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Achilles tendon enthesis behavior under cyclic compressive loading: Consequences of unloading and early remobilization

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ABSTRACT

The Achilles tendon enthesis (ATE) anchors the Achilles tendon into the calcaneus through fibrocartilaginous tissue. The latter is enriched in type II collagen and proteoglycans (PGs), both of which give the enthesis its capacity to withstand compressive stress. Because unloading and reloading induce remodeling of the ATE fibrocartilage (Camy et al., 2022), chronic changes in the mechanical load could modify the mechanical response under compressive stress. Therefore, we investigated the ATE fatigue behavior in mice, under cyclic compressive loading, after 14 days of hindlimb suspension and 6 days of reloading. In addition, we performed a qualitative histological study of PGs in ATE fibrocartilage. The mechanical behavior of ATE was impaired in unloaded mice. A significant loss of 27 % in Δd (difference between the maximum and minimum displacements) was observed at the end of the test. In addition, the hysteresis area decreased by approximately 27 % and the stiffness increased by over 45 %. The increased stiffness and loss of viscosity were thrice and almost twice those of the control, respectively. In the reloaded entheses, where the loss of Δd was not significant, we found a significant 28 % decrease in the hysteresis area and a 26 % increase in stiffness, both of which were higher regarding the control condition. These load-dependent changes in the mechanical response seem partly related to changes in PGs in the uncalcified part of the ATE. These findings highlight the importance of managing compressive loading on ATE when performing prophylactic and rehabilitation exercises.

1. Introduction

The Achilles tendon enthesis (ATE) anchors the Achilles tendon into the calcaneus through a fibrocartilaginous tissue (Roffino et al., 2021). The enthesis is subjected to a complex internal stress state during movement. In addition to tensile strain, in both humans (Chimenti et al., 2016) and mice (Mora et al., 2022), compressive strain has been described during dorsiflexion of the ankle where the tendon overlays the postero-superior portion of the calcaneus. At this site, it has been suggested that the periosteal fibrocartilage, through a gradient in elastic

modulus, enables the ATE to resist compressive loading of the Achilles tendon when it presses against the calcaneus during ankle dorsiflexion (Tits et al., 2023). In addition to periosteal fibrocartilage, the ATE also features fibrocartilage which anchors the tendon to the bone and thus probably contributes to the ability of the enthesis to resist compressive forces. This is composed of an uncalcified part adjacent to the tendon (uncalcified fibrocartilage, UFC) and a calcified zone adjacent to the bone (calcified fibrocartilage, CFC) (Roffino et al., 2021). From a molecular perspective, aggrecan and type II collagen, two typical macromolecules of the fibrocartilage, have been identified in this type of

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enthesis (Camy et al., 2022; Thomopoulos et al., 2003; Waggett et al., 1998). Some studies have suggested that these macromolecules contribute to the enthesis resistance to compressive stresses (Benjamin et al., 2006; Friese et al., 2020; Roffino et al., 2021). Owing to its binding with hyaluronic acid, aggrecan is a proteoglycan (PG) enriched in negative charges that allow osmotic pressure inside the fibrocartilage and promote water retention. This contributes to the viscoelastic properties of fibrocartilage (Benjamin & Ralphs, 1998; Benjamin & Ralphs, 2004). Meanwhile, type II collagen fibers are organized in a network perpendicular to the compressive solicitation axis. Through its interaction with PGs, the collagen II network restrains water, thereby reinforcing fibrocartilage resistance to compressive stress (Sivan et al., 2014; Sizeland et al., 2020).

It has been reported that the enthesis fibrocartilage is altered in response to chronic variations in mechanical load. When loading is reduced, a PG loss occurs, leading to a decrease in the entire fibrocartilage area. These changes have been reported in various models of unloading, such as muscular paralysis induced by botulinum toxin injections (Abraham et al., 2021) and stress shielding (Mutsuzaki et al., 2015). Jarvinen et al. (1999) reported a decrease in both the UFC and CFC areas after three weeks of immobilization in rat patellar enthesis. However, at the ATE, only the area of the UFC was reduced in response to 14 days of simulated microgravity in mice due to considerable PGs loss (Camy et al., 2022). Interestingly, de novo expression of PGs in the UFC was observed after six days of remobilization following the unloading period (Camy et al., 2022). Thus, considering the supposed role of fibrocartilage, changes in both fibrocartilage composition and area likely affect the enthesis capability to withstand compressive stress under unloading and reloading conditions.

