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Tendinopathy and tendon material response to load: What can we learn from small Animal studies

Patrick M. Williamson, MS^{1,2}, Benjamin R. Freedman, PhD^{3,4}, Nicholas Kwok², Indeevar Beeram², Jan Pennings², Jeremy Johnson², Daron Hamparian², Emily Cohen², Jenna L. Galloway, PhD⁵, Arun J. Ramappa, MD^{6,*}, Joseph P. DeAngelis, MD^{6,*}, Ara Nazarian, Ph.D.^{2,6,7,*}

¹ Boston University, Mechanical Engineering Department, Boston, MA, USA

² Musculoskeletal Translational Innovation Initiative, Carl J. Shapiro Department of Orthopaedic Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

³ Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA, USA

⁴ John A Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, USA

⁵ Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA, USA

⁶ Carl J. Shapiro Department of Orthopaedic Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

⁷ Department of Orthopaedic Surgery, Yerevan State Medical University, Yerevan, Armenia

Abstract

Tendinopathy is a debilitating disease that causes as much as 30% of all musculoskeletal consultations. Existing treatments for tendinopathy have variable efficacy, possibly due to incomplete characterization of the underlying pathophysiology. Mechanical load can have both beneficial and detrimental effects on tendon, as the overall tendon response depends on the degree, frequency, timing, and magnitude of the load. The clinical continuum model of tendinopathy offers insight into the late stages of tendinopathy, but it does not capture the subclinical tendinopathic changes that begin before pain or loss of function. Small animal models that use high tendon loading to mimic human tendinopathy may be able to fill this knowledge gap. The goal of this review is to summarize the insights from *in-vivo* animal studies of mechanically-

Corresponding Author: Ara Nazarian, Ph.D., Musculoskeletal Translational Innovation Initiative, Beth Israel Deaconess Medical Center and Harvard Medical School, 330 Brookline Avenue, RN115, Boston, M, 02115. anazaria@bidmc.harvard.edu, p: 617.667.8512.

*These authors have contributed equally as senior authors.

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induced tendinopathy and higher loading regimens into the mechanical, microstructural, and biological features that help characterize the continuum between normal tendon and tendinopathy.

Graphical Abstract

Keywords

Tendon; Tendinopathy; Small Animal; Mechanics; Biology

1. Introduction

The prevalence of tendinopathy has been reported up to 3.8%[1] in the general population with 30% of visits for musculoskeletal pain in general practice resulting from tendinopathy[2, 3]. Clinically, tendinopathy is characterized by tendon pain during activity, localized tenderness upon palpation, swelling, and impaired performance[4]. Treatment strategies for tendinopathy vary[2, 5], and efficacy may depend on the tendon injured[6]. Exercise rehabilitation is broadly accepted as a primary strategy, but the most effective treatment protocol depends on the patient factors such as pain, kinesiophobia (the fear of motion), range of motion, and muscle capacity[7]. Prevention, diagnosis, and treatment of tendinopathy depend on improving our understanding of the pathophysiology and etiology of tendon injury.

Models of early-stage tendinopathy are limited because human data are restricted to late-stage tendinopathy, as clinical presentation occurs after the initiation of pain or restricted function. Although novel measurement techniques permit analysis of earlier timepoints[8–10], the lack of clinical presentation during early-stage tendinopathy limits investigation into the underlying etiology. Tendinopathy is thought to be caused by repeated overload of the tendon with insufficient recovery time, leading to an inadequate healing response[7] and incomplete recovery of preinjury material strength and function[2, 11]. A sudden exposure to elevated activity may expose the tendon to high risk for damage[12].

The leading clinical model of tendinopathy formulated by Cook *et al* suggests that the local mechanical environment around tendon cells provokes a cascade of biochemical responses that alter the extracellular matrix (ECM)[4, 13–17], and incorporates pain and the concept of “reactive-on-degenerative” tendon[18]. The Cook model describes overuse tendinopathy as a continuum with three stages: reactive tendinopathy, tendon disrepair, and degenerative

tendinopathy[13]. Reactive tendinopathy is the initial response to overload characterized by tendon thickening and a non-inflammatory proliferative response, and the second stage, tendon disrepair, includes microstructural disruption[13]. Ultrasound imaging in humans demonstrates that reactive and disrepair tendons may revert to normal tendons as defined by the absence of hypoechoic regions and/or the reduction of fusiform swelling following loading reduction[10, 19–21]. The final stage is degenerative tendinopathy, characterized by permanent, maximum accumulation of fatigue damage, in which localized areas of cell death and structural degeneration appear[13]. Although purely overloading tendon is considered an unlikely cause of tendinopathy[22], it is clear that tendon loading is a contributing factor that affects the healing response[12].

An increase in tendon-focused research has improved our understanding of tendinopathy. This increase has led to definitions of tendinopathy that implicate a wide variety of factors including accumulated damage[2, 22, 23], altered ECM[15, 24], decreased time to heal[2, 25–27], and increased cell number and cell rounding[13, 28, 29]. More recently, research has identified other possible factors, such as metabolic disease[12, 30], age[22, 31], tendon compression[32, 33], tendon location or function[34], and genetics[22] as possible additions to this list. One perspective regarding the reparability of tendon separates tendon into two discrete parts, intrinsic and extrinsic compartments, that perform different, but coordinated functions during tendon healing[12]. These factors likely all contribute to the development and progression of tendinopathy, but how these factors culminate in altered tendon function and tendon's inability to recover remains unclear.

Studies performed in animals are commonly designed to mimic central mechanical and structural features of tendinopathy identified from human tendon samples[35–37]. Animal studies aim to recapitulate similar features of tendinopathy in humans, but also enable more mechanistic insight into disease mechanism and careful control of experimental parameters. Onset of tendinopathy typically involves progression of microdamage with repeated loading, which has motivated study of tendons experiencing higher loading (e.g., Achilles tendon (AT) [38], patellar tendon (PT)[39], and rotator cuff [40]). Although tendon is an active tissue that normally responds to various loads[17, 41], the thresholds that define the difference between normal tendon response[2] and the initiation of a healing or reparative response are not well-defined[16]. Some animal studies use an injection, such as TGF- β 1 to mimic the biological response of tendinopathy[42, 43], but these studies are not covered in this review. *In-vivo* small animal models of mechanically-induced tendinopathy offer an attractive framework to examine the cascade of processes that occur throughout tendon pathology and repair[44], and may be invaluable for understanding the interplay between mechanics, biology, and structure.

