise in preserving chondrocytes and improving kOA-like outcomes (Martins et al., 2019). However, exercise had a statistically significant impact on the number of chondrocytes in the tibial hyaline cartilage for this study.

Our study and the other studies show that the effects of exercise on chondrocyte count depend on many variables, including the type of exercise, the selected animal model (OA, RA, or kOA-like), and the kind of cartilage being studied (fibrocartilage or hyaline cartilage). Even though our study positively impacted the knee's fibrocartilage and tibial hyaline cartilage, other studies on OA, RA, and hyaline cartilage also reported favourable outcomes in different conditions. These variations emphasize the need for particular workout suggestions based on the state and type of cartilage. Additionally, they emphasize the potential advantages of exercise in maintaining the health of chondrocytes and cartilage in various arthritis-related disorders, but the underlying mechanisms may vary. More study is required to clarify these pathways and direct practical applicability for human patients.

The study has particular strengths, such as the use of young male rats as a controlled animal model, allowing researchers to evaluate the effects of exercise on knee joint cartilage histology without the difficulties of using human participants. In addition, the young rats were more likely to engage in physical activity. The investigator also conducted a thorough microscopic evaluation of the knee joint's cartilage that advances the knowledge of exercise effects, essential for developing exercise regimens and therapies for cartilage health. The study's conclusions are exclusive to the small sample of young male rats, which restricts the applicability of the findings to human populations. Also, there was no difference in age, gender, or species among the young male rats utilized in the study, making it difficult to extrapolate the results to a larger population.

Furthermore, the study's short length may not reflect the long-term effects of endurance resistance exercise on the viability of the identified changes. Since there was no activity-free control group in the study, it was not easy to separate the effects of exercise from other possible factors impacting cartilage health. Future studies may include functional tests that give a complete picture of how exercise affects cartilage with evidence-based trials with larger samples that might transfer into clinical recommendations for human exercise programs.

Endurance resistance exercise does increase the cellularity of knee joint cartilages. This increase is more prominently and specifically seen in fibrocartilage and tibial hyaline cartilage. This study implicates the protective role of resistance exercise on the morphology of these cartilages.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as indicated: **KY:** Concept and study design, acquisition, analysis and interpretation of data, drafting the manuscript. **KJJB:** Concept and study design, acquisition, analysis and interpretation of data. **SIF:** Concept and study design, critical review and approval of the final version to be published. **JR:** Concept and study design, drafting the manuscript. **AAK:** Critical review, approval of the final version to be published. **SNNS:** Critical review, approval of the final version to be published.

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