ORIGINAL ARTICLE

Nr 2023;13 (4):517-523

Evaluation of the Effect of Melatonin on the Calcaneal Tendon of Ovariectomized Wistar Rats

Bethânia Lissa da Silva Rosa[1], Gustavo Henrique Silva Pompêo[1], Caroline Melo Ramos[2], Juliana Helena Ferreira[2], Silvia Graciela Ruginsk[2], Petrus Pires Marques[3], Flávia Da Ré Guerra[1]

[1] *Department of Anatomy, Institute of Biomedical Science, Federal University of Alfenas – UNIFAL-MG, Alfenas, Brazil*

[2] *Department of Physiology, Institute of Biomedical Science, Federal University of Alfenas – UNIFAL-MG, Alfenas, Brazil*

[3] *Department of Medicine, José do Rosário Vellano University-Unifenas, Alfenas, Brazil*

CORRESPONDING AUTHOR:
Flávia Da Ré Guerra
Department of Anatomy
Institute of Biomedical Science
Federal University of Alfenas
UNIFAL-MG
37130-001 Alfenas, Minas Gerais, Brazil
E-mail: unifal.flavia@gmail.com

DOI:
10.32098/mltj.04.2023.01

LEVEL OF EVIDENCE: 4

SUMMARY

Background. The Achilles tendon is the most injured tendon in humans. There is a close relationship between changes in the structural composition of the extracellular matrix and tendon tissue related to age and menopause because estrogen deficiency can lead to decreased collagen synthesis and a consequent decrease in the strength and elasticity of tendons, indicating a greater propensity for injuries and ruptures. However, there is still a lack of scientific evidence on the relationship between decreased estrogen levels during menopause and the involvement of this structure. This study sought to evaluate the effect of melatonin treatment on the Achilles tendon of ovariectomized Wistar rats and investigate the influence of the treatment on tissue structure, extracellular matrix, and biomechanics.

Materials and methods. Light microscopy and polarized light microscopy were used to observe the tendon's morphology using HE and toluidine blue dyes. Tissue biomechanics aspects were tested to assess possible functional changes in organ.

Results. Significant changes arose from the ovariectomy processes and melatonin treatment, including histological changes, GAG profile alterations, and biomechanical properties. **Conclusions.** Melatonin reversed some effects of ovariectomy, but also led to changes in the tendons. Further studies are needed to better understand the relationship between these factors and to identify dose-dependent effects as well as their relationship with the pathophysiology of tendinopathies.

KEY WORDS

Melatonin; Achilles tendon; menopause; ovariectomy; tendinopathy.

INTRODUCTION

Understanding the structural organization of the extracellular matrix of tendons remains under scientific investigation and is related to tissue dynamics with various physiological, mechanical, and metabolic variables. As a structure of the musculoskeletal system, it is essential for proper functioning, converting mechanical stimuli into biochemical signals that trigger cell signaling responsible for tenocyte activation and response. This occurs through an extracellular matrix rich in type I collagen, which ensures its stiffness and elasticity (1, 2).

Knowledge of the effects of menopause on female physiology has already been established and it can be associated with various bone, muscular, sexual, vascular, and metabolic disorders. The tendons can be affected by a varier of ways, including changes in composition of GAGs and fibers (3). In addition, there is an increased incidence of tendon injuries and ruptures among elderly women compared to males of the same age (4, 5). The calcaneal tendon, also known as Achilles tendon, is the most frequently affected by ruptures throughout the human body, with slow and often incom-

plete recovery, with significant and varied rates of recurrence depending on the chosen treatment method (6).
Despite the existence of estrogen replacement therapies that aim to reduce or eliminate the effects of menopause, there is much controversy about the effectiveness and safety of these treatments, including the risk of cancer (7, 8). Similarly, the effects of estrogen supplementation on tendons have little beneficial evidence and many contradictions, indicating the need for more studies to understand this relationship (9).
Due to this inconsistency in data and aiming for a safer alternative for prevention and possible treatment, some studies have indicated that melatonin, an endogenous hormone involved in the circadian cycle, is a promising substance (10). Its role in menopause is still debated, but evidence shows an important role in the decline of melatonin secretion and the onset of menopause after the age of 40 (11). In addition, melatonin was shown to have effects over chondrocytes and osteoblasts (12), and an important antioxidant action (13), a function that is important in inhibiting the etiopathogenesis of tendinopathies and has been shown to be extremely safe and nontoxic, even at high doses (14).