The goal of this review is to summarize the insights gained from *in-vivo* animal studies of mechanically-induced tendinopathy. In addition, we examine the impacts that higher loading regimens have on the mechanical, microstructural, and biological features in tendons. High loading was defined as any loading regimen aimed to represent an increase from normal activity. A better understanding of the interplay between these realms could lead to improved patient management, especially in the presence of painful tendon.

2. Mechanical Changes with High Loading

2.1 Tendinopathy Induction – Is Overloading Enough?

Loading can have restorative or detrimental effects on tendon based on multiple factors including magnitude, frequency, duration, and the specific tendon of interest. Physical therapy regimens with eccentric exercises (which lengthen the muscle as resistance increases) have been shown to improve patient symptoms and are central to the treatment of tendinopathy in humans [45–47]. Although eccentric training alone may not improve symptoms for all patients, it is thought that muscle alteration that reduces tendon load due to eccentric training may improve tendon health[48]. Similarly, animal models have shown possible therapeutic effects of eccentric exercise models of tendon loading[49, 50]. However, given that tendon overuse (defined as higher load level and frequency) is accepted as a causative factor for tendinopathy,[12] it is counterintuitive that additional loading through eccentric exercise would improve tendon health.

Although altered response due to higher loading has been explored in multiple studies, a precise threshold between normal use and overuse has proven elusive[15]. The inability to define a threshold may be due to the natural variation of tendon structure for a given tendon within a single species. These differences could explain the varying degree of damage induced by a consistent loading protocol[51, 52]. In comparison to human studies, animal models of tendon loading offer the ability to more precisely control loading and document the effect of the loading regimen on tendon health.

2.2 Effect of High Loading on Cross-Sectional Area

The best characterized macroscopic property of tendinopathy is variation in cross-sectional area (CSA). Normally, healthy tendon adapts to loading without change in CSA[53], but with tendinopathy, tendon CSA increases in order to decrease tendon stress[54]. Reactive tendons may exhibit increased CSA, but further thickening of degenerative tendons is variable, suggesting that the tendon's response during the late stage of tendinopathy affects CSA differently compared to the earlier stages[13].

Mechanical overuse models in small animals with multiple timepoints have demonstrated sustained significant increases in CSA for two to sixteen weeks of declined treadmill and eccentric loading (Supraspinatus tendon (SS)[55–57], AT [58, 59]). These results suggest that tendons become reactive with only two weeks of overuse loading. However, some studies examining the long term effects of overuse loading using single timepoints at 12 weeks or later have shown no significant change in CSA (AT: inclined treadmill [60] running wheel/ vibration [61], SS: horizontal treadmill [62], calcaneal tendon (CT), superficial flexor tendon (SFT), and deep flexor tendon (DFT): climbing[63]). Unlike reactive tendons, which display homogenous thickening, human tendons in disrepair and degeneration stages exhibit inhomogeneous thickening,[13] which suggest that single timepoint studies may have captured tendon degeneration.

It remains unclear if loading direction, frequency, and magnitude variation affect changes in CSA at different stages of tendinopathy. Studies that applied normal tendon loading (not tendinopathy) have seen different CSA responses for different types of loading. One study

found that 5 weeks of eccentric or concentric loading had no effect on rat AT or PT CSA, but increased CSA of tricipital tendons[50]. In separate studies using vibrational loading, different levels of vibration strength training did not affect AT tendon CSA [61]. Eccentric training (AT[50, 58]) and downhill treadmill running (SS[55–57]) provoke an increase in tendon CSA in rats. Although tendon CSA is not altered during uphill treadmill running or climbing (CT, SFT, DFT[63] and AT[60, 61]), the results are mixed for horizontal treadmill running. (AT[49, 64] and SS[62]). The relationship between type and degree of loading as it relates to CSA change may signal the start of tendinopathy, but overall this single measurement alone is insufficient to confirm the presence of tendinopathy or its stage.

2.3 Effect of High Loading on Sub-Failure Mechanical Response

Healthy tendon is known to stiffen in response to normal levels of loading[13], to allow better load transmission without additional change in strain[65]. Tendinopathy often occurs in the absence of trauma, implying a gradual accumulation of micro-injuries[12, 66]. In addition to increased CSA, tendinopathic tendon is expected to stiffen[13], implicating subrupture fatigue damage in the initiation and progression of tendinopathy[2, 23, 67]. Studies applying direct loading to PTs show that higher loading intended to provoke tendinopathy increases tendon modulus[51, 52, 68], but downhill treadmill running decrease tendon modulus (AT[69] and SS[56, 57]). Time may also be a factor, as SS elastic modulus decreases at 4 weeks of downhill running in one study[57], but not until 8 weeks in another[56].

Some studies have investigated the change of tendon stiffness following cyclic loading. The loading and unloading stiffnesses of rat PT exhibit cycle-dependent changes[51]. Initially, unloading stiffness decreases, but decreases with further loading[51]. The initial stiffness reduction is hypothesized to result from an early stage of damage where fibers under greater tension are loaded, whereas the subsequent increase above initial stiffness may result from impaired tendon recoil, in which less tendon deformation is recovered during a loading cycle[51]. Comparable results were found employing a similar protocol, in which loading stiffness increased by more than 24% during the last 100 cycles of a fatigue regimen compared to the initial measurement[70].

Hysteresis is a viscoelastic mechanical property that quantifies the damping capacity of the tissue[51, 71], and is attributed to changes in non-collagenous components in the interfibrillar space[72] and loss of fibril crimp[51]. Studies of fatigue loading of rat PT have examined hysteresis and quantified the change in damping capacity of tendon during the accumulation of subrupture fatigue damage. With high levels of fatigue damage, there is a loss of hysteresis in rat PT[51, 67, 68] and murine PT[70]. Further, the initial hysteresis loss (the change in hysteresis measured at the end of the fatigue loading regimen) is correlated with the stiffness measured seven days later[51], suggesting that the tendon responds to loss of damping capacity during loading by increasing stiffness.

Stiffness and cross-sectional area change do not fully characterize the multifactorial mechanical response of tendinopathic tendon. Further studies are needed to characterize the changes to the complex viscoelastic properties of tendinopathic tendon that include the response to different loading rates and non-zero mean stress for cyclic tests [73].

2.4 Effect of High Loading on Tendon Failure

The ultimate tensile strength (UTS) of tendon reflects the maximum load that a tendon can bear prior to failure. Clinically, loading is thought to increase tendon strength to accommodate higher load. This notion is largely supported by small animal studies[49, 50, 62–64, 74]. Downhill treadmill exercise uniformly decreases UTS of the rat SS[56] and AT[75] after overuse loading, but some forms of exercise do not alter the UTS in one or more groups[50, 61, 63, 64, 74, 76]. Direct fatigue loading to rat PT did not alter failure load[70]. These results suggest that UTS alone may not be an indicator of tendon damage accumulation, and that detrimental tendon overload is unique for different tendons. With the exception of a recent study that used a specimen-specific bone fixation method[77], load-to-failure testing has been criticized for errors imposed by clamping methods.

