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ORIGINAL ARTICLES

CASE REPORT

NOTES AND NEWS

Effects of different frequencies of loading on healing in partial rupture of the Achilles tendon in a rat model

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Abstract

Objective: To determine the histological effects of different frequencies of loading in a healing Achilles tendon following partial rupture.
Design: Experimental laboratory study.
Setting: University of Zimbabwe, Department of Physiology, Animal house.
Subjects: Sixty female Sprague-Dawley rats
Intervention: Partial tenotomies of the right Achilles tendon were performed surgically. From day 1 post operatively, the animals were allocated to treadmill running at different frequencies (once (OD), two (BD), three (TDS) and four(QID) times daily) up to 21 days. Histological sides of the tendons were made at days 7,14 and 21 and interpreted by a blinded pathologist
Main Outcome Measures: Collagen fibre orientation, inflammatory cell populations, fibroblast morphology and neoangiogenesis were observed and scored using the Grande Biomechanical Histological Correlation Score.
Results: Mean weight was 209.67g ±30.14. The best and worst arrangements of collagen were in the QID group (73%) and OD group (46.7%) respectively. These differences were not statistically significant (p=0.487). The BD group had the most mature fibroblast nuclei and the QID tendons had the least mature (p=0.577). Inflammatory cell populations were independent of loading frequency (p=0.132).
Conclusion: Changing the frequency of the same type of loading in a healing tendon does not have an effect on the healing process in partially ruptured Achilles tendons during the inflammatory and proliferative phases.

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Introduction

Nearly five hundred years after the first description of a conservative protocol of management by Ambroise Pare (c1510-1590), optimal rehabilitation of Achilles Tendon Rupture (ATR) continues to elude clinicians

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and researchers alike.[1] The Achilles tendon is the largest tendon in the human body.[2] It is also the most injured tendon and accounts for a considerable proportion of all surgical tendon repairs.[3] The incidence of ATR in non-athletes is on the increase globally, possibly from the increase in sport participation by the generally aging population.[3-6] This injury has a bimodal distribution with the first peak occurring in men in their third to fourth decade while the second peak occurs in post-menopausal women in their seventh to eighth decade.[2] Management of this injury is a long process because the tendon is a metabolically relatively inactive and a vascular structure.[7] This feature (though useful for its function) impedes healing after rupture. The treatment is controversial, because the literature and practice are equivocal on the best way of managing the injury.[3,5] Recent studies, both human and animal, have shown that regardless of whether the injury is managed surgically or otherwise, prolonged immobilisation as was previously advocated, is detrimental to successful healing, prolongs recovery time and results in inferior functional outcomes when compared to early mobilisation.[8] This new paradigm of early functional rehabilitation aims for a well-balanced weight-bearing protocol to substitute cast immobilisation.[9] While the benefits of early weight bearing have been debated, the current challenge in implementing these data in practice is in the precise prescription of weight-bearing exercise. This weight-bearing exercise should be enough to induce mechanotransduction processes that lead to healing yet not so much as to cause fatigue, impair healing or cause further harm to the injured tendon. To date, the precise effects of specific dosages of loading on viscoelastic tissues are not clearly understood.[10] As such, establishing specific dosage parameters for early functional loading of the healing Achilles tendon are still undefined. This limits the clinical application of mechanical loading in the management of Achilles tendon rupture. The objective of the current study was to establish the histological effects of different frequencies of loading on healing in partial rupture of the Achilles tendon using an animal model in Sprague-Dawley rats. We hypothesised that no histological differences would be observed across the different loading frequencies.

Ethical considerations.

Ethical clearance to conduct this research was obtained from the Joint (Parirenyatwa and College of Health Sciences) Research and Ethic Committee (JREC) at the University of Zimbabwe. All institutional and international guidelines to the care of laboratory animals were adhered with.

Materials and Methods

Sixty female Sprague Dawley rats were conditioned to a treadmill apparatus (LE8700, PANLAB, Barcelona) for ten days before surgical hemi-transection of the right Achilles tendon was carried out. The animals were anaesthetised with 5% halothane gas via face mask. Once anaesthetised, the skin on the right hind limb was swabbed with antiseptic solution and shaved. A longitudinal incision of the lateral aspect of the limb was made. Care was taken to avoid the course of the sural nerve. The Achilles tendon complex was exposed. The lateral portion of the tendon was nicked with a blunt scalpel until it was partially severed. This created a frayed tear. The ends were left unsutured. The overlying skin was then sutured with continuous suturing using dissolvable suture material. The animals received post surgical analgesic with Carprofen, 100mg orally for the first 48 hours. On day 1 post-surgery, the rats were randomly assigned to four groups. These groups performed the treadmill exercise for five minutes at a time, once (OD), twice (BD), thrice (TDS) and four times (QID) a day. At days 7, 14 and 21, five animals from each group were selected at random for sacrifice. They were given an overdose of 5% halothane gas then euthanised with a 1ml intracardial injection of pentobarbitone. They were observed until they expired.

