

Models for the study of tendinopathy

R.C. Dirks^{1,2} and S.J. Warden^{1,2,3}

¹Center for Translational Musculoskeletal Research, School of Health and Rehabilitation Sciences, Indiana University, Indianapolis, IN, USA;

²Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, USA;

³Department of Physical Therapy, School of Health and Rehabilitation Sciences, Indiana University, Indianapolis, IN, USA

Abstract

Tendinopathy refers to the clinical presentation of activity-related pain, focal tendon tenderness, and intratendinous imaging changes. The underlying pathology was once thought to be due to inflammation ('tendinitis'), but is now considered to predominantly result from degeneration ('tendinosis'). While some progress has been made in understanding tendinosis, the condition remains poorly understood and a need exists for suitable exploratory preclinical models. It is unlikely that one suitable model exists because of the complexity of the underlying pathology and myriad of possible causes. This paper provides an overview of current models utilized in tendinopathy research. It progresses hierarchically from *in vitro* and *ex vivo* models to *in vivo* models. For each model, rationale for use, pertinent findings, and advantages and disadvantages are discussed. By improving on these models, new methods for the prevention and treatment of tendinopathy may be explored with the ultimate outcome being a reduction in the occurrence and effects of the condition in humans.

Keywords: Animal Models, Overuse, Tendinitis, Tendinopathy, Tendinosis

Introduction

Tendinopathy refers to the clinical presentation of activity-related pain, focal tendon tenderness and intratendinous imaging changes. The underlying pathology was historically believed to be one of inflammation, and thus the condition was termed 'tendinitis'. While the initiating stimulus may begin with inflammation, numerous studies have shown the established pathology to consist of tendon degeneration with a complete absence of inflammatory cells. Consequently, the term 'tendinosis' has been recommended to describe the typical histopathological appearance of the underlying pathology.

Studies of the pathogenesis of tendinosis in humans are difficult as desirable outcome measures necessitate tissue procurement. While tissue can be obtained surgically or via biopsy, these procedures are typically restricted to individuals with ad-

vanced pathology and it is difficult to obtain comparative normal tendon samples from control subjects. To further understanding of tendinosis and its underlying causes, suitable models are required. Models may range from *in vitro* to *in vivo* and allow for invasive studies of the whole tendon, its surrounding tissues, and the molecules involved in the pathology. Molecular studies can be done *in vitro*, which allows for specific cell populations to be studied and thus local effects of various factors to be observed. Tendons may be removed from an organism for *ex vivo* studies which allow investigators to study the effect of different factors on the entire tendon rather than just specific cell types. Finally, studies can be conducted using animals in order to analyze tendinosis *in vivo* with the input of cell signaling occurring within the organism. By using these models, the changes occurring in tendinosis can be studied more thoroughly which may help lead to better treatment options for patients.

This paper provides an overview of current models utilized in tendinosis research. It progresses hierarchically from *in vitro* and *ex vivo* models to *in vivo* models. For each model, rationale for use, pertinent findings, and their advantages and disadvantages are discussed.

In vitro models

In vitro literally means 'in glass' and typically refers to studies on isolated cells grown in an artificial environment. *In vitro*

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Corresponding author: Stuart J. Warden, P.T., Ph.D., Department of Physical Therapy, School of Health and Rehabilitation Sciences, Indiana University, 1140 W. Michigan St., CF-326, Indianapolis, IN 46202, USA
E-mail: stwarden@iupui.edu

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studies in tendinosis research are used to study cellular responses to stimuli believed to be involved in the pathogenesis of the condition, with the principal stimuli be mechanical.

Cyclical stretching

Rationale: As mechanical overload of a tendon is considered a primary risk factor for the development of tendinosis, *in vitro* studies typically expose isolated tenocytes/fibroblasts to cyclical stretching. The loading is in an attempt to explore the effect of cellular deformation on the production of potential cellular and molecular mediators¹.