An unloaded tendon is exposed to a mechanical environment that is similar to ligaments - structures that require static strength from increased collagen matrix deposition[71]. Although not explicitly covered in this review, tendon's response to unloading may coincide with the reactive-on-degenerative component to Cook's model[18], where portions of tendon are stress-shielded[78]. In both cases, the portion of tendon under chronically lower loading develops decreased tendon damping capacity, thereby increasing risk of further damage[71].

Although Legerlotz *et al*[61] reported the animals' body mass and food intake as a success-measure during training, most small animal studies applying higher load to tendon do not report functional deficits and pain. Given the relevance of pain and function to human tendinopathy, inclusion of these measurements in future small animal studies would improve our understanding of the etiology of tendinopathy.

In small animal studies applying high tendon load, the tendon CSA, modulus, and failure load increase, Table 1. yet, it is unclear whether these changes correspond with the natural response to higher load or the initiation of tendinopathy. The high loading threshold that triggers positive tissue adaptation distinct from tendinopathy remains controversial. Further, macroscopic mechanical properties are insufficient to confirm tendinopathy induction or define the load type and magnitude that initiates a tendinopathic response. In animal studies, pairing multiscale mechanical properties with pain, functional outcomes, structural changes, and biological response may provide a more comprehensive, quantitative measure of the state of tendon structure and the adaptive or pathological responses to higher loading.

3. Microstructural Changes with High Loading

Tendons can adopt a wide range of structural configurations[12], and recent research suggests that tendon structure may be related to the function of the muscle-tendon unit (i.e. force transmission versus energy storage)[79]. It is widely accepted that tendon's structure adapts to mechanical load, which manifests as changes to the collagenous and non-collagenous matrix. Small animal studies applying high mechanical loading have shown temporal structural changes with different loading regimens.

3.1 Effect of High Loading on Collagen

A key component to tendon's mechanical response to load is alteration of the collagenous matrix[80]. Collagen structure has been measured in excised human tissue primarily using histological methods, whereas animal studies offer the ability to measure structure nondestructively using electron microscopy and second harmonic imaging alongside collagen-related gene expression[81].

Changes in collagen microstructure have been observed with different types and levels of overuse training. An increase in collagen fiber content was demonstrated at 30 days for resistance training, and at 45 days for both resistance and endurance training[82]. Interestingly, resistance training (which involves higher intensity) shows an earlier increase suggesting the potential for faster progression of tendinopathy[82]. Increased collagen disorganization has been shown with the application of both increased repetition and increased force[83]. These changes suggest that microstructural damage accumulates faster not only due to higher loading duration, but also higher tendon stress.

Advantageous changes to collagen structure may occur as early as during the first week of loading. Within the first week of treadmill loading, flexor digitorum longus collagen fibril diameter, number, and overall fibril CSA increased relative to control[84, 85]. Similarly, for CT, the thickness of the peritendinous sheath increased, perhaps increasing load bearing capacity[86]. Increased Col I expression with high loading has been shown for fatigue loaded PT,[87] and SS[88]and AT[89] after treadmill running.

Multiple detrimental alterations to collagen microstructure occur as early as week 3 or 4 post loading; studies have observed increased fibril disorganization[57, 58, 90], microtears within collagen fibers[25, 27, 91, 92], decreased fibril diameter, number, and relative fibril CSA[84], and increased proportion of type III collagen fibers (reticular)[91]. Furthermore, from a microstructural perspective, collagen damage was invoked using electrical stimulation of the flexor digitorum profundus [27]. Changes to collagen in response to loading has also been studied in tendon fascicles *ex-vivo*, though this isn't covered in our review[93–98].

3.2 Effect of High Loading on Glycosaminoglycans (GAGS)

The function of the non-collagenous tendon matrix has been of great interest recently. Human PT biopsies from patients with PT tendinopathy have indicated that increased GAG and proteoglycan content may be associated with tendon dysfunction[99–101]. Small animal studies offer a mechanism to explore the effects of high loading on GAG and proteoglycan content.

In rat models, glycosaminoglycan content increases with resistance training[102] and treadmill running[89, 91, 103, 104], which has been suggested to improve interfibrillar sliding[89, 105]. Furthermore, the relative proportions of GAGs may change with downhill running, as one study observed an increase in dermatan sulfate and chondroitin sulfate, a decrease in heparan sulfate, and increased proportion of chondroitin sulfate relative to dermatan sulfate.[106] Notably, Marqueti, *et al* demonstrated increased GAG content and increased proteoglycan content (proximal CT) for older animals at 12 weeks of resistance

training[102], which suggests that resistance loading can help protect aging tendons from damage. Also, pathological GAG deposition and calcific tendinopathy may also be possible [107, 108].

Overall, high mechanical loading in small animal tendons leads to microstructural changes that differ depending on the magnitude and duration of loading, but overall includes increased Col I production, increased Col III/ Col I proportion, increased matrix disorganization, and altered proportions of matrix proteins.

4. Biological Changes with High Loading

4.1 Overview

Advances in imaging, sequencing, and transgenic mouse models have enabled mechanistic evaluation for studying the effect of mechanical loading on tendon biology. Recent studies have revealed interplay between complex changes in cell behavior, protein expression, gene expression, and vascular changes with the onset of high mechanical loading. Within the model systems commonly evaluated, *in-vivo* models can provide the most clinically relevant information about diseased biological mechanisms, due to the capacity to mechanically stimulate tissues within a living organism. The most common *in-vivo* loading models to study biological changes following high mechanical loading include treadmill activity[35, 109], forelimb reaching and pulling[83, 110], and electrical stimulation[111]. Since specific loading regimens in rodent models do not precisely control for the stresses and strains exhibited by animals during activities such as treadmill overuse, mechanical loading of tendon *in-situ* or *ex-vivo* can be advantageous due to the ability to precisely control applied stresses and strains while preserving the native architecture of the ECM[112]. For example, direct mechanical actuation is applied to tendons while animals are under anesthesia[68] or freshly harvested and maintained in a bioreactor[113].

4.2 Effect of High Loading on Cell Behavior

For decades, histology from tendinopathic human tendons has been well characterized, which has enabled benchmarks for load induced tendinopathy models in rodents. After high mechanical loading in rodent overuse models, cell number has been shown to increase in multiple tendons: the SS[55, 56, 103, 114], AT[60, 86, 115, 116], and FDL[110, 117]. Additionally, with high loading a rounder cell morphology is reported in the SS[56, 57, 103], FDL[110], and AT[115, 116]. Tendon cells may also show greater surface area[118], loose chromatin (which allows for increased replication)[82], and increased cytoplasm volume[82]. Multiscale strain transfer is also attenuated following high cyclic loading[113].