MATERIALS AND METHODS

The present study is part of a research project titled "*Effects of melatonin on metabolic parameters in ovariectomized Wistar rats*" conducted from August 2021 to November 2022. It was approved by the Animal Ethics Committee of the Federal University of Alfenas, Alfenas, Minas Gerais, Brazil (registration number 0010/2020 – Date of approval: April 14, 2021).

Animals

The animals used were Wistar rats, initially housed in collective cages (4-5 animals per box) in a maintenance vivarium in a temperature-controlled room maintained at 21 ± 2 °C and maintained on a controlled 12-hour light-dark cycle. After acclimatization for 2-3 days, the animals were divided into ovariectomized bilateral or SHAM (fictitious surgery) groups and then transferred to individual boxes, where they were kept for a 3-week treatment period and received water and standard chow *ad libitum*.
Animals were divided into the following experimental groups: SHAM control (SHAM-C), SHAM treated with melatonin (SHAM-M), ovariectomized control (OVX-C), and ovariectomized treated with melatonin (OVX-M). Each group consisted of 8 individuals, totaling 36 animals.
Researchers responsible for the animal care were aware of how animals were been treated, given the nature of the treatment (surgeries and melatonin treatment). Since the cages were labeled using numeric code. the researchers responsible for euthanasia, tendon removal and sequent experiments were not aware of group distribution.

Bilateral ovariectomy and sham surgery

The rats were anesthetized with a mixture of Xylazine Hydrochloride (10 mg/Kg) and Ketamine Hydrochloride (90 mg/Kg) in a volume of 0.1 ml solution for 100 g body mass. The reflexes of the animals were tested to initiate surgical procedures and to verify the effectiveness of the anesthetic. Subsequently, the dorsal region's hair was trimmed, and the animal was placed in lateral decubitus position. A longitudinal incision of approximately 1.5 cm was made between the last rib and thigh. Using forceps, the muscular plane was overcome, opening the peritoneal cavity. The ovary was located and exposed outside the cavity, and with the aid of suture lines, circulation was interrupted and the ovary was removed. The muscular layer was sutured with a stitch and skin with two to three stitches.
The same procedure was repeated for contralateral organs. A group of animals underwent fictitious surgery (SHAM) to simulate surgical stress without removing the ovaries, a group of animals underwent fictitious surgery. For this purpose, the entire surgical process is similar, except for the removal of the ovary; there is only displacement of the ovary outside the cavity to simulate the same procedure as accurately as possible.
Uterine mass was used as one of the indicators for validating the experimental model. With low circulating levels of 17-β-estradiol in postmenopausal women, there is a tendency for reduced uterine trophism, leading to a smaller size and mass of the uterus compared to the females of reproductive age (15). There was no effect of melatonin treatment on uterine mass, as had already been demonstrated in a previous study using intraperitoneal administration of melatonin to 8-week-old ovariectomized Wistar rats (16).

Animal treatment

The control animals received the vehicle solution (0.9% saline) by oral gavage at a volume of 1 ml/kg, while the treated animals received melatonin at a dose of 10 mg/kg by oral gavage (16). Treatment began on the day following surgery, lasted for 3 weeks, and was carried out daily at the same time, just before the start of the night period (5 pm).

Gavage

The orogastric gavage procedure was performed by introducing a curved flexible tube (or needle) with a rounded tip attached to a syringe, with the solution in the animal's mouth and carefully pushed through the esophagus into the stomach.

Euthanasia and collection of the calcaneal tendon

Three weeks after surgery and treatment, animals were euthanized with a lethal dose of anesthetic (4% isoflurane by inhalation) to collect the calcaneal tendon. Animals showing visible signs of distress, injury or abnormal behavior would be excluded from the experimentations.

Biomechanical analysis

The tests were performed using a material testing machine (TAXT Plus, Stable Micro Systems). The tendons were maintained in physiological solution until the time of the test. Before the test, the length, width, and thickness of the tendons were measured using a caliper, with the latter two determined at the midpoint of the distal region of each tendon, and the cross-sectional area was calculated from these measurements. The tendons were placed in the machine, and each end was attached to adapters fitted into the machine. During the test, the tendons were subjected to a gradual increase in load at a constant displacement rate of 20 mm/min using a 1 kN load cell until the tendon was ruptured. From these data, the maximum stress and strain were calculated, and a stress-strain curve was constructed.