In this context, we studied the effects of both unloading and returning to normal load conditions on the mechanical behavior of the ATE under compressive stress. A hindlimb suspension mouse model was used to unload the ATE (Morey-Holton & Globus, 1998). The mechanical response of ATE was investigated using a cyclic compressive test. To explain the potential changes in mechanical behavior, we characterized the PGs of fibrocartilage using a qualitative histological study. Our hypotheses were that (i) unloading would alter the mechanical behavior of the ATE in compression owing to a loss of PGs in the tissue, and (ii) that return to normal load conditions would have a protective effect on enthesis resistance to compressive stress by restoring PGs in the fibrocartilage.

2. Material and methods

2.1. Animals and experimental procedure

Twenty one 14-week-old male C57BL/6J mice (26.00 g \pm 0.99) were used for this experiment. This study was approved by the Committee on the Ethics of Animal Experiments of Languedoc Roussillon in accordance with the guidelines of the French National Research Council for the Care and Use of Laboratory Animals (APAFIS#28764–2020122115407491). Mice were housed in single cages in temperature-controlled rooms (22 °C) under a 12-h light–dark cycle and were fed a standard diet ad libitum. Care was taken to minimize animal discomfort and suffering.

Fourteen mice were hindlimb-unloaded for 14 days through tail suspension (Morey-Holton, 1982). Following the suspension, seven mice were euthanized (HU group). The remaining seven mice were not suspended and allowed to move freely in their cages before euthanization (Hindlimb Unloaded and Reloaded, HUR group). Mice in the control group (CTL, n = 7) were allowed to move freely in their cages throughout the experiment.

2.2. Cyclic compressive test

The left ankles of the animals were dissected, including the Achilles tendon, tibiotalus and talocalcaneal joints. The plantar tendon was

removed. The samples were frozen in liquid nitrogen and stored at -80 °C until mechanical testing. The samples were defrosted at room temperature before the cyclic compressive test. The Achilles tendon was detached from the tibia and clamped using a Mayo-Hegar needle holder. The sample needle holder set was positioned in a holding device sculpted in a sawbone and attached with Prolene 2/0 sutures. We bound the sample needle holder set to the support with modelling clay to reinforce and guaranteed the immobility of the set during the test. The whole device was positioned in the lower grip of a Universal testing machine at an angle of 45° relative to the horizontal (Fig. 1). This angle enabled to load mainly the ATE enthesis (UFC and CFC) while minimizing the mechanical characterization of the neighbouring structures (Achilles tendon, periosteal fibrocartilage and plantar fascia). This was verified during pretest by performing a static compressive test to failure (SD1). The test was performed on an Instron 5566A universal testing machine fitted with a 100 N load cell and equipped on the upper grip with a flat hexagonal-shaped tip of 1 mm circumradius. With a tip larger than the entire enthesis, the compressive force could be applied uniformly over the UFC and CFC assuring a macroscopic mechanical characterization of the ATE. A loading and unloading compressive test of 2,500 cycles from 0 to 5 N was applied at a 0.2-mm/s speed. The 2,500 cycles corresponded to the compressive solicitations undergone by the ATE for one day in a laboratory mouse (de Visser et al., 2006; Mendes et al., 2015) and 5 N represented 40 % failure strength assessed during the pre-tests. The samples were regularly hydrated with PBS throughout the experiment with phosphate buffer solution (PBS). The load and displacement data were acquired using Dewesoft software coupled with a 100 Hz acquisition device. Two Veho cameras were used to check the location of the applied load and absence of sample movement during the test.

Data analysis was performed using the MATLAB software. For each cycle, the change in the displacement of the enthesis (Δd), corresponding to the difference between the maximum and minimum displacements, was calculated. Hysteresis, which corresponds to the area between the load and unload curves, was determined using the *trapz* function. We ensured to characterize the enthesis in its elastic domain, and that there was no plastic deformation or irreversible damage. The percentage of variation in stiffness values was calculated for the CTL samples in the first 20 cycles and a 1 % mean percentage of variation in stiffness (SD2) was found. Then, the stiffness, corresponding to the slope of the curve, was calculated before the end of the loading phase. To characterize the changes in these parameters during the test, the loss or gain percentages were calculated between the beginning (100 cycles) and middle (1250 cycles) of the test and between the beginning and end (2,500 cycles) of the test.