Broadly speaking, characterizing cell types within tendon has been an active area of research in the tendon community[119]. Morphologically, with high loading, both fibroblast and chondrocyte appearance become more abundant with time[103]. Loaded tendons may also have an increased quantity of myofibroblasts, as characterized by positive staining for α -smooth muscle actin (α -SMA), production of α -SMA stress fibers, and generation of large traction forces.[118] Although knowledge for specific cell markers in mechanical loading-induced tendinopathy model systems is lacking, available data suggest cells in

the SS tendon-derived cells following high loading are positive for CD90.1, CD3.1, and CD94[103, 120]. Given the evidence that staining for macrophages, mast cells, T- cells, and myofibroblasts in subscapularis (SB) tendons in patients with torn SS[121] has provided, groups have been motivated to study immune cell populations[103].

The presence of apoptosis in tendinopathy remains controversial. Interest in studying the interplay of apoptosis and tendinopathy originates from human studies identifying elevated apoptosis in intact SB tendons in patients with torn SS[122]. In rodent models of high mechanical loading, apoptosis has been induced in both explant models of the PT[68] and rodent overuse of the AT[91], but not in the SS[103]. Tendinopathic SS from rats had elevated heat shock proteins and caspases with imbalance in antiapoptotic (heat shock protein) and proapoptotic (caspase) genes[123]. However, rats following 16 weeks of loading did not have evidence of apoptosis, but had increased IGF1[123], which can promote tenocyte proliferation[124, 125], collagen synthesis in tendon[126] and musculotendinous tissues[127], and tissue biomechanics following injury[128, 129].

Taken together, high loading models in rodents can create hypercellularity, cell roundness, apoptosis, elevated abundance of myofibroblasts, and a chondrogenic phenotype in most tendon types. Therapies to modulate cell rounding, multiscale strain transfer, aberrant differentiation, immune cell populations, and apoptosis in response to high loading are ripe areas for future study.

4.3 Effect of High Loading on Protein and Gene Expression

Advances in mechanobiology have uncovered relationships between forces applied to the ECM, multiscale strain transfer to the nucleus, and cell processes (e.g., transcription, inflammation, migration, proliferation, and differentiation)[43, 97, 130–136]. Here, we highlight specific changes within tendon following high mechanical loading in rodent studies. As high mechanical loads are applied, changes to tendon structure and mechanical properties may occur in concert with the generation of interstitial pressures surrounding the tendon and alterations in blood flow. These mechanosensory factors may activate mechano-transductive pathways[137] and promote non-tenogenic behavior in tendon cells[138, 139], secretion of non-tendinous proteins, release of growth factors and cytokines resulting in non-tendon like phenotypes, increase in the type III/I collagen ratio, and an imbalanced secretion of MMPs and tissue inhibitors of MMPs (TIMPs)[106, 140, 141]. Indeed, increased loading on the rat SS elevated expression of MMPs and TIMPs[142].

High treadmill activity affects tendon stem/progenitor cell differentiation in a tendon-dependent manner, as stem cell markers (Oct4, SEA1, Oct54, and NS) are upregulated in the PT, but not the AT[143]. Aberrant differentiation following loading may provide a mechanism to end-stage tendinopathy which progresses to calcific tendinopathy, with upregulation of genes associated with bone development and resorption[116, 144]. Other non-tendon like markers such as those associated with cartilage development have been identified through the presence of GAG staining in the AT[91, 115, 116, 145] and SS[103]. Overused shoulders in rodents express cartilage markers in the short term [140], that return to baseline after rest[146]. Proteoglycans and heparin affine regulatory peptide (chondrogenesis factor) are elevated with overuse[106].

Several cytokines have been implicated in load-induced tendinopathy (e.g., IL-6, IL-15, IL-11, IL-18). Rodent studies have demonstrated IL-6 increases in concert with continued loading[14, 117, 147] and aging[117] at both the protein and gene levels[120]. Inflammation related cytokines such as IL-11, IL-15, IL-18, and TNF α were also upregulated in overused tendons[120]. Interestingly, IL-33 expression may promote type-3 collagen synthesis and contribute to scar formation; however, this has only been shown in a tendon injury model[36]. In rat forelimb grasping models, repeated grasping has led to elevated TNF α , IL1 α , and IL1 β in the flexor digitorum muscle, tendon, and radius[83, 110]. High force tasks also increase spinal cord sensitization through substance P[83].

Several growth factors have been implicated in tendinopathy (e.g., TGF- β 87, CTGF99, IGF-1[14], periostin-like factor[110], osteoactivin[148], PDGF[147], VEGF[88, 102, 141], FGF[149]). IGF-1 is known to stimulate collagen protein synthesis with loading in humans[14] and rodents[103], and is commonly identified to increase in tendinopathy[49, 88, 102], potentially to combat effects of apoptosis and heat shock signaling[123]. TGF- β and CTGF are known to stimulate collagen synthesis in human tendons[14], and *in-vitro* via stimulation of tendon fibroblasts[150]. Animal studies have demonstrated load-[102, 149] and repetition-[83] induced increased tendon tissue concentration of the isoform TGF- β 1, and in gene expression, but this remains controversial[49, 97, 140]. Interestingly, this effect may be age dependent, as increased levels are observed in young rats, but decreased levels in old rats with loading[102]. Rodent studies of high loading also suggest elevated CTGF protein[110, 147] and gene expression[102], but this remains unclear[49, 97].

Taken together, high loading promotes non-tenogenic differentiation and protein expression (e.g., GAGs), and secretion of several proinflammatory cytokines and growth factors that occur in concert with tendon degeneration. Future studies are needed to mechanistically evaluate the role of each factor on tendon degeneration and whether their modulation can prevent or accelerate recovery from tendinopathy.

4.4 Effect of High Loading on Vascularization

Clinical studies of patients in the late stages of tendinopathy suggest neovascularization[151, 152] and intratendinous blood flow[153–157]. For these reasons, vascularization has been studied in several rodent models. Certain AT and SS overuse models have highlighted elevated vascularity[111, 141, 153]. Increased vascularity occurs in concert with elevated cellularity in the peritendinous sheath[86]. However, in another overuse rat running model, tendon neovascularization has not been observed continuously[103].