Findings: Cyclical deformation of tenocytes/fibroblasts has been found to cause a number of effects including an increase in the production of prostaglandin E₂ (PGE₂)^{2,3}, cyclo-oxygenase-1 (COX-1)^{3,4}, COX-2, cytosolic phospholipase A₁ (cPLA₁) and secretory phospholipase A₂ (sPLA₂)⁴, with stretching frequency being the best way to impact COX-2 expression and PGE₂ production⁴. Cyclical stretching has also been shown to increase leukotriene B₄ (LTB₄) production and modify the expression of 5-lipoxygenase (5-LO)¹.

The fibroblasts used in cyclical stretching can be pre- or co-treated with factors in order to explore additive and/or synergistic effects. Yang⁵ included interleukin-1 β (IL-1 β) in the growth media to cause inflammatory responses in human patellar tendon fibroblasts. When IL-1 β was present, 4% stretching decreased the expression of COX-2 and matrix metalloproteinase-1 (MMP-1) as well as PGE₂ production, but 8% stretching caused an increase in MMP-1 expression. Therefore, lesser stretching seemed to be anti-inflammatory while greater stretching led to a pro-inflammatory effect.

Advantages and disadvantages: *In vitro* models have the benefit of being able to easily and rapidly explore many different cellular processes, such as DNA synthesis, mitosis, gene expression, and cell differentiation^{2,6-15}. Also, the cells can be arranged and stretched in a way that is similar to the shape, alignment, and stretching that occurs *in vivo*³, which is important because changing the shape of the cell may lead to different cellular responses¹⁶. However, there are inherent disadvantages to *in vitro* models, some of which can be corrected by advancing the model, while others will always remain. For instance, deformation and strain of the dish and substrate used are not completely transferred to the cells, making stretching durations and frequencies imprecise. Hopefully, future improvements in the equipment used and experimental set-up will minimize or eliminate these problems. Unfortunately, a limitation that will persist with *in vitro* models is the study of isolated cell types in an environment devoid of extracellular matrix and neurovascular supply. The study of cells outside their normal environment ultimately limits the translatability of findings.

***Ex vivo* models**

To study tendon processes and responses in whole tissues rather than isolated cells, several *ex vivo* models have been developed. These allow for interactions between tendon cells and the extracellular matrix to remain intact providing a more nat-

ural environment while still providing great control over experimental conditions. The three major means of perturbing and studying tendon tissue *ex vivo* are cyclical loading, creep loading, and stress deprivation.

Cyclical loading

Rationale: Like *in vitro* cyclical stretching of isolated cells, *ex vivo* cyclical loading was developed to assess the effect of repetitive loading on tendon, yet with the extracellular matrix intact¹⁷.

Findings: After 24 hours of cyclical loading, tendons have been shown to exhibit decreased ultimate failure strength and a significant increase in midsubstance lengths¹⁷. Tendons undergoing higher loading became significantly weaker than control groups and cellular turnover and the amount of collagenase increases with increased load magnitude and duration suggesting tendon degradation¹⁸. Tensile loads can also be applied in order to cause a deformation of cell nuclei, which may lead to differences in the intracellular signals¹⁹. To study these signals, levels of gene expression can be examined. For example, Devkota¹⁸ found increased levels of PGE₂ in the growth media, which were further increased as the loading duration was increased. MMP-1 mRNA levels can also be altered by adjusting the amplitude and frequency of loading, as low strain and frequency causes a significant inhibition of MMP-1 expression, while higher strain and frequency completely inhibits MMP-1 expression²⁰.

Advantages and disadvantages: When using this method, it is important to use strains above 5%, as strains below 5% seem to cause little or no damage^{21,22}. This method does not include the complexity of an *in vivo* model because like the *in vitro* cellular stretching, complete repair processes cannot occur due to a lack of vascularization and systemic signaling molecules. In addition, mechanical strain differences between the grips and the midsubstance of the tendons have been found, so the stress may be greater at the edges of the tendon instead of being distributed evenly throughout¹⁷. This model also has the disadvantage of requiring a media for the tendons. Devkota¹⁸ replaced the media every 3 days for tissue maintenance, which can cause misleading results as the substances in the media accumulate over the 3 days, making the highest levels immediately before the media is changed. However, as this method has become more widely used, several advances of been made to improve the technique. One of these advances is using a closed-loop force-feedback system that is capable of loading six tissue samples with independent force and displacement control. This system can be used to account for factors such as creep and force relaxation while controlling specific load parameters such as peak strain, peak stress, strain rate, stress rate, loading frequency, and force-time integral, which can be used to determine the mechanisms of tendon injury²³.