2.3. Statistical analysis

The effects of condition (CTL, HU, HUR) and number of cycles (100, 1,250, 2,500 cycles) on the Δd and the hysteresis and the stiffness values were assessed with a two-way mixed ANOVA on the RStudio software. The multiple comparisons were assessed pairwise comparisons were performed using a *t*-test with the Bonferroni correction. The significance level was set at $p < 0.05$.

2.4. Histological experiment

For four mice per group, the right ankles were dissected following the same protocol as that for the cyclic compressive test. The samples were fixed in 4 % paraformaldehyde solution for 24 h, decalcified, dehydrated, and embedded in paraffin, following the same protocol as previously described (Camy et al., 2022). 7- μ m-thick sagittal sections of the ATE were stained with Alcian blue (pH 2.5). At this pH, this staining highlights the sulfated (small structural PGs) and unsulphated PGs (coupled with hyaluronic acid) (Sorvari & Sorvari, 1969). After staining, the sections were dehydrated and mounted in a resinous medium for

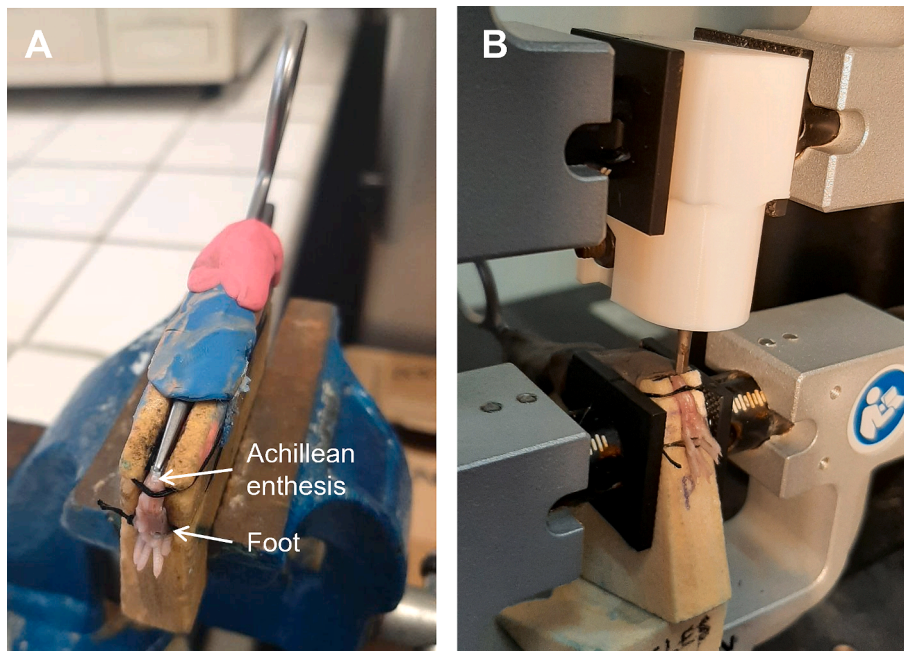


Fig. 1. Experimental loading device used to perform a cyclic compressive test on mice ATE. A: Sample-needle holder set positioned on the sawbone. B: Experimental device positioned in the testing machine.

qualitative microscopic analysis. The same samples was then photographed under transmitted and polarized light. The delineation between the UFc and CFc and the delineation between the CFc and subchondral bone were determined on polarised light image and transferred to the transmitted light image using Adobe Photoshop software (SD3).

3. Results

3.1. Mechanical testing

Fig. 2 illustrates the hysteresis loops acquired during the cyclic compressive test for the CTL, HU, and HUR conditions from which Δd , stiffness, and hysteresis area were calculated.