Neovascularization in tendon following overuse may be driven by VEGF[158], as it is known to increase the permeability of vessels[159]. Following treadmill overuse, VEGF increases after 3 days, but decreases after one week and remains high over 16 weeks[141]. This suggests that new blood vessel formation occurs early in the tendon disrepair stage and continues throughout[141]. These data are supported by another study using the same loading protocol, which highlights a low expression of VEGF after one week and an increase after 8 weeks[88]. In addition to VEGF, studies report increased expression of nitric oxide (NO) that may promote synthesis of collagen fibers and induce the replacement of injured cells[160].

4.5 Summary High Loading's Effect on Tendon Biology

Although human models of disease provide the most accurate clinical representation of tendinopathy, they are limited by the availability of tissue sources and often require large study numbers. Therefore, animal studies can offer advantages due to being readily available.

Taken together, rodent high loading studies have highlighted several effects of loading on tendon pathology. Certain high loading models have recapitulated similar end-stage tendinopathy phenotypes commonly seen in humans. Overall, these studies provide insight into temporal patterns in gene and protein expression, aberrant differentiation pathways, cytokine secretion, and GF expression that are associated with the disease process. Harnessing these attributes in future studies will greatly add to our understanding and better enable identification of new treatments. It is noted that studies using tendon explants from large animals (e.g., [135, 161–167]) and cells seeded on scaffolds (e.g., [133, 168–172]) have also provided insight into mechanisms of tendinopathy, but are not discussed here in this review focused on learnings from small animals.

5. Future Perspectives

The precise etiology of tendinopathy has yet to be characterized, though from small animal studies of high loading, it is clear that a number of factors contribute to the progression of detrimental tendinopathic symptoms that may carry different weights depending on the tendon location and function. Small animal studies prescribing high tendon loading regimens show that tendon CSA, modulus, hysteresis change, and failure load may increase or decrease depending on the tendon location and the duration, frequency, and magnitude of loading. Collagen III production increases in response to high loading in small animals. GAG content increases with resistance and treadmill loading in rats, which may have protective qualities for tendon. Small animal studies provide insight into temporal patterns associated with the disease process, where growth factors, inflammation, and neovascularization are current areas of study. To date, none of these small animal studies directly contradict the clinical perspective that reducing load after potential overuse and subsequent physical therapy with eccentric exercise may have positive effects on tendinopathic symptoms. It is important to note that results from rodent tendon studies cannot be directly translated to humans. In specific instances, these animals have been shown to have increased healing rates compared to humans, [173, 174] which may include a higher capacity to respond to loading and prevent tendinopathy than humans.

As the inception of tendinopathy is multifactorial and seems to depend on the tendon studied, small animal model systems aiming to mimic the human tendinopathic condition should pay specific attention to the tendon location, age, and activity level of the patient population of clinical interest. Doing so will allow for prescription of loading that matches that seen in the patient and iterative feedback from the clinical setting to improve our understanding of the etiology and progress of the disease. This may provide indicators of how a specific tendon responds to the type and degree of loading seen in the patient.

An improved understanding of healthy tendon metabolism and homeostasis would allow a clearer baseline to detect tendon pathology. Mechanotransduction at the cellular and subcellular levels has been used to evaluate tensional homeostasis [175, 176], tendon development [177], gene expression [135], and collagen turnover [178, 179]. Knowledge from this setting have motivated more pointed studies in the small animal setting to better define the factors that define the temporal and load amplitude thresholds for overloading.

Future small animal studies applying high mechanical load to induce tendinopathy should consider quantifying pain [180–182] and function – such as measurements of dynamic weight bearing [183] and motion analysis [184] used in animal models to study different tendon diseases. Further, quantitative tendon imaging, especially using high-frequency ultrasound can be used to evaluate and quantify tendinopathy and treatment in small animals [185–187]. This may help indicate the onset of tendinopathic changes, aid in the staging of tendinopathy in small animals, and improve comparisons to clinical data. Also, it may be of interest to explore temporal changes in structure and biology during the early onset and throughout the training regimen. Further, studies that use data from these small animal studies to model the temporal material response as a function of the changes seen in the structure and the biological response may expose areas of interest along the continuum of tendinopathy.

This review offers an update on the realm of mechanically-induced tendinopathy with the hope that we, as a field, can make further progress to directly affect patient management. The studies of mechanically-induced tendinopathy included in this manuscript offer insight into the potential indicators of tendinopathy that differentiate therapeutic versus detrimental mechanical load. Though clinical measurement tools haven't been established in humans, increased apoptosis may indicate the need for anti-apoptotic drugs. Increased matrix degeneration suggests that better imaging techniques are needed to assess matrix degeneration earlier. Recent studies using shear wave propagation [188] and ultrasound [189, 190] have shown promise in this area. For clinical translation, it may be necessary to correlate results from the clinical measurement techniques to the more invasive measurements that can be made in the small animal model setting.

Generally, small animal models that mimic the human condition offer a high throughput setting to establish the efficacy of biomaterial therapeutics that improve detriments associated with tendinopathy. Biomimetic biomaterials may be designed to deliver biomolecules to injured tendon to, for example, promote cell or matrix regeneration [191, 192], or mediate inflammation [35, 193]. Also, biomimetic biomaterials may be engineered to mimic the elasticity and material strength of tendon, [194, 195] or improve tendon-to-bone healing. [196] Overall, small animal studies of mechanically-induced tendinopathy have motivated more basic science research to improve our understanding of tendon's response to loading to further improve the field's diagnostic and therapeutic capability.

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Statement of Significance

This review summarizes the insights gained from in-vivo animal studies of mechanically-induced tendinopathy by evaluating the effect high loading regimens have on the mechanical, structural, and biological features of tendinopathy. A better understanding of the interplay between these realms could lead to improved patient management, especially in the presence of painful tendon.

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Mechanical Load Application

Measurements

Figure 1. Mechanical Load Applied and Measurements of Induced Change in Small Animal Models

Studies use different methods to apply load to tendon experimentally, such as direct tendon loading applied to the patellar tendon, reaching and grasping tasks aimed at loading the flexor digitorum tendon, active (via muscle stimulation) or passive ankle dorsiflexion aimed to load the Achilles, and treadmill running in different configurations mainly loading the Achilles or rotator cuff tendons. Mechanical, structural, and biological changes to the tendon are measured to quantify the effects of loading. F – Force; $t1$ – timepoint 1; $t2$ – timepoint 2;

Figure 2. High Tendon Loading Leads to Mechanical, Structural, and Biological Changes in Small Animal Models

High loading small animal studies induce multi-scale changes to tendon that are similar to tendinopathy. Increased cell rounding, apoptosis, vascularization, aberrant differentiation, inflammation, and altered growth factor production (increased TGF- β , IGF, and CTGF) are seen in some studies. Mechanically, increased hysteresis, and higher modulus may result. Structurally, increased collagen disorganization and altered non-collagenous matrix components are likely. Overall, between tendon types and loading protocols, there is no clear consensus for how high loading affects tendon. CSA – Cross-sectional Area; IL-6 –

Interleukin-6; TGF- β – Transforming Growth Factor Beta; IGF – Insulin-like Growth Factor;
CTGF - Connective Tissue Growth Factor

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Table 1.