Creep loading

Rationale: In addition to repetitive loading, sustained loading of a tendon may also result in tendon injury, so research has been conducted using prolonged loading of the tendon.

Findings: Wren²² studied and compared the effects of both cyclical and creep loading of human Achilles tendons and

found that both result in significant damage. For creep tests, the applied stress did not have a significant relationship with the time to failure. Instead, initial strain was a predictor, while with cyclical loading there was a significant relationship between the peak stress and the time to failure, as well as between initial strain and time to failure. Therefore in creep loading, higher initial strains create more damage, leading to an earlier failure of the tendon.

Advantages and disadvantages: When comparing the creep and cyclically loaded tendons, both have similar disadvantages, and both developed tendon damage; therefore, either could be used to study tendinopathy.

Stress deprivation

Rationale: Studies using *in vivo* immobilization or *in vitro* stress deprivation of tendons have resulted in increased MMP-1 mRNA expression^{20,24,25}; therefore, stress deprivation could decrease the tensile properties of tendon, making it an appropriate model for tendinosis studies.

Findings: After undergoing stress deprivation, tendons have decreased tensile modulus and ultimate tensile stress, as well as increased expression of MMP-1 mRNA, but have no significant changes in collagen diameter. Because changes in tensile properties exist without changes in collagen, the tensile properties are not likely related to the changes in collagen²⁶.

Advantages and disadvantages: This model does cause some aspects of human tendinopathy, but because collagen is not altered, it should not be used when studying the effects of collagen on tendinopathies.

Chemical *in vivo* models

While the previous methods are beneficial in understanding specific cellular responses, it is also essential to look at the tendons *in vivo* in order to gain a more complete understanding of tendinopathy. To do this, researchers have developed a variety of methods for inducing tendon conditions in animal models, including chemical and mechanical interference. A variety of models are necessary because each model may only represent certain aspects of the pathogenesis and features of tendinopathy. Because there are no animals that have the same tendon characteristics as humans, these studies can be done in many different animals and researchers may choose different animals for specific reasons, such as knowing the genetic sequence of an animal²⁷. There are disadvantages of both chemical and mechanical induction of tendinopathies. For example, chemically induced models cause acute changes, which may not accurately represent the chronic changes of human tendinopathy. However, chemical injections do have the advantages of being less labor intensive and producing consistent tendon damages.

Collagenase injection

Rationale: Because tendinosis is a degenerative disorder, it likely involves the breakdown of collagen within the tendon. In order to mimic this mechanism, bacterial collagenase has

been injected into tendons to induce degeneration of the collagen, and thus the tendon.

Findings: Foland²⁸ was one of the first researchers to use collagenase and did so in an effort to affect tendons in horses. Collagenase was injected into 3 sites of the superficial digital flexor tendon. The injected tendons developed lesions and type III collagen was formed along with the normally occurring type I collagen. Others have used collagenase injections in rat supraspinatus tendon²⁹, which results in an increased number of cells, more round, plump, metabolically active fibroblasts, disruption of collagen organization, and an increase in tissue vascularity. After looking at the effects during specific time intervals, the changes that occurred after collagenase injection did not become normal after 12 weeks²⁹. Lui³⁰ conducted a similar experiment using the patellar tendon of rats and found the same effects; however, they observed the tendons for a longer period of time and determined that the tendons had healed at 32 weeks post-injection. Both groups also found a lack of inflammatory cells in the injected tendon, but inflammatory responses have been observed at two weeks post-injection of collagenase into the patellar tendons of rats³¹. In addition to inflammation, Fu³¹ found matrix calcification at 16 weeks post-injection, which corresponds to cartilaginous metaplasia and ossification. Also, cells had separated from their pericellular matrix by a lacunar space. Along with all of these observations, collagen degradation followed by new collagen synthesis and increased cross-linkage was observed after collagenase injection into the patellar tendon of rabbits.