The two-way mixed ANOVA analysis revealed a main effect of the number of cycles (SD4 A, B and C) but no effect of the condition (SD4 D, E and F), nor an interaction between the two factors (SD4 G, H and I). The significant effect of the number of cycles was observed for the parameters Δd ($p < 0.001$), hysteresis area ($p < 0.001$), and stiffness ($p < 0.001$). The fatigue behavior of the ATE during compressive cyclic loading was marked by a decrease in both Δd and hysteresis area and an increase in stiffness. We investigated whether the effect of the number of cycles was found in all experimental conditions using pairwise comparisons (SD4). For the CTL condition, the statistical analysis did not show any significant difference regarding the number of cycle for the Δd (**Fig. 3A**), hysteresis area (**Fig. 3D**), or stiffness (**Fig. 3G**). For all parameters, variations occurred mainly between 100 and 1,250 cycles. For the HU condition, while the hysteresis area decreased by around 27% (**Fig. 3E**) and stiffness increased by over 45% at the end of the test (**Fig. 3H**), only around 27% loss of Δd between the beginning and the end of the test was statistically significant ($p < 0.05$) (**Fig. 3B**). Except for the hysteresis area, both Δd and stiffness variations occurred equally in the first and second parts of the test. For the HUR condition, pairwise comparisons showed a significant decrease in the hysteresis area (**Fig. 3F**, $p < 0.05$, at 1250 cycles; $p < 0.01$ at 2,500 cycles) and an increase in stiffness (**Fig. 3I**, $p < 0.05$, at 1250 cycles; $p < 0.01$, at 2,500 cycles) at both 1,250 and 2,500 cycles. However, the loss of Δd was not statistically significant (**Fig. 3C**). Variations in Δd , hysteresis area, and stiffness occurred during the first part of the cyclic compressive test.

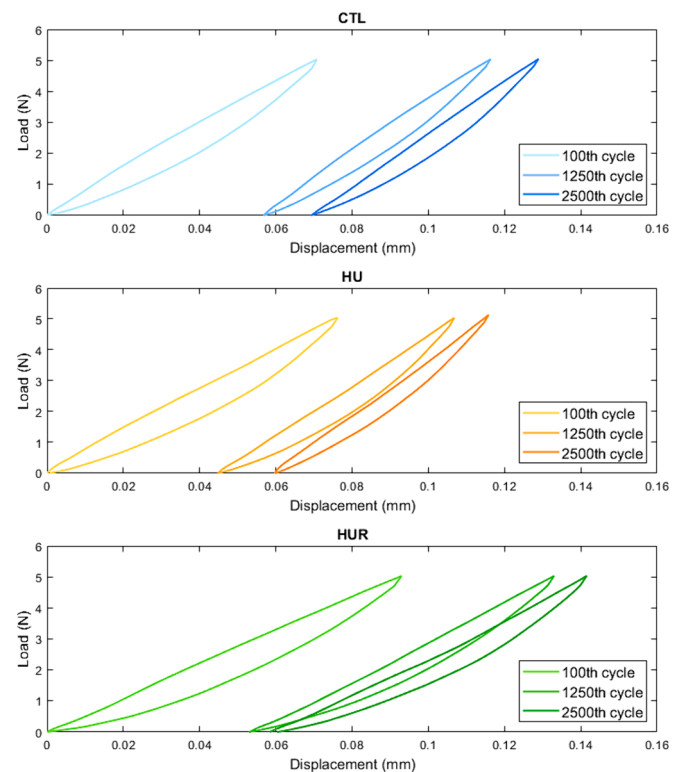


Fig. 2. Hysteresis loops acquired during the cyclic compressive test at 100, 1,250, and 2,500 cycles. Blue: CTL. Orange: HU. Green: HUR. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Although no statistically significant effect of the condition was observed, some trends are visible through compressive cyclic testing. First, the loss in Δd and hysteresis area were 1.7 times higher in the HU group than in the CTL group. The increase in stiffness was three times higher. In addition, HUR entheses whose loss of Δd was similar to the