Bilateral transfemoral amputations of both hind limbs were then performed. The left hind limbs were taken as internal controls. The Achilles tendon complex with both attachments intact were shaved of excess muscle and fascia and preserved in 10% formaldehyde solution for 24 hours in labelled jars awaiting histological analysis. At histology, the serial slides of the samples were made using a 5μm microtome. They were stained with haematoxylin and eosin and Sirius red. Collagen fibre orientation, fibroblast concentration and fibroblast nuclei shape, neoangiogenesis and inflammatory cell populations were observed at x10 and x40 power with a light microscope. A blinded pathologist interpreted the slides and scored the parameters using a modification of the Grande Histological Biomechanical Correlation Score.

Statistical analysis.

The data was analysed using STATA version 10. We used the chi-square test to test for associations between the frequency of loading and the observed effects. In some instances the Fisher's exact test was used. Means were calculated using the Student's t-test. All values were reported as Mean (SD) [range] unless otherwise stated. Statistical significance was pegged at the 0.05 level.

Results

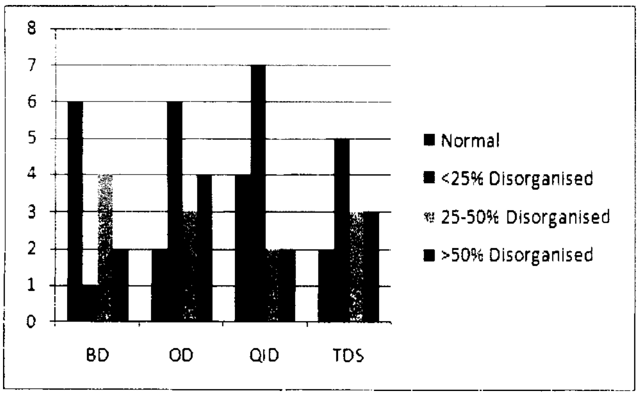
The weight of the rats were normally distributed, mean 209.67g (SD=30.14). Twelve slides were deemed unreadable and were removed from the analysis. All the tendons analysed showed signs of healing despite the early weight bearing beginning day one post surgery ($p<0.05$).

Figure I: Normal tangential collagen.

Effect on collagen fibre orientation

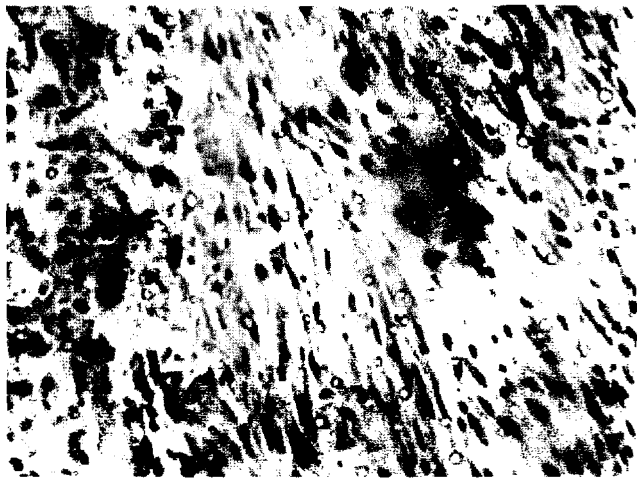
Normal tangential collagen (figure I) was observed in 20% of the experimental specimen (n=15). Forty percent of these were in the BD group. Performing the treadmill protocol once daily yielded the most disorganised collagen fibre orientation.

Figure II: Effect of different frequencies of loading on collagen fibre orientation.



The best overall collagen fibre arrangement was observed in the QID group in which 73% of the tendons had collagen fibre arrangement that were <25% disorganised or better. This group also had 25% normal orientation of collagen fibres. The most abnormal collagen fibre orientation was observed in the O.D group where 46.7% of the tendons were graded >25% disorganised (figure II, figure III). These observations were however, not statistically significant (p=0.487).