Advantages and disadvantages: While this method results in many characteristics similar to that of human tendinopathy, including hypercellularity, loss of matrix organization, increased vascularity, and a lack of inflammatory cell inflammation³²⁻³⁷, it does have some drawbacks. Because it is bacterial collagenase that is injected, there may be differences from human collagenase, which may result in altered response³⁸. Also, it causes a healing response after injection, but tendinopathy patients often have an impaired healing process³⁹.

Cytokine injection

Rationale: Because tendinopathies may begin with inflammation that is followed by degeneration, cytokine injections have been used in order to cause an inflammatory response, which may be similar to that found in the onset of tendinopathy.

Findings: Stone³⁸ injected species-specific cytokines into rabbit patellar tendons³⁸. After four weeks, the tendons had increased cellularity, which then decreased toward normal after 16 weeks, but there were no changes in the matrix or in cross-sectional area. The cytokine injected tendons had a significant decrease in ultimate load at 16 weeks; however, because there was no matrix damage or collagen degradation, the cytokine injection only caused a slight tendon injury that was reversible. Therefore, the results of cytokine injection are much less severe than those of collagenase injection, making it a less reliable model of tendinopathy.

Advantages and disadvantages: Unlike collagenase injections, cytokine injections are specific to the species³⁸, so any differences

caused by injections from another species are eliminated. While cytokines do increase cellularity and decrease failure loads, the injection is composed of a collection of cytokines, so it is unknown what is contributing to the changes, as well as if they are in concurrence with what occurs in human tendinopathy.

Prostaglandin injection

Rationale: *In vitro* studies have shown that repetitive mechanical loading of human tendon fibroblasts increases the production of prostaglandins^{2,3}. Also, *in vivo* studies have shown that exercise increases the levels of prostaglandins in the peritendinous space⁴⁰. Therefore, prostaglandins may be an important step in the development of tendinopathy.

Findings: Sullo⁴¹ administered weekly injections of PGE₁ into the Achilles tendons of rats. After one week, the treated tendons had significantly higher water content and wet weight than the control tendons, as well as acute inflammation. At three weeks, half of the injected tendons had fibrosis of the paratenon along with adhesions and degeneration, while the other half still had acute inflammation. After five weeks of injection, all of the tendons had fibrosis of the paratenon and adhesions and degeneration, so repeated PGE₁ injections initially caused inflammation that gradually changed to degeneration. Khan⁴² injected different doses of PGE₂ into the patellar tendon of rabbits once a week for four weeks. Both doses (50 ng and 500 ng) caused an increase in cellularity, disorganization and degeneration of the collagen matrix and a decreased diameter of the collagen fibrils. The tendons that were injected with the higher dose of 500 ng demonstrated more degradation than those injected with only 50 ng, so injecting higher doses of prostaglandins could be a useful method of creating tendinopathy in an animal model.

Advantages and disadvantages: Injection of prostaglandins does cause many outcomes similar to human tendinopathy, including hypercellularity, deformations of the tendon such as disorganization and degeneration, and loosely organized and thinner collagen fibrils⁴²; however, further studies must be done to fully understand the mechanisms of prostaglandins. Shorter studies have been done, but the long-term effects of prostaglandin injections have not been studied, nor have different injection schedules. Finally, the biochemical properties of prostaglandin injected tendons have not been studied and doing so would help to evaluate the effects of prostaglandins.

Fluoroquinolone injection

Rationale: After observing that quinolone antibacterial agents can cause tendinopathy in human patients⁴³⁻⁴⁶, several researchers have injected fluoroquinolones into animals to try to create an animal model of tendinopathy.