Mechanical Changes Induced by High Loading in Small Animal Models

Study Information				Mechanical Measurements				
Animal	Tendon	Loading Mechanism	Comparison	CSA	Modulus	Hysteresis	Failure Load	Functional Outcome
Rats								
SD F Adult [68]	PT	Direct Tendon Loading	7200 Cycles vs 100 Cycles	-			-	-
SD F Adult [51]	PT	Direct Tendon Loading	7200 Cycles vs Other Groups	-			-	-
SD F Adult [87]	PT	Direct Tendon Loading	High Load Endpoint vs Baseline	-			-	-
SD M Adult [58]	AT	Eccentric Contraction	Loaded Tendon vs Unloaded Contralateral Thickness* Not CSA		-	-	-	-
SD Adult * Gender not specified [50]	AT	Eccentric and Concentric Training	Eccentric (E) vs Untrained (U)	3	-	-	3	-
"	AT, PT, or Tri	"	Concentric (C) vs Untrained (U)	3	-	-	3	-
"	PT	"	Eccentric (E) vs Untrained (U)	3	-	-		-
"	Tri	"	Eccentric (E) vs Untrained (U)		-	-		-
SD Adult * Gender Not Specified [75]	AT	Downhill Bipedal Running	Trained vs Untrained	-		-		-
SD M * Age Not Reported [55]	SS	Downhill Treadmill Running + Compression or Injury	Overuse (OV) vs Cage Activity			-		-
"	SS	"	Overuse plus Extrinsic Injury (OV+E) vs Cage Activity		3	-	3	-
SD Adult * Gender not Specified [57]	SS	Downhill Treadmill Running	Overuse (OV) vs Cage Activity			-		-
"	SS	"	Overuse Plus Extrinsic Injury (OV+E) vs Cage Activity			-		-
"	SS	"	Extrinsic Injury vs Contralateral	3	3	-	3	-
SD F Adult [61]	AT	Running Wheel	Trained vs Non-Active Controls	3	3	3	3	
"	"	Vibration Strength-Trained	Vibration Strength-Trained (LVST) vs Non-Active Controls	3	3	3	3	3
"	"	High-Vibration Strength-Trained	High-Vibration Strength-Trained (HVST) vs Non-Active Controls	3	3	3	3	3
"	"	High Strength-Trained	High Strength-Trained (HST; n = 6) vs Non-Active Controls	3	3	3	3	3
W M Old and Adult [63]	CT	Climbing	Old Trained vs Old Sedentary	3	3	-	3	-

Study Information				Mechanical Measurements				
Animal	Tendon	Loading Mechanism	Comparison	CSA	Modulus	Hysteresis	Failure Load	Functional Outcome
"	SFT	Climbing	Old Trained vs Old Sedentary	3	3	-		-
"	DFT	Climbing	Old Trained vs Old Sedentary	3	3	-	3	-
"	CT	Climbing	Young Trained vs Young Sedentary	3	3	-		-
"	SFT	Climbing	Young Trained vs Young Sedentary	3	3	-	3	-
"	DFT	Climbing	Young Trained vs Young Sedentary	3	3	-		-
SD M Adult [49]	AT	Treadmill Running (Horizontal)	Running vs Controls	3		-		-
W M Adult [64]	AT	Treadmill Running (Horizontal)	ST - Speed Exercise Running vs Cage Control		-	-		-
"	"	"	IT - Endurance Exercise Running vs Cage Control		-	-	3	-
"	"	"	LT - Endurance Exercise Running vs Cage Control	3	-	-	3	-
SD M Adult [76]	TAT	Treadmill Running (Horizontal)	Running vs Cage Control Sedentary Controls	-	3	-	3	-
"	FDLT	"	Running vs Sedentary Controls	-	3	-	3	-
SD M Adult [62]	SS	Treadmill Running (Horizontal)	Exercise vs Cage Controls	3	-	-		-
W M Adult [74]	AT	Strength Training	Strength Training vs Control	-	-	-	3	-
"	AT	Swimming	Swimming vs Control	-	-	-		-
Mice								
C57BL/6J F Adult [70]	PT	Direct Tendon Loading	4 N Peak or 1 h vs Contralateral Control	-	3	-	3	-
"	PT	"	6 N Peak for 1 h vs Contralateral Control	-		-	3	-
"	PT	"	4 N Peak for 2 h vs Contralateral Control	-		-	3	-
"	PT	"	4 N or 6N Peak or 1 h - Baseline vs Endpoint	-			-	-
"	PT	"	4 N Peak for 2 h - Baseline vs Endpoint	-			-	-
C57BL/6 M Old and Adult [197]	Plant	Treadmill Running (Horizontal)	Running vs Sedentary			3	-	-

Color Legend: Red - Increase; Blue - Decrease; Tan – No Change; Gray – Not Reported

SD – Sprague Dawley; W – Wistar; M – Male; F – Female; Coll – Collagen; Coll Org – Collagen Organization; GAG – glycosaminoglycan; Ag -Aggrecan; V – Versican; D – Decorin; Bn – Biglycan; Fn- Fibronectin; E – Elastin; Fmod – Fibromodulin; TNc – Tenascin-C; L - Leucin

Table 2.