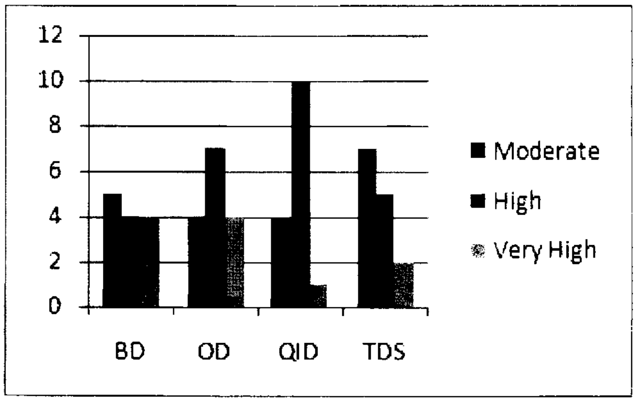
Figure III: Collagen fibre orientation that is 25%-50% disorganised.



Effect on fibroblast concentrations and nuclei shape

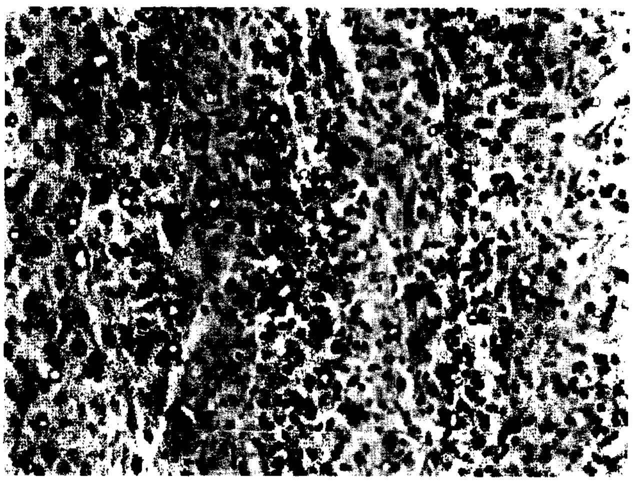
Very high fibroblast counts were observed in 11 (n=57) specimens from the experimental limb. High fibroblast counts were observed in 26 specimens and of these ten were from the QID group (figure IV). Immature, plump fibroblast nuclei were observed in 81% of the tendons (46/57). The highest number of immature fibroblast nuclei was observed in the QID while the BD had the most mature nuclei. No spindle shaped nuclei were observed in the TDS group. These observations were not statistically significant (p=0.577).

Figure IV: Effect of different frequencies of loading on fibroblast concentration.



Effect on inflammatory cell populations: Thirty five (61%) of the experimental tendons had very low neutrophil and lymphocyte counts while all the control limbs (n=48) had very low neutrophil counts. By day 21, the neutrophil counts were very low (p=0.003). The lowest neutrophil and lymphocyte counts were observed in the QID group and the highest neutrophil and lymphocyte counts were in the BD and TDS groups respectively (figure V). Inflammatory counts were independent of the frequency of loading (p=0.132).

Figure V: Very high inflammatory cell concentration.



No eosinophils, macrophages or basophils were observed in the tendons. Generally, inflammation was acute on chronic in the experimental limb and non-existent in the control limb. Isolated hyaline cartilage nodules were observed in two slides that were unremarkable on further analysis.

Effect on neoangiogenesis: Vascularisation with either capillaries (n=26) or arterioles (n=24) was observed in 90% of the tendons. Tendons from the QID group demonstrated the most advanced neoangiogenesis as all the tendons were with capillaries at the very least. Neoangiogenesis was not dependant on the frequency of loading (p=0.435).

Discussion

Achilles tendon rehabilitation is a long process fraught with controversy on the ideal management protocols. This study sought to establish the effects of known frequencies of loading on collagen fibre orientation, inflammatory cell populations, fibroblast morphology and neoangiogenesis in the inflammatory and proliferative phases of Achilles tendon healing following partial rupture.