Findings: Kato⁴⁷ gave oral doses of either pefloxacin (PFLX) or ofloxacin (OFLX). A single administration of either 300 or 900 mg/kg of PFLX or 900 mg/kg OFLX caused inflammatory cell infiltration in the inner sheath of the Achilles tendon and caused disorganized collagen bundles. The fibroblasts had fragmented nuclei and were beginning to undergo cell death. After two weeks of administration, there was reduced severity in the

rats given OFLX, but not PFLX, and none of the tendons ruptured as they sometimes do in humans receiving quinolone treatment^{44,46}. Kashida and Kato⁴⁸ then compared the effects of ten different fluoroquinolones and found variability in their effects, with fleroxacin and pefloxacin being the most toxic and causing lesions in the Achilles tendon when rats were given 100 mg/kg. Many fluoroquinolones had less toxicity and only induced lesions at 300 mg/kg or 900 mg/kg, while norfloxacin, ciprofloxacin, and tosufloxacin had no effect, even at 900 mg/kg. Pefloxacin and fleroxacin have also been a target of other studies for inducing Achilles tendinopathy. These studies have found that pefloxacin causes inhibition of proteoglycan synthesis and induces oxidative damage to collagen⁴⁹. When rats are given single oral doses of fleroxacin as low as 30 mg/kg, they experience pathological changes which became more severe in rats given higher doses of up to 600 mg/kg. The tenocytes undergo degenerative changes and become detached from the extracellular matrix. The collagen fibril diameter decreases while the distance between fibrils increases; therefore, even low doses of fleroxacin are capable of causing changes in the Achilles tendon⁵⁰.

Advantages and disadvantages: Using fluoroquinolones, specifically pefloxacin and fleroxacin, in rats to induce features of tendinopathy is beneficial because both humans and rats exhibit common effects including lesions, and edema⁴⁸. However, there are some differences that could be quite important in developing a fluoroquinolone model to mimic human injury. First, the injection of fluoroquinolones in rats does not lead to tendon rupture as it does in humans⁴⁴ and a lower dosage in humans seems to cause more severe effects than in rats, so many factors that exist only in humans may contribute to the onset of tendinopathy, and thus the rat model is less effective.

Mechanical *in vivo* models

Because tendinopathy typically occurs in athletes and active individuals⁵¹, it is widely believed to occur from overuse. In order to understand the effect of overuse on the tendon, mechanical models have been developed. While mechanical methods cause chronic tendinopathy rather than acute tendinopathy, they do require more time and effort for producing tendon changes. Also, these changes can heal once the mechanical overuse has ended⁵², which is inconsistent with human tendinopathy.

Electrical muscle stimulation

Rationale: Stimulating the muscle to produce contractions that result in flexion and extension movements, and thus loading of the tendon⁵³.

Findings: This method has produced a number of results that resemble human tendinopathy. Backman⁵³ stimulated the triceps surae muscle of rabbits, which resulted in degeneration of the tendon, an increased number of capillaries and the presence of inflammatory cells. This technique was later repeated, and after three weeks, the tenocytes became abnormal and the

collagen was irregular with disrupted bundles. These changes became even more pronounced after six weeks; however, the non-exercised leg also had tendinopathic changes⁵⁴. Nakama⁵⁵⁻⁵⁷ stimulated the flexor digitorum profundus (FDP) muscle of rabbits in order to study the changes of the FDP tendon. After stimulation at 10 reps/min, the loaded tendons had a significantly greater percentage of tear area, tear density, and mean tear size, especially in the outer regions of the tendon⁵⁵. By increasing the repetition rate to 60 reps/min, the tear area percent, density and size were all significantly greater than at a rate of 10 reps/min. The lower rate caused large tears, but the higher rate caused a greater variation of tear sizes⁵⁷. The outer regions of the tendon also contained higher densities of VEGF and VEGFR-1; however, the tendons did not have an increase in capillaries or inflammatory cells⁵⁶. Asundi⁵⁸ repeated this study in order to examine the mRNA levels of many tendinopathy associated proteins, and found no differences in the levels of mRNA between the loaded and contralateral limb.