Morphological analysis

Processing for structural analysis in light microscopy

After dissection, the Achilles tendons were fixed in a 4% formalin solution in Millonig buffer pH 7.4 for 18 h at room temperature. The pieces were then washed in buffer, dehydrated in alcohol baths, followed by transparency with xylene baths, and embedded in paraffin (Histosec). Sections of 7 μm were prepared. For an overall view of the tissue, some sections were stained with hematoxylin and eosin (HE). Toluidine Blue staining was used for the detection of proteoglycans (0.025% in McIlvaine buffer, pH 4.0). The slides were left to stain for 10 min, quickly washed in water, and air-dried. They were then briefly immersed in xylene and mounted on Canada balsam. Observations and documentation were performed using an Olympus BX 60 microscope equipped with a photographic camera.

Polarization microscopy: birefringence

Serial longitudinal sections (7 μm thickness) were deparaffinized, stained with Picrosirius, and analyzed under polarization microscopy using an Olympus BX 60 microscope equipped with a polarizer and analyzer. The sections, considering the major axis of the tendon, were positioned at 45° between the crossed polarizer and the analyzer.

Statistical analysis

All results are expressed as the mean ± standard deviation. Two-way analysis of variance (two-way ANOVA) followed by Tukey's *post-hoc* test was performed for comparisons between groups. Surgery (OVX and SHAM groups) and treatment (vehicle or melatonin groups) were considered as variables. The level of significance was set at $p < 0.05$.

RESULTS

Biomechanical analysis

Figure 1 summarizes the results of the biomechanical analysis.

The force necessary to rupture the tendon is designated as the failure load (F). Using this parameter and tendon displacement, it was possible to calculate the Stress in MPa ($\sigma = F/A$), strain in % (ε = displacement/initial length), and Young's modulus ($E = \sigma/\varepsilon$) (17). Values are presented as mean with standard deviation bars and asterisks indicate the level of significance: *$p < 0.05$; **$p < 0.005$.

Morphological analysis

As shown in **figure 2**, tissue organization was observed through the distribution of tenocytes. In the SHAM groups (A and B), the organization remained both in the control group and in the group treated with melatonin. In contrast, the OVX groups (C and D) presented a high-

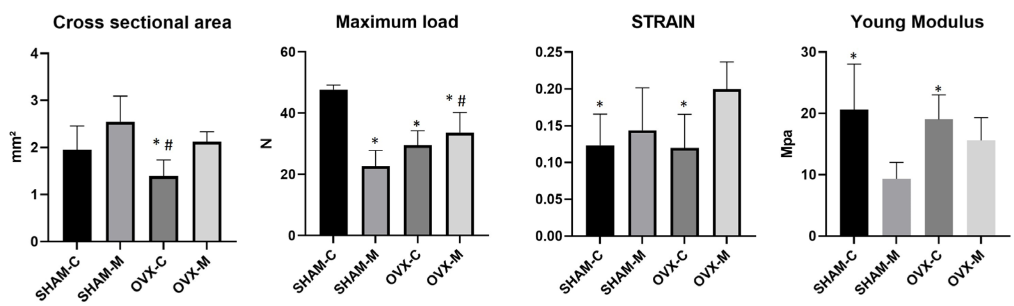

Figure 1. Summary of biomechanical results. All bars represent mean ± standard deviation. Stress is the force necessary for tendon rupture considering its cross-sectional area. Strain is a measure of the extent to which a tendon can stretch before losing its biomechanical characteristics or rupture. Young's modulus is the result of the division between these two parameters. *$p < 0.05$; **$p < 0.005$.

er level of tissue disorganization, corroborating the findings described in the literature (2, 7) and without notable differences between the treated and control groups.