Angiogenesis plays a key part in tendon healing by re-establishing circulation and limiting ischemic necrosis at the injury site while permitting repair. This process is initiated by the release of factors such as Vascular Endothelial Growth Factor (VEGF), bradykinin and NO from inflammatory cells. In this study we observed no differences in neoangiogenesis across the different exercise groups at days 7, 14 and 21. As did Palmes *et al.*[11] Salate *et al*[12] however, observed an effect on neoangiogenesis with laser therapy in partially ruptured rat Achilles tendons on days 3 and 5 post rupture. In this study we may have failed to observe a significant effect on neoangiogenesis because by day 7, the number of new blood vessels decreases and returns to normal.[13] This result was reflected by other researchers.[11,14] On the other hand, Nakama *et al*[15] observed increases in VEGF and capillary densities in loaded rabbit tendons at 14 weeks. As the latter was a tendinopathy model, the observed new capillaries may have been pathological.

In this study, the control tendons had very few inflammatory cells; a sign that there was no underlying inflammatory process. The different frequencies of loading had no effect on the population of inflammatory cells in the experimental limb but the changes in concentration of neutrophils with time were significant. This demonstrates that after the initial inflammatory response which is most observable in the first week, the inflammation resolved and did not persist which is a good indicator of healing. Studies on the inflammatory response in tendons show that inflammation begins soon after the onset of exercise and persists for a short while after[16]. Human tendon fibroblasts produce inflammatory cell mediators in response to stretching which are crucial to the healing process.[17] These mediators lead to release of vasoactive and chemotactic factors which recruit fibroblasts and begin collagen synthesis.[18]

Fibroblasts are the dominant cell type in tendons and are responsible for the changes that result from mechanical loading by altering Extracellular Matrix (ECM) proteins.[18] Mechanical loading enhances repair by stimulating fibroblast activity for instance through increasing collagen synthesis. During the proliferative phase of healing, we expected to observe fibroplasia and fibrillogenesis in the tendon. This was not observed in the tendons in this study. As a result, a concomitant low production of collagen could be expected.

Collagen fibres are the main structural component of tendon. They provide the tensile strength of the tendon and are at their greatest mechanical advantage when they are arranged parallel to the axis of greatest tension. A highly linear collagen fibre arrangement translates to a tendon with a high Ultimate Tensile Strength (UTS).[19]

In this protocol, changing the frequency of loading had no effect on the collagen fibre orientation of healing tendons. Similar results were observed by Boyer *et al*[20] who noted no differences in the healing response of canine flexor tendons regardless of the training group they were assigned to. Other results from an in vitro study by Bosch et al[21] also support these observations. In their study, they demonstrated that collagen synthesis is dependent not on the amount or type of loading but on the post loading resting time. The authors postulate that with rest times of ≥24 hours between bouts of exercise it is highly likely that collagen synthesis would increase as a consequence of increased fibroblast proliferation. This is echoed by Miller *et al*[22] who demonstrated that adaptation of the Extracellular Matrix (ECM) occurs acutely within 24 hours. An article from Kjaer *et al*[23] reflects the same view. They argue that acute exercise leads to Matrix Metalloprotease (MMP) regulated collagen catabolism which is then followed by collagen synthesis. This effect lasts for up to 3 days in humans. Kjaer *et al* conclude that increases in the collagen synthesis arise from a mechanically induced anabolic pathway using MAP kinase which is an enzyme controlled receptor. As with any enzyme system, the principles of enzyme- substrate saturation should apply. As such, the intracellular proteins produced because of the initial loading episode are likely to form and maintain receptor-ligand bonding for long periods of time during which another loading episode would stimulate more intracellular proteins that would not have an effect because of the unavailability of binding sites. In this instance, the authors (Kjaer *et al*) noted that the resting values of these proteins were approximated at around 72 hours post-initial loading. Also, repetitions of acute exercise bouts would lead to even more collagen catabolism and hence may actually have an inhibitory effect on healing. In another study on in vivo application of static loads in chicken flexor tendons, Slack *et al*[24] established that increased collagen synthesis could still be detected 72 hours after a single bout of mechanical loading. This gives credence to the notion of "tendon memory" as postulated by Andersson *et al*[25] who also found no added benefit to repeating the same type of exercise within a 24 hour period. They suggested that the tendons "remembered" the initial loading episode and hence did not respond to subsequent loading of the same type.

In this work we assumed that any loading episode shorter than 15 minutes as previously done by Andersson *et al* would be short enough to have an effect if repeated. From these results it appears it is enough that the tendon has perceived a loading stimulus, however short. However, as did the prior group, in our study we failed to take into consideration the improvements in tendon parameters with time. We maintained the same exercise protocol throughout the experiment and while it may have been correctly dosed at the start of the experiment it may well have become suboptimal as the experiment progressed. A more efficient way would have been to use undulating periodisation in the frequency of the treadmill running.