Structural Changes induced by High Loading in Small Animal Studies

Study Information				Structural Measurements												
Animal	Tendon	Loading Mechanism	Comparison	Coll Type		Coll Org	Non-Coll Tendon Matrix									
				Coll 1	Coll 3		GAG	Ag	V	D	Bn	Fn	E	Fmod	TNc	L
Rats																
SD F * Age not reported [87]	PT	Direct Tendon Loading	Mid-level Fatigue (Day 1 post-Fatigue)													
‘ ‘	PT	‘ ‘	Mid-level Fatigue (Day 3 post-Fatigue)													
‘ ‘	PT	‘ ‘	High-level Fatigue (Day 1 post-Fatigue)													
‘ ‘	PT	‘ ‘	High-level Fatigue (Day 3 post-Fatigue)													
SD F * Age not reported [98]	PT	Direct Tendon Loading	High-level Fatigue vs Control (Sham and Naive)													
SD Adult F * Age not reported [198]	PT	Direct Tendon Loading	Tendon Elongation Increased by 0.6% or 1.7% vs Control													
SD F * Age not reported [199]	AT	Concentric, Eccentric or Isometric Training by Nerve Stimulation	Concentric (CON), Eccentric (ECC), or Isometric (ISO) Loading vs Contralateral Control													
SD M * Age not reported [140]	SS	Downhill Treadmill Running	Running vs Baseline													
SD M * Age not reported [55]	SS	Downhill Treadmill Running + Compression or Injury	Overuse + Intrinsic or Extrinsic													
SD (n = 14); Adult [75]	AT	Downhill Bipedal Running	Running vs Cage Control													
SD (n=20); M Adult [88]	SS	Downhill Treadmill Running	Acute Exercise vs Control													

Study Information				Structural Measurements												
Animal	Tendon	Loading Mechanism	Comparison	Coll Type			Non-Coll Tendon Matrix									
				Coll 1	Coll 3	Coll Org	GAG	Ag	V	D	Bn	Fn	E	Fmod	TNc	L
' '	SS	' '	Chronic Exercise vs Control													
SD M * Age not reported [103]	SS	Downhill Treadmill Running	Running vs Cage Control													
SD Adult * Gender not reported [57]	SS	Downhill Treadmill Running	Extrinsic Compression (E), Overuse (O/V), or Both vs Control													
SD M * Age not reported [56]	SS	Downhill Treadmill Running	Exercise vs Cage Control													
SD M Adult [145]	AT	Uphill Treadmill Running	Runners vs Sedentary Controls													
SD Adult * Gender not reported [60]	AT	Uphill Treadmill Running	Running vs Non-running Control													
SD M * Age not reported [49]	AT	Uphill Treadmill Running	Running vs Control													
SD M Adult [200]	PT	Uphill Treadmill Running	LR11 vs Control													
' '	PT	' '	HR11 vs Control													
W Adult and Old [102]	CT	Resistance Training	Young Trained (YT) vs Young Sedentary (YS)													
' '	CT	' '	Old Trained (OT) vs Old Sedentary (OS)													
W M Adult [115]	CT	Uphill Treadmill Running	Running vs Cage Control													
W M Adult [91]	AT	Uphill Treadmill Running	Running vs Cage Control													
SD M Adult [116]	AT	Uphill Treadmill Running	Runnnng vs Cage Control													
SD M Adult [89]	AT	Treadmill Running (Horizontal)	Runner (RUN) vs Sedentary (SED)													

Study Information				Structural Measurements													
Animal	Tendon	Loading Mechanism	Comparison	Coll Type		Coll Org	Non-Coll Tendon Matrix										
				Coll 1	Coll 3		GAG	Ag	V	D	Bn	Fn	E	Fmod	TNc	L	
SD F Adult [92]	FDT	Reaching + Grasping	Reach Limb vs Non-Reach Limb														
SD F Adult [83]	FDT	Reaching + Pulling	12-week HRLF vs Normal Control (NC)														
‘ ‘	FDT	‘ ‘	12-week LRHF vs Normal Control (NC)														
‘ ‘	FDT	‘ ‘	12-week HRHF vs LRLF														
SD F Adult [110]	FDT	Reaching + Grasping	HRHF vs Control														
SD F Adult [147]	FDT	(HRLF) High Repetition Low Force Grip Tasks	High Repetition Low Force Task (HRLF) vs Trained Only Controls (TR0 and TR24)														
SD F Old and Adult [117]	FDT	High Repetition, Low Force (HRLF) Reaching and Handle-Pulling Task	Old HRLF vs Control														
‘ ‘	FDT	‘ ‘	Young HRLF vs Control														
‘ ‘	SS	‘ ‘	Old HRLF vs Control														
‘ ‘	SS	‘ ‘	Young HRLF vs Control														
SD M Adult [111]	AT	Electrical Muscle Stimulation	Eccentric vs Untrained Controls														
Rabbit																	
NZW F Adult [201]	AT	Electrical Muscle Stimulation	Loaded vs Contralateral Control														
Mice																	
C57BL/6 J F Adult [70]	PT	Direct Tendon Loading	Loading Regimens vs Contralateral Control														
C57BL/6 M Old and Adult [197]	Plant	Uphill Treadmill Running	Old Trained vs Old Cage Control														

Study Information				Structural Measurements												
Animal	Tendon	Loading Mechanism	Comparison	Coll Type			Non-Coll Tendon Matrix									
				Coll 1	Coll 3	Coll Org	GAG	Ag	V	D	Bn	Fn	E	Fmod	TNc	L
''	''	''	Old Trained vs Young Cage Control													
C57BL/6 J F Adult [143]	PT or AT	Treadmill Running (Horizontal)	Moderate or Intense Training vs Cage Control													

Color Legend: Red - Increase; Blue - Decrease; Tan – No Change; Gray – Not Reported

SD – Sprague Dawley; W – Wistar; M – Male; F – Female; CSA – Cross-sectional Area

Table 3.

Biological Changes Induced by Small Animal Studies.

Study Information				Biological Measurements						
Animal	Tendon	Loading Mechanism	Comparison	Cell Rounding	Abberant Differentiation	Inflammation	Apoptosis	Growth Factors	Matrix Degradation Enzymes	Vascularization
Rat										
SD F Adult [68]	PT	Direct Tendon Loading	High-level Fatigue vs Naive Control (3 Days Post-Fatigue)							
, ,	, ,	, ,	High-level Fatigue vs Naive Control (7 Days Post-Fatigue)							
SD F * Age not reported [98]	PT	Direct Tendon Loading	High-level Fatigue vs Control (Sham and Naive)							
SD F Adult * Age not reported[198]	PT	Direct Tendon Loading	Tendon Elongation Increased by 0.6% vs Control							
, ,	PT	, ,	Tendon Elongation Increased by 1.7% vs Control							
SD F * Age not reported [199]	AT	Concentric, Eccentric or Isometric Training by Nerve Stimulation	Concentric (CON) or Isometric (ISO) Loading vs Contralateral Control							
, ,	AT	, ,	Eccentric (ECC) Loading vs Contralateral Control							
SD F Adult * Age not reported [202]	AT	Concentric, Eccentric or Isometric Training by Nerve Stimulation	Concentric (CON), Eccentric (ECC), or Isometric (ISO) Loading vs Contralateral Control							
SD Adult *Gender not specified [50]	Tri, PT, or AT	Eccentric and Concentric Training	Concentric Loading vs Untrained Controls							
, ,	Tri, PT, or AT	, ,	Eccentric Loading vs Untrained Controls							