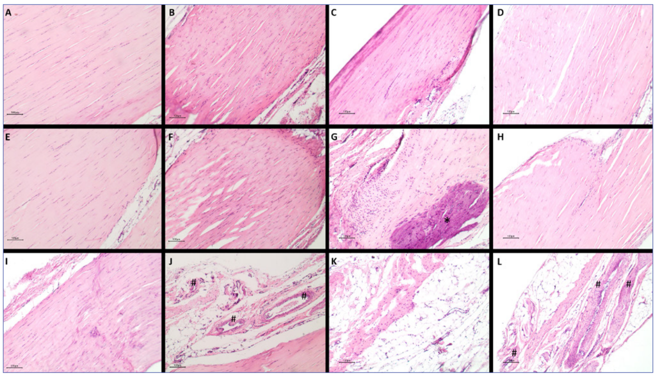

Figure 2. Photographs showing extracellular matrix organization and cell disposition. The columns were arranged as follows: **(A)** SHAM-C; **(B)** SHAM-M; **(C)** OVX-C; **(D)** OVX-M. In the first row there is a tension region in the tendons. In the SHAM-C group, the cells were arranged in an organized manner between the collagen fibers of the tendon matrix. In the OVX-C and OVX-M groups, disorganization was observed in the arrangement of the cells in the tissue. The second row comprises the compression regions of tendons. Note that the OVX-C group presented apparent hypercellularity and disorganization of the cell arrangement when compared to the other groups. The third row comprises the epitenon. Note the reduced thickness of the epitenon in the SHAM-C group, compatible with what is expected from a healthy one without signs of injury. In the other groups, there was an increase in epitenon compared to the control group. In the melatonin-treated groups (SHAM-M and OVX-M), epitenon had a considerable number of blood vessels.

*Indicates the region of enthesis; #indicates big-caliber vases.

The birefringence of collagen is an important characteristic in polarization analysis, as shown in **figure 3**, because this organization establishes a relationship with the direction of vibration and resonance of electrons in peptide bonds along the fiber, which depends on the molecular geometry, collagen concentration, and refractive indices. The picrosirius dye allows for better visualization of this phenomenon by making such a configuration strongly pink red, while the more green-brown spectrum identifies areas of lower birefringence or disorganization.

The results showed a higher degree of tissue organization in the two groups treated with melatonin (SHAM and OVX), which may indicate an important role in maintaining the structure and functionality of the tendon.

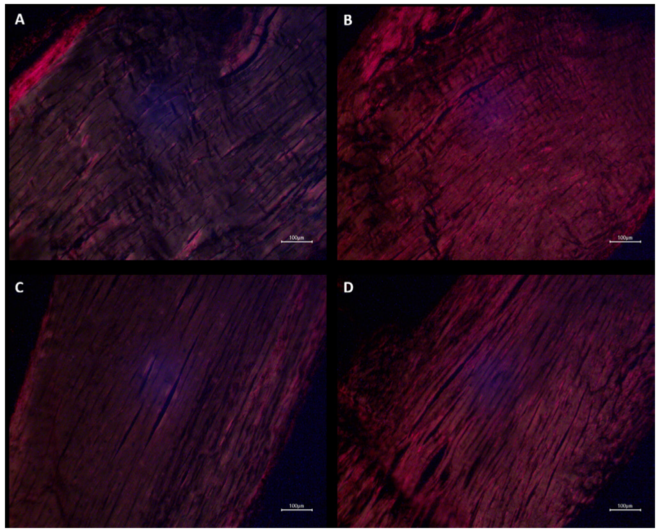

Figure 3. Photographs of the tension region of tendons using Picrosirius die, in polarization microscopy. **(A)** SHAM-C; **(B)** SHAM-M; **(C)** OVX-C: **(D)** OVX-M. Observe the Orange-red color of the melatonin-treated animals indicating a greater degree of collagen fibers organization.

Magnification 100x.

As shown in **figure 4**, there was an increase in GAGs in the groups treated with melatonin (B and D), whereas the control groups had a lower amount of these substances in their matrix. In the compression regions, we observed a higher concentration of GAGs in SHAM-C and OVX-M (**figure 4B,H**). Because GAGs are anionic, they stain with cationic dyes such as toluidine blue and acquire a blue color. Large amounts of material lead to the phenomenon of metachromasia, which turns them purple (18). All treatments (SHAM-M, OVX-C and OVX-M) led to some type of alteration in the distribution pattern of GAGs in the tension and compression regions.