These results collectively suggest that after application of a single loading episode there is no need to repeat the same loading protocol within 24 hours as it would have no added effect to collagen synthesis, alignment and eventual healing. Contrary to this assertion, Takai *et al*[26] showed that the frequency of controlled motion of healing flexor tendons is a significant factor in accelerating the healing response with higher frequencies being more beneficial. Their results show improved tendon structure at three weeks and six weeks post operatively in the high frequency group. Key differences though from the current study are that they performed passive movement of the tendons. This type of movement has a different cellular signalling pathway from active movement.[26] In addition, they observed for changes at 21 and 42 days. At these time points in the healing process the changes are well demarcated and are easily distinguishable. The present study observed for similar variables but earlier and with shorter periods between, at a time when more sensitive tests other than histology could have identified the differences. Histological analysis however, has been used successfully in other tendon studies.[27-29]

In general, the results of this study show evidence of different histological effects in healing tendons exposed to varying frequencies of mechanical loading even during the early stages of healing. These observations however, were not statistically significant. As such, changing the frequency of application of the same type of mechanical loading does not have an effect on healing in partial rupture of the Achilles tendon during the inflammatory and proliferative phases. This highlights nature's intolerance for monotony and should act as a guide to the prescription of exercise in clinical practice.

The study also supports the beneficial effect of early weight bearing post tendon rupture. This is in agreement with other studies that have found that early weight bearing is not detrimental to tendon healing.[25,27,30-31] It does however, disagree with other authors[32,33] who found no benefit to loading a healing Achilles tendon in the early stages of healing. These results though should be interpreted cautiously as this was study was conducted in a rat model. Rats are quadruped animals and can better adjust weight bearing through an injured limb better than a bipedal human, for whom these data will be inferred. In addition, this study was conducted in during the early stages of healing in a partial rupture of the Achilles tendon as a result it is difficult to extrapolate the conclusions to full ruptures in long term healing. In general, methodological consistency is low across studies on tendon studies. Some studies were conducted in vitro[18,20] others though in vivo were in canines[34] while others used the rabbit anterior cruciate ligament[33] and some though in rodent Achilles tendon observed changes at different time points during early healing[25] or at different stages of healing altogether.[35] This limits the generalisability of the data obtained from these studies.

Conclusion

The use of early functional weight-bearing has now become widely accepted as a valid and safe option in tendon rehabilitation. However, doubts still exist on the prescription of weight-bearing exercise. In this study we examined the effects of different frequencies of weight-bearing (loading) exercise on early healing in partial rupture of the Achilles tendon. Our results demonstrate that changing the frequency of the same type of loading within a 24 hour period has no bearing on the healing during the inflammatory and proliferative phases. We concur with previous work that intimated on a tendon memory. Further studies to define the components and longevity of this tendon memory are needed to better inform clinical practice on how often a healing Achilles tendon should be loaded following partial rupture. In clinical practice, these results also show the need to vary the type of exercise prescribed during early rehabilitation in order to optimise tendon healing.

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An echocardiography audit to determine and characterise rheumatic heart disease lesions seen in 2012

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Abstract

Introduction: Rheumatic heart disease is still a common problem in Zimbabwe. It has a significant mortality rate due to heart failure, stroke or endocarditis. Timely surgical interventions can reduce mortality. An echocardiography audit was performed to determine the proportion of patients referred for echocardiography who had a diagnosis of rheumatic heart disease, the pattern of valvular involvement and the presence of surgical indications on echocardiography.
Objective: To determine the number of echocardiograms done in 2012 and the proportion with a diagnosis of rheumatic heart disease. To determine which valve lesions were present and whether there were any echocardiographic indications for surgical intervention.
Method: A record review of all echocardiograms performed by the investigator during the period January to December 2012 was performed. A data collection form was used to extract the data and the findings were tabulated and analysed.
Results: Three hundred and eight (308) echocardiograms were performed by the investigator during the year of review. 236 of these were abnormal and rheumatic heart disease was diagnosed in 16% of them. The commonest valve lesion was mitral regurgitation and half of the patients had surgical indications. The common complications were pulmonary hypertension and left atrial enlargement.
Conclusion: This audit shows that rheumatic heart disease is still quite common in patients referred for echocardiography. Lesions are severe and the majority of patients are in need of surgical intervention.

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