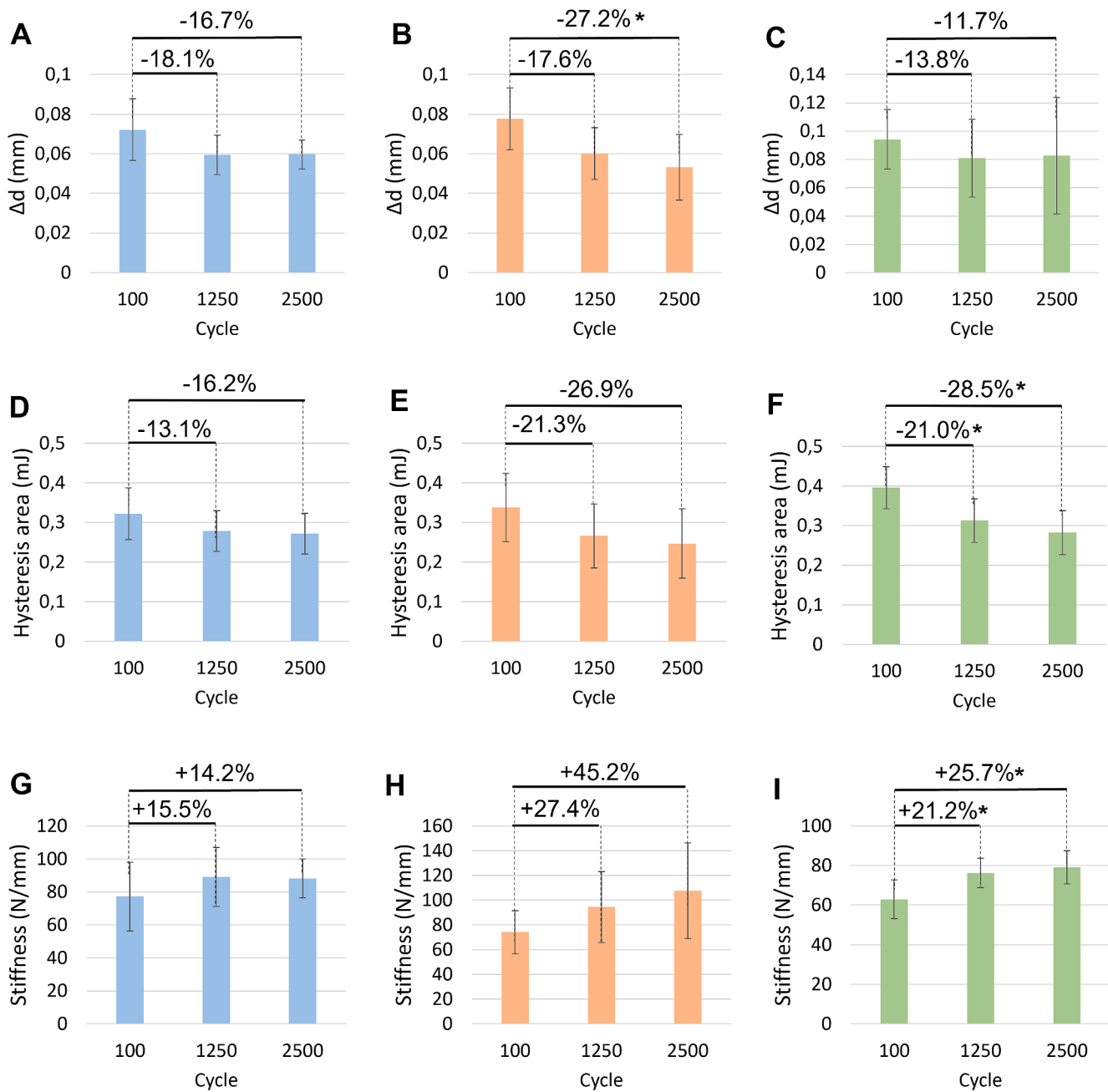


Fig. 3. Δd (A, B, C), hysteresis area (D, E, F) and stiffness (G, H, I) values at 100, 1,250, and 2,500 cycles for CTL (blue: A, D, G), HU (red: B, E, H), and HUR (green: C, F, I) conditions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

CTL entheses at the end of the test, exhibited (similar to the HU entheses), a loss of hysteresis area and an increase in stiffness that were higher during the cyclic compression test compared with the CTL condition.

3.2. Histological study

No difference in Alcian blue staining was observed between the conditions in the CFC of ATE. In contrast, changes in mechanical loading affected the staining intensity and distribution of stained areas in the UFc, reflecting changes in the PG content (Fig. 4). In HU animals, we observed a severe decrease in staining intensity, particularly in the deep part of the enthesis, suggesting a drastic loss of PG in this region (Fig. 4B). In HUR animals, we observed intense staining uniformly distributed in the UFc, demonstrating a higher PG content in the reloaded entheses than in the other conditions. In contrast to the CTL

and HU entheses, the superficial part of the HUR enthesis was strongly stained (Fig. 4C).