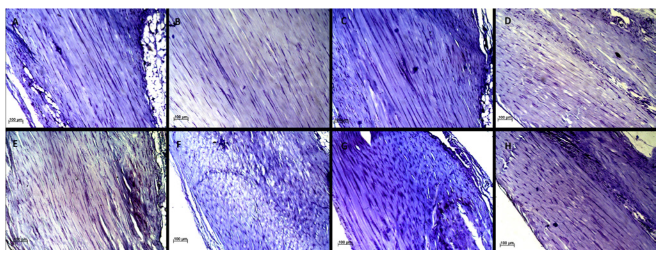

Figure 4. Photographs of toluidine blue slides. The first row contains the tension region of the tendons, and the second row contains the compression region. In the columns, there are different groups: **(A)** SHAM-C; **(B)** SHAM-M; **(C)** OVX-C; (D) OVX-M. The blue color indicates anionic substances in the tendon (mainly GAGs). The purple sections are due to metachromasia, a phenomenon that occurs in toluidine blue when, and there are large quantities of anionic substances.

Magnification 100x.

DISCUSSION

Collagen is the main constituent of the extracellular matrix of tendons, and it is not only the composition of type I collagen that determines tissue characteristics, but also its specific spatial organization and parallel alignment of fibers. In tendinopathies, the tissue presents with disorganized collagen fibers, increased amounts of proteoglycans and glycosaminoglycans, hypercellularity, neovascularization, and an increased extracellular matrix without collagen. Estrogen also plays an important role in tendon health as it helps maintain the integrity of connective tissue. Deficiency of estrogen, as occurs in menopause, can lead to a decrease in collagen synthesis and consequently, a decrease in tendon strength and elasticity (6, 8).

Effect of ovariectomy over tendons

The group of ovariectomized animals (OVX-C), simulating a menopause situation, presented considerable alterations in the analyzed tendon parameters. Cell disorganization was detected in both the tension and compression regions, as well as an increase in epitenon (**figure 2C,G,K**) when compared to the control group (SHAM-C), even though the general orientation of collagen fibers, revealed by polarization, seems to have remained similar (**figure 3**). It has already been shown that estrogen promotes collagen synthesis, acting on tendons among other tissues (3). There is a relationship between low estrogen concentration and the reduction of tendon resistance, the reduction of the type I collagen ratio with other subtypes, and an increase in cell proliferation and density (6, 9). For the same reason, we can observe the difference in the enthesis composition of the OVX-C group compared to the others, regarding hypercellularity and hypervascularization, since the cellular population of this region is more metabolically active, with the presence of fibroblasts and vascular cells, which also respond to the decrease in estrogen levels, following the same pattern (18).

It was possible to perceive a visible reduction in the content of GAGs in the compression region (**figure 4C,G**). GAGs are important components of the tendon extracellular matrix (1-5% of dry weight) as they assist in tissue mechanics, provide elasticity, and promote the attraction of water to the environment, making the tendon more hydrated (19). The distribution of these proteoglycans varies along the tendon, being present in greater quantity in the compression regions, acting in lubrication and adhesion of collagen molecules (16). The results may be related to a possible inflammatory process, in which there is an increase in signaling substances that favor the degradation of the extracellular matrix and, consequently, the degradation of these GAGs (23).

Estrogens influence greatly the tendons, which can explain the lower rate of tendon injuries in pre-menopausal women. Also, the hormonal effects of the menopause lead to a decrease in collagen turnover, tendon elasticity and MMP overexpression (20). This could explain the decrease in the cross-sectional area of the tendon (**figure 1**). Curiously, the process led to a significant increase in elasticity (Young's modulus).

Effect of ovariectomy with melatonin supplementation over tendons

The ovariectomized animals that received melatonin supplementation (OVX-M) showed some differences compared to those that did not receive it (OVX-C). The treatment used in this study was not able to completely reverse disorganization; however, there was an improvement in the compression region. The epitenon remained enlarged but highly vascularized (**figure 2D,H,L**). The levels of GAGs were more similar to those in the control group in the compression region, but higher in the tension region (**figure 4D,H**). The epitenon is one of the structures responsible for innervation and blood supply of the tendon as a whole, and primarily regulates metabolism in this region in response to changes in tissue physiology (2). Thus, in processes of healing or tendinopathy, similar alterations occur: increased vascularization, expression of neuronal mediators, and increased innervation, and as this tissue mechanics is not completely understood, it is difficult to differentiate between healing and initial pathological processes (18, 21). The increase in VEGF concentration during the initial inflammatory process is the main cause of hypervascularization in the region, since its effects allow more mediators and inflammatory cells to arrive at the site (14).