Study Information				Biological Measurements						
Animal	Tendon	Loading Mechanism	Comparison	Cell Rounding	Abberant Differentiation	Inflammation	Apoptosis	Growth Factors	Matrix Degradation Enzymes	Vascularization
SD M * Age not reported [140]	SS	Downhill Treadmill Running	Running vs Baseline			3			3	(midsubstance)
SD M * Age not reported [55]	SS	Downhill Treadmill Running + Compression or Injury	Overuse + Intrinsic or Overuse + Extrinsic vs Control							
SD M Adult [120]	SS	Downhill Treadmill Running	Running vs control							
SD M Adult [149]	SS	Downhill Treadmill Running	Exercise vs Control			Of Note was the differential expression of inflammation-related genes, representing 12% (11 genes) of the significantly upregulated genes and 19% (7 genes) of significantly downregulated genes.				
SD Adult [75]	AT	Downhill Bipedal Running	Running vs Cage Control							
SD M Adult [88]	SS	Downhill Treadmill Running	Acute Exercise vs Control				3			(NR)
' '	SS	' '	Chronic Exercise vs Control			3	Casp3 downreg after 1 Week, no mention of down/upreg after 8 wks			(NR)
SD M * Age not reported [103]	SS	Downhill Treadmill Running	Running vs Cage Control			3	3			(NR)
SD Adult * Gender not reported [57]	SS	Downhill Treadmill Running	Extrinsic Compression (E), Overuse (O/V) or Both vs Control							
SD M * Age not reported [56]	SS	Downhill Treadmill Running	Exercise vs Cage Control							
SD M Adult [145]	AT	Uphill Treadmill Running	Runners vs Sedentary Controls							
W M Adult [86]	CT	Vertical Jump	Vertical Jump (VJ) vs Control			3				("peritendinous sheath")

Study Information				Biological Measurements						
Animal	Tendon	Loading Mechanism	Comparison	Cell Rounding	Abberant Differentiation	Inflammation	Apoptosis	Growth Factors	Matrix Degradation Enzymes	Vascularization
‘ ‘	CT	Uphill Treadmill Running	Treadmill Running (TR) vs Control			3				(“peritendinous sheath”)
SD Adult *Gender not reported [60]	AT	Uphill Treadmill Running	Running vs Non-Running Control			3				(midsubstance)
SD M * Age not reported [49]	AT	Uphill Treadmill Running	Running vs Control	3				TGF- β , CTGF: Down IGF: Up	3	
SD M Adult [200]	PT	Uphill Treadmill Running	LR11 vs Control							
‘ ‘	PT	‘ ‘	HR11 vs Control							
W M Adult and Old [102]	CT	Resistance Training (Climbing with Weights)	Young Trained (YT) vs Young Sedentary (YS)							(NR)
‘ ‘	CT	‘ ‘	Old Trained (OT) vs Old Sedentary (OS)							(NR)
W (n = 24); M Adult [115]	CT	Uphill Treadmill Running	Running vs Cage Control							(NR)
W M Adult [91]	AT	Uphill Treadmill Running	Running vs Cage Control		3	3				(NR)
SD M Adult [116]	AT	Uphill Treadmill Running	Runnnng vs Cage Control							
SD F Adult [92]	FDT	Reaching + Grasping Task	Reach Limb vs Non-Reach Llmb							
SD F Adult [83]	FDT	Reaching + Pulling	12-Week HRLF vs Normal Control (NC)						3	
‘ ‘	FDT	‘ ‘	12-Week LRHF vs Normal Control (NC)				3		3	
‘ ‘	FDT	‘ ‘	12-Week HRHF vs LRLF						3	
SD F Adult [110]	FDT	Reaching + Grasping	HRHF vs Control							(“peritendon”)
SD F Adult [148]	FDT	High Repetition Negligible Force (HRNF) Food Retrieval Task	HRNF vs FRC (3 Week Timepoint)							

Study Information				Biological Measurements						
Animal	Tendon	Loading Mechanism	Comparison	Cell Rounding	Abberant Differentiation	Inflammation	Apoptosis	Growth Factors	Matrix Degradation Enzymes	Vascularization
''	''	''	HRNF vs FRC (6 Week Timepoint)			123				
SD F Adult[147]	FDT	(HRLF) High Repetition Low Force Grip Tasks	HRLF vs Control							
SD F Old and Adult [117]	FDT	High Repetition, Low Force (HRLF) Reaching + Handle-Pulling Task	Old HRLF vs Control					3		("endotendon")
''	FDT	''	Young HRLF vs Control					3		("endotendon")
''	SS	''	Old HRLF vs Control							("endotendon")
''	SS	''	Young HRLF vs Control			3		3		("endotendon")
SD M Adult [89]	AT	Treadmill Running (Horizontal)	Runner (RUN) vs Sedentary (SED)			no chane			MMP13 decreases	
SD and W Both Sexes * Age not reported [203]	AT	Treadmill Running (Horizontal)	Contralateral-Impaired vs Control							
''	Tibialis Anterior	''	Running vs Untrained Control							
SD M Adult [111]	AT	Electrical Muscle Stimulation	Eccentric vs Untrained Controls							("epitendon")
W M Adult [86]	CT	Swimming	Adapted Swimming ADP vs Control	3		3				("peritendinous sheath")
Rabbit										
NZW F Adult [204]	AT	Electrical Muscle Stimulation	Trained vs Control (At Week 1)							both
''	AT	''	Trained vs Control (At Week 3 or 6)							both
NZW F Adult [201]	AT	Electrical Muscle Stimulation	Loaded vs Contralateral Control	3	3			IGF-II expression significantly Lower		
NZW Both Sexes Adult [205]	AT	Electrical Muscle Stimulation	Loaded vs Baseline							(paratenon)
Mice										

Study Information				Biological Measurements						
Animal	Tendon	Loading Mechanism	Comparison	Cell Rounding	Abberant Differentiation	Inflammation	Apoptosis	Growth Factors	Matrix Degradation Enzymes	Vascularization
C57BL/6 M Old and Adult [197]	Plant	Uphill Treadmill Running	Old Trained vs Old Cage Control							
''	''	''	Old Trained vs Young Cage Control							
C57BL/6J F Adult [118]	PT	Treadmill Running (Horizontal)	Running vs Cage Control							
C57BL/6J F Adult [143]	PT or AT	Treadmill Running	Moderate Training vs Cage Control							(paratenon)
''	PT or AT	''	Intense Training vs Cage Control							(paratenon)

Color Legend: Red - Increase; Blue - Decrease; Tan – No Change; Gray – Not Reported

SD – Sprague Dawley; W – Wistar; M – Male; F – Female;