Advantages and disadvantages: As with all *in vivo* models, the availability of cellular signaling, extracellular interactions, and a vascular system are all beneficial since they can result in different molecular pathways and repair mechanisms being induced. Because of this, electrical stimulation results in molecular factors that can be observed and also cause degenerative changes that are found in human patients⁵⁶. Also, muscle stimulation is advantageous because the load can be controlled on anesthetized animals. However, like all models, electrical stimulation does have some weaknesses. For example, some studies⁵⁴ have found changes in the contralateral tendons along with the stimulated tendon, which eliminates the internal control. Also, the stimulation causes different effects in different areas of the tendon, as Nakama⁵⁵ found differences in the distribution of tears in the tendon and Asundi⁵⁸ studied cellular changes of the whole tendon and found that although some areas had changes, they were diluted by the regions that experienced no change. Therefore, the protein and enzyme expression of the entire tendon must be studied in order to determine if this is an accurate model. In addition, overuse models may not be relevant to all cases of human tendinopathy as inactive individuals may also develop tendinopathy^{59,60}.

Downhill treadmill running

Rationale: Like the *in vitro* and *ex vivo* models, treadmill running also causes repetitive overuse of the tendons that is reproducible and thus allows a wide range of outcomes to be investigated.

Findings: Running rats downhill has been shown to induce several different changes in the supraspinatus tendon. After running rats on a treadmill at 17 m/min on a 10° decline for one hour a day, 5 days a week for a varying number of weeks, Soslowsky⁶¹ was able to induce changes in the supraspinatus tendon. These changes included increased cellularity and deformation of cell shape, which remained consistent from 4 to 16 weeks. Also, the collagen fibers became misaligned and the tendon had a greater cross-sectional area after 16 weeks of running. The maximum stress was significantly decreased, as was

the tissue modulus. Perry⁶² repeated this method in order to study inflammatory and angiogenic markers in the supraspinatus tendon and found that FLAP and COX-2 had maximum gene expression at eight weeks, but decreased toward normal levels at 16 weeks; however, COX-2 levels were somewhat low at all time points. VEGF also increased and remained increased even after 16 weeks. Other groups have used the same downhill protocol and found similar results in the supraspinatus tendon, specifically tenocyte rounding and proliferation, collagen disarray, hypervascularity, glycosaminoglycan accumulation, and collagen fragmentation⁶³. Many researchers have also looked at the expression of genes after downhill treadmill running and have found the expression of cytokines IL-18, IL-15, and IL-6⁶⁴, increased expression of mediators of apoptosis, increased levels of heat shock proteins⁶⁵, as well as increased expression of genes expressed in cartilage and a decrease in genes expressed in tendons⁶⁶. Jelinsky⁵² also found that the collagen content was slightly decreased after the rats ran and then rested; therefore, 2 weeks of rest seems to be enough time to restore normal gene expression after the animals spend 2 or 4 weeks running on the treadmill. In addition to the supraspinatus tendon, downhill treadmill running has also been used in an effort to induce tendinopathy in the Achilles tendon of rats; however, this resulted in no significant changes in the tendon⁶⁷.

Advantages and disadvantages: In general, treadmill running is a useful model since it causes repeated injury of the tendon which prevents normal repair from occurring, and thus leads to degeneration⁶⁸. The method does cause weight shifting and load redistribution as the animal runs¹⁸, and the pathologic changes to the tendon may only exist while the rat is exposed to running loads⁶⁸. Treadmill running does have the disadvantage of requiring a lot of time and effort because it can be difficult to force the animals to run fast enough and for long periods of time. Also, downhill running seems to target only certain tendons, such as the supraspinatus tendon of rats. When studying the supraspinatus tendon, downhill running leads to significant changes in the histology, geometry, and mechanical properties of the tendon⁶¹ and causes more load on the tendon than cyclical loading³⁸. While running downhill does induce changes in the supraspinatus tendon, it has no effect on the Achilles tendon⁶⁷.