As mentioned previously, ovariectomy reduced the girth of the tendon. Melatonin treatment reversed this effect (**figure 1**), returning to the same values as the control. Melatonin supplementation caused several effects on tendons in ovariectomized animals, reducing the stress they could resist while increasing the strain values, which led to a significant reduction in Young's modulus. This means that although the tendon became more resistant to stress forces, it became stiffer and possibly more prone to rupture.

The cross-sectional area of the tendon can be directly related to its tensile strength and maximum load supported (22, 23). This is because tendon cross-sectional area is directly related to the amount of extracellular matrix and collagen present in the tissue, and collagen is the main protein responsible for tendon mechanical strength (24).

The stress increase could be due to the increased collagen organization (**figure 3**), but other changes could be responsible for the lower elasticity, like GAG profile changes, essential to help the tendons respond adequately to the forces applied (25).

Effect of melatonin treatment on non-ovariectomized animals

The SHAM-M group included animals that received melatonin, but did not undergo ovariectomy. In such animals, the tendons seemed to remain organized, but with increased and more vascularized epitenons (**figure 2B,F,J**). The treatment caused an increase in collagen fiber organization, which could be observed with or without ovariectomy (**figure 3B,D**). In addition, there was an inversion in the expected GAGs profile for the tension and compression areas (**figure 4B,F**). Even with these observed changes, melatonin alone did not change the biomechanical parameters of control animals (SHAM-C) in the present study.

CONCLUSIONS

Ovariectomy led to significant structural changes in the calcaneal tendons, which were reflected in their biomechanical behavior. Melatonin was able to reverse some of these changes, but future studies could test the safety of such treatment more deeply, since melatonin alone was able to alter some tendon properties. More differences could arise from ovariectomy or melatonin use considering a longer period of treatment and the long turnover of collagen. Awareness should also be raised regarding the widespread use of melatonin worldwide in healthy individuals for the treatment of sleep disorders.

FUNDINGS

This research was funded by the National Science and Technology Development Council (CNPq), Brazil.

DATA AVAILABILITY

The data supporting the findings of this study are available within the article itself. Any request for additional content can be requested to the corresponding author.

CONTRIBUTIONS

BS, GP, CR, JF: investigation, writing – original draft. SR, FG: project administration, funding acquisition, resources, formal analysis. PM: formal analysis, translating, writing – review & editing.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

1. Thomopoulos S, Parks WC, Rifkin DB, Derwin KA. Mechanisms of tendon injury and repair. Journal Orthop Res. 2015;33(6):832-9. doi: 10.1002/jor.22806.
2. Nourissat G, Berenbaum F, Duprez D. Tendon injury: from biology to tendon repair. Nat Rev Rheumatol. 2015;11(4):223-33. doi: 10.1038/nrrheum.2015.26.
3. Oliva F, Piccirilli E, Berardi AC, Frizziero A, Tarantino U, Maffulli N. Hormones and tendinopathies: the current evidence. Br Med Bull. 2016;117(1):39-58. doi: 10.1093/bmb/ldv054.
4. Gulati V, Jaggard M, Al-Nammari SS, et al. Management of achilles tendon injury: A current concepts systematic review. World J Orthop. 2015;6(4):380-6. doi: 10.5312/wjo.v6.i4.380.
5. Frizziero A, Vittadini F, Gasparre G, Masiero S. Impact of oestrogen deficiency and aging on tendon: concise review. Muscles Ligaments Tendons J. 2014;4(3):324-8.
6. Finni T, Kovanen V, Ronkainen PHA, et al. Combination of hormone replacement therapy and high physical activity is associated with differences in Achilles tendon size in monozygotic female twin pairs. J Appl Physiol. 2009;106(4):1332-7. doi: 10.1152/japplphysiol.91439.2008.
7. Hansen M, Kongsgaard M, Holm L, et al. Effect of estrogen on tendon collagen synthesis, tendon structural characteristics, and biomechanical properties in postmenopausal women. J Appl Physiol. 2009;106(4):1385-93. doi: 10.1152/japplphysiol.90935.2008.
8. Leblanc DR, Schneider M, Angele P, Vollmer G, Docheva D. The effect of estrogen on tendon and ligament metabolism and function. J Steroid Biochem Mol Biol. 2017;172:106-16. doi: 10.1016/j.jsbmb.2017.06.008.
9. Ganderton C, Semciw A, Cook J, Pizzari T. The effect of female sex hormone supplementation on tendon in pre and postmenopausal women: A systematic review. J Musculoskelet Neuronal Interact. 2016;16(2):92-104. Available at: http://www.ismni.org/jmni/pdf/64/jmni_16_092.pdf.
10. Song C, Wang J, Kim B, et al. Insights into the Role of Circadian Rhythms in Bone Metabolism: A Promising Intervention Target? Biomed Res Int. 2018;2018:9156478. doi: 10.1155/2018/9156478.
11. Walecka-Kapica E, Chojnacki J, Stępień A, Wachowska-Kelly P, Klupińska G, Chojnacki C. Melatonin and female hormone secretion in postmenopausal overweight women. Int J Mol Sci. 2015;16(1):1030-42. doi: 10.3390/ijms16011030.
12. Oliva F, Piccirilli E, Berardi AC, Frizziero A, Tarantino U, Maffulli N. Hormones and tendinopathies: the current evidence. Br Med Bull. 2016;117(1):39-58. doi: 10.1093/bmb/ldv054.
13. Parandavar N, Hojat M, Abdali K, Keshtgar S, Emamghoreishi M, Yeganeh BS. The effect of melatonin on the lipid levels in menopausal women: A double-blind, controlled, clinical