4. Discussion

Although fibrocartilage is supposed to protect the ATE from compressive stress during foot dorsiflexion (Shaw & Benjamin, 2007), to the best of our knowledge, the capacity of the achillean enthesis to withstand compression has never been studied. However, the characterization of the mechanical behavior of ATE under compression is especially important, as changes in the amount of fibrocartilage have been reported in unloaded entheses (Abraham et al., 2021; Camy et al., 2022; Deymier et al., 2020; Jarvinen et al., 1999; Mutsuzaki et al., 2015). Therefore, the first goal of our work was to compare the mechanical responses of CTL and HU entheses under a fatigue compressive test for

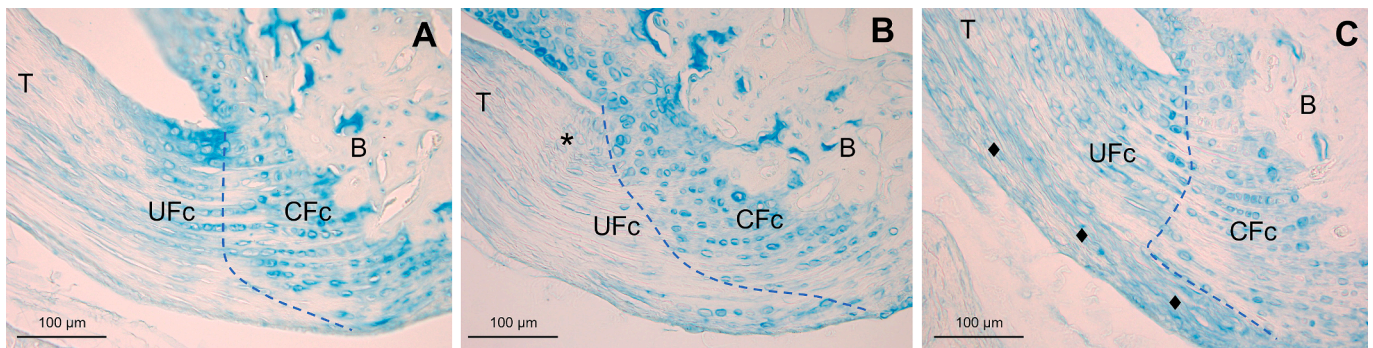


Fig. 4. Alcian blue staining of sagittal sections of CTL (A), HU (B), and HUR (C) ATEs. HU entheses show a drastic reduction in staining intensity in the deep part of UFc (*) compared to the CTL entheses. In HUR entheses, a strong, uniform staining can be observed in the UFc with an intense staining in the superficial part of the enthesis (◆) compared to CTL et HU conditions. T: tendon. UFc: uncalcified fibrocartilage. CFc: calcified fibrocartilage. B: bone. Dashline: tidemark. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2,500 cycles, corresponding to one day of ankle mechanical solicitations for a laboratory mouse (de Visser et al., 2006; Mendes et al., 2015).

The present study showed that cyclic compressive stress impaired the mechanical behavior of the CTL Achilles tendon enthesis, characterized by a loss of Δd , loss of hysteresis area, and increase in stiffness, especially during the first part of the test. This mechanical response was similar to that observed in other cartilaginous tissues subjected to cyclic compression. Several studies have reported a reduction in the thickness of articular cartilage (Kaab et al., 1998; Kaplan et al., 2017; Wong et al., 1999; Zevenbergen et al., 2018), intervertebral discs (Mazuoka et al., 2007), and meniscus (Maritz et al., 2020) in both humans and animals. Moreover, an increase in the stiffness of ovine intervertebral discs (Johannessen et al., 2004) and articular cartilage (Kaplan et al., 2017) has also been reported.