Uphill treadmill running

Rationale: Because running rats on a downhill incline did not induce tendinopathy in all tendons in rats⁶⁷, Glazebrook⁶⁸ studied the effects of running rats uphill on the Achilles tendon.

Findings: This method resulted in disorganized collagen fibers with more numerous and rounded nuclei found in rows between the collagen fibers. Also, the tendons from running rats had neovascularization and a greater number of fibroblasts.

Advantages and disadvantages: This method seems more advantageous than downhill treadmill running when studying the Achilles tendon because uphill running appears to require more eccentric muscle contraction, which results in the exertion of greater forces⁶⁹, and thus an increase in overall damage to the tendon⁷⁰⁻⁷².

Fatigue loading

Rationale: Tendinopathy is typically caused by repetitive loading, and the genes associated with tendinopathy may be altered in response to changes in load; therefore, studying the effects of specific load may provide insight into the mechanisms of tendinopathy.

Findings: Fatigue loading is performed by cyclically loading the patellar tendon of rats between 1 and 35 N at a set frequency of 1 Hz until a specific amount of tendon elongation is reached. Using this method, Sun⁷³ found that stretching the tendons to a 1.7% increase in elongation causes microstructural damage and an upregulation of MMP-13 and IL-1 β while a 0.6% increase in elongation resulted in only minor changes in microstructure and a downregulation of MMP-13 and IL-1 β . Fung⁷⁴ also used this method to study the damage accumulation and found that at low fatigue (0.6%), the tendons did show some kinked collagen fibers. At moderate fatigue (1.7%), the amount of matrix disruptions was increased and the spaces between fibers were widened. At high fatigues (3.5%), the spaces between fibers were even wider, along with severe matrix disruption. High fatigues also caused an increase of Col I, Col III, and Col V expression.

Advantages and disadvantages: This model has the advantage of being able to induce controlled and reproducible amounts of damage within the patellar tendon of rodents in a short time frame. However, it does have limitations in that the tendon damage is introduced acutely with a single loading bout which contrasts chronic damage accumulation in human subjects, and the loading protocol requires surgical exposure of the tendon and its surrounding structures^{73,74}.

Disuse

Rationale: It is important to understand the effects of disuse since inactive individuals can experience tendinopathy^{59,60}.

Findings: Nakagawa⁷⁵ tail suspended rats for five weeks as a disuse model, which resulted in a decrease in collagen fiber surface area and an increase in proportion of thin to thick collagen fibers. However, the tail-suspension resulted in an overall lack of growth of the rats during their growing period, which may have been the cause of the lack of growth of the collagen fibers in the tendon.

Advantages and disadvantages: Understanding the effects of disuse on tendons is important since not all human injuries are a result of mechanical injury. The study by Nakagawa⁷⁵ did cause changes in the tendon, but these could have been due to confounding variables, so further studies should be conducted.

Conclusions

Because tendon injuries affect a wide range of individuals, it is important for researchers to begin gaining an understanding of the mechanisms so proper treatments can be developed. In order to understand the mechanisms, establishing effective models of tendinopathy has become very important. As more research has been done, it has become apparent that one perfect

model of tendinopathy does not exist. Instead, the understanding of tendinopathy must come from a wide range of models. *In vitro* models are important in understanding the molecular mechanisms occurring within the individual tendon cells, but are unable to account for the effect the rest of the body's cells have on the injured tendon. *Ex vivo* models are able to include other characteristics of tendinopathy that can result from the arrangement of cells in the entire tendon along with the connective tissue that also exists in tendon; however, like *in vitro* testing, the signaling and responses from other parts of the body cannot be observed. *In vivo* models provide researchers with the ability to examine how various repair mechanisms and signals from other areas of the body interact with the injured tendon, but because the interaction of all factors are very complex, it is more difficult to determine some of the basic molecular pathways occurring, which could be more easily studied in the *in vitro* models. When these three main categories of models are further broken down, one can see that a wide array of studies is being conducted, each having their own benefits and drawbacks. Therefore, finding appropriate treatments for tendinopathy will likely be a culmination of the results from these models, which can then be applied to patients based on their unique characteristics.

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