trial. J Educ Health Promot. 2018;7:144. doi: 10.4103/jehp.jehp_187_17.
14. Kocadal O, Pepe M, Akyurek N, et al. The Evaluation of Exogenous Melatonin Administration in Supraspinatus Overuse Tendinopathy in an Experimental Rat Model. Clin Shoulder Elb. 2019;22(2):79-86. doi: 10.5397/cise.2019.22.2.79.
15. Dutta M, Bayan H. An age related study of morphology of human uterus. Nat Journal Clin Anatom. 2016;5(1):28-35. doi: 10.1055/s-0039-3401584.
16. Contini Mdel C, Millen N, González M, Mahieu S. Melatonin prevents oxidative stress in ovariectomized rats treated with aluminium. Biol Trace Elem Res. 2011;144(1-3):924-43. doi: 10.1007/s12011-011-9060-7.
17. Vieira CP, De Oliveira LP, Da Ré Guerra F, Dos Santos De Almeida M, Marcondes MC, Pimentel ER. Glycine improves biochemical and biomechanical properties following inflammation of the achilles tendon. Anat Rec (Hoboken). 2015;298(3):538-45. doi: 10.1002/ar.23041.
18. Ackermann PW, Hart DA, editors. Metabolic Influences on Risk for Tendon Disorders. Cham: Springer International Publishing; 2016.
19. Yoon JH, Halper J. Tendon proteoglycans: biochemistry and function. J Musculoskelet Neuronal Interact. 2005;5(1):22-34. Available at: http://www.ismni.org/jmni/pdf/19/05YOON.pdf.
20. Maffulli N, Cuozzo F, Migliorini F, Oliva F. The tendon unit: biochemical, biomechanical, hormonal influences. J Orthop Surg Res. 2023;18(1):311. doi: 10.1186/s13018-023-03796-4.
21. Galatz LM, Gerstenfeld L, Heber-Katz E, Rodeo SA. Tendon regeneration and scar formation: The concept of scarless healing. J Orthop Res. 2015;33(6):823-31. doi: 10.1002/jor.22853.
22. Arampatzis A, De Monte G, Karamanidis K, Morey-Klapsing G, Stafilidis S, Brüggemann GP. Influence of the muscle-tendon unit's mechanical and morphological properties on running economy. J Exp Biol. 2006;209(Pt 17):3345-57. doi: 10.1242/jeb.02340.
23. Kubo K, Kanehisa H, Fukunaga T. Effects of resistance and stretching training programmes on the viscoelastic properties of human tendon structures in vivo. J Physiol. 2002;538(Pt 1):219-26. doi: 10.1113/jphysiol.2001.012703.
24. Kannus P. Structure of the tendon connective tissue. Scand J Med Sci Sports. 2000;10(6):312-20. doi: 10.1034/j.1600-0838.2000.010006312.x.
25. Fenwick SA, Hazleman BL, Riley GP. The vasculature and its role in the damaged and healing tendon. Arthritis Res. 2002;4(4):252-60. doi: 10.1186/ar416.