It has been suggested that the deterioration in the mechanical behavior of the articular cartilage and intervertebral disc under cyclic compression was the result of a fluid shift in the tissue. Cartilaginous tissues mainly consist of type II collagen and aggrecan. As the latter interacts non-covalently with hyaluronic acid, this proteoglycan is strongly negatively charged. This results in the attraction of cations, which, in turn, bring water into the tissue via osmosis (Vincent and Wann, 2019). This hydration provides swelling pressure that is restrained by the collagen network. Type II collagen and proteoglycans create swollen hydrated tissues that resist compression (Han et al., 2011). When compressive stress is applied to cartilaginous tissue, a fluid shift occurs outside the tissue. In an unconfined cyclic compressive test, Mazuoka et al. (2007) found a correlation between water loss and height loss in rat caudal intervertebral discs. Some authors have suggested that water extrusion leads to collagenous matrix packing in articular cartilage, causing a loss of cartilage thickness (Bader et al., 1992; Wong et al., 1999). Moreover, water loss can decrease tissue viscosity (Bader et al., 1992). Thus, in view of these data, the loss of Δd we observed on the ATE under cyclic compressive loading would be the result of water extrusion since this interface is enriched in type II collagen as well as in proteoglycans and glycoaminoglycans such as aggrecan and hyaluronic acid (Friese et al., 2020; Jensen et al., 2018; Waggett et al., 1998). Water extrusion could explain the stiffening of the enthesis induced by collagen matrix packing, as well as the loss of viscosity, as reflected by the decrease in the hysteresis area. In these CTL entheses, with a fixed maximal load of 5 N applied, changes in the mechanical behavior occurred mainly in the first part of the test. This demonstrates that equilibrium seems to have been reached during the first 1,250 cycles, where no further deformation or fluid flow occurred.

After 14 days of simulated microgravity, we observed a severe decrease in PG content compared to that in the CTL group. Consequently, the fibrocartilage of the ATE may be less hydrated, which could explain the deleterious effects on the mechanical behavior of the ATE under cyclic compressive stress. Indeed, with unloading, a significant

loss of Δd occurred after both 1,250 and 2,500 cycles. Interestingly, the loss of PGs by enzymatic digestion in the intervertebral disc results in an increase in both height loss and stiffness of the tissue after cyclic compressive loading (Bardir et al., 2010). In addition, the enzymatic digestion of PGs leads to a decreased damping coefficient after cyclic compressive stress, which highlights the loss of viscosity of the articular cartilage (Bader et al., 1992). Thus, the increased loss of Δd after 14 days of hindlimb unloading, which was almost twice higher than control, could be a result of the loss of PG content. The latter may explain the increased stiffness and loss of viscosity, which were three times and almost twice as high, respectively, as those of the control. It is also possible that the collagen fiber disorganization described in the superficial part of the ATE under the same experimental conditions (Camy et al. 2022) plays a role in the deterioration of the fatigue behavior of the HU entheses. If the enthesis responds like articular cartilage, where water extrusion and collagen packing occur rapidly with cyclic compressive loading (Bader et al., 1992), the loss of PGs in HU entheses, as well as collagen disorganization, could not be responsible for the impairment of Δd during the second part of the test. However, as we performed our tests on both the UFc and CFc, the mineral matrix of the CFc may also play a role in this delayed impairment.

Finally, although the Δd we observed was smaller than the enthesis thickness in the loading direction, a possible effect of subchondral bone on mechanical response of the ATE during our test cannot be ruled out due to the porous structure of bone and fibrocartilage (Tits et al., 2023). Although reports in literature have demonstrated that the calcaneus microstructure is affected by unloading conditions during 1 month spaceflight (Gerbaix et al., 2017), we recently showed with micro-indentation experiment that a 14-day simulated spaceflight had no effect on the Young's modulus and hardness of subchondral bone (Camy et al., 2024). These results suggest that the changes in the mechanical behavior of the ATE observed here are not attributed to the mechanical properties of the subchondral bone. Further investigations must be conducted about the impact of unloading on the subchondral bone microstructure.

The second purpose of our study was to investigate the effects of the return to normal load on the ATE mechanical behavior under compressive stress. After six days of reloading, while the biological characteristics of the enthesis were not recovered, some aspects of the mechanical response were restored since the loss in Δd was similar to CTL entheses at 2,500 cycles, and fatigue behavior was mainly deteriorated in the first 1,250 cycles of the test. Compared to the CTL condition, we observed an abnormally high PG content in the UFc, probably owing to the return of compressive stress with reloading. The work of Walsh and Lotz (2004), who found a positive correlation between the compressive stress level applied on the intervertebral disc and PG content, supports this hypothesis. In a previous study, we observed over-expression of PGs in the UFc with reloading. This was accompanied by

an increase in collagen II expression (Camy et al., 2022). With a potential increase in type II collagen expression and amplified PG content, especially on the superficial part of the ATE, where a cyclic compressive load was applied, we can assume that the interface is protected against repeated compression through improved tissue water retention. This protective effect probably accounts for the similar capacity of the HUR entheses to resist repeated compressive stress as that of the CTL entheses.

Despite the same capacity of the reloaded entheses to withstand cyclic compressive stress as that of the CTL entheses, the viscoelastic properties of the entheses were not restored with early remobilization. A significant increase in stiffness was observed after 1,250 and 2,500 cycles. In addition, the increase in stiffness was still twice that of the CTL entheses after cyclic compressive loading. In addition to the involvement of PGs and collagens in the evolution of ATE stiffness under compression, the mineral fraction may also contribute to the stiffening of the enthesis. In a previous study, we found an increase in the CFC area of the enthesis after six days of reloading, especially on the superficial part of the enthesis (Camy et al., 2022). Interestingly, in porcine vascular tissues subjected to uniaxial unconfined compression test, it was demonstrated that the elastic modulus is greater in calcified aortic tissues than in healthy aortic tissues (Walraevens et al., 2008). This result is consistent with the contribution of the mineral matrix to the stiffening of HUR entheses. Finally, for the HUR entheses, we observed a significant reduction in the hysteresis area after 1,250 and 2,500 cycles. The viscosity loss, similar to that of the HU entheses, was almost twice that of the CTL entheses. Collagen fiber remodeling may explain this abnormally high loss of viscosity. Reloading induces highly crimped collagen fibers in the UFc of the ATE (Camy et al., 2022), which could result from this highly crimped collagen network having a lower capacity to restrain water. This therefore reduces the swelling pressure on the fibrocartilage and resulting in a greater loss in viscosity in response to cyclic compressive loading.

Both our histological and mechanical studies are subject to some limitations. As the entheses subjected to the mechanical test could not be prepared according to the histological standards (fresh sample, not frozen beforehand and fixed immediately after sampling), we chose to carry out the proteoglycan study on the controlateral foot. As a result, it was not possible to make direct correlations between the biological response and the mechanical response for the same enthesis. We decided to prioritize the quality of tissue processing, as this guarantees high-quality staining without artefacts. Regarding the mechanical study, although a some uncertainty in the placement of the testing device could have occurred despite a visual control by camera, it is unlikely that we characterized neighbouring structures since we optimized our set-up to focus the applied compressive forces only on the ATE by positioning our samples at an 45° angle. However, as fibrocartilage is an anisotropic structure considering the orientation of collagen fibers at the interface (Sartori and Stark, 2020), even a slight angular variation can lead to some variability in the values of the parameters we assessed.

5. Conclusion

In conclusion, this study is the first to characterize the fatigue behavior of the ATE under compressive loading. This shows that chronic mechanical unloading impairs the fatigue behavior of the interface and strongly suggests that this worsening mechanical response results from the remodeling of the fibrocartilage extracellular matrix. Furthermore, our study demonstrated that a short period of free remobilization was insufficient to completely restore fatigue behavior under cyclic compression. Although fibrocartilage is supposed to protect the ATE against wear and tear from tensile, shear, and compressive stresses (Shaw & Benjamin, 2007) by mitigating stress concentrations (Bunker et al., 2014; Smith et al., 2012), an impairment in fatigue behavior under cyclic compressive loading could weaken the interface. In humans, where compressive stress has been demonstrated in ATE (Chimenti

et al., 2016; Lyman et al., 2004), individuals may be exposed to chronic periods of inactivity and skeletal muscle disuse owing to a sedentary lifestyle, immobilization following an injury, and bed confinement. Spaceflight, which places astronauts under hypokinetic and hypodynamic conditions, also reduces muscle activity (Booth et al., 2017). Clinically, our study highlights the need to manage compressive stress through ankle dorsiflexion angle when countermeasure exercises are performed or during early remobilization to minimize the risk of fibrocartilage injury.

CRediT authorship contribution statement

Claire Camy: Writing – original draft, Validation, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Aurélien Maurel-Pantel:** Methodology. **Marylène Lallemand:** Methodology, Investigation, Conceptualization. **Théo Fovet:** Methodology. **Thomas Brioché:** Methodology. **Cécile Genovesio:** Methodology. **Angèle Chopard:** Validation, Supervision, Conceptualization. **Martine Pithioux:** Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sandrine Roffino:** Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jbiomech.2024.112231>.

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