HHS Public Access

Author manuscript

J Orthop Res. Author manuscript; available in PMC 2019 February 01.

Published in final edited form as:

J Orthop Res. 2018 February ; 36(2): 557–565. doi:10.1002/jor.23761.

Mechanobiology of Young and Aging Tendons: *In Vivo* Studies with Treadmill Running

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Abstract

Tendons are unique in the sense that they are constantly subjected to large mechanical loads and that they contain tendon-specific cells, including tenocytes and tendon stem/progenitor cells. The responses of these cells to mechanical loads can be anabolic or catabolic and as a result, change the biological properties of the tendon itself that may be beneficial or detrimental. On the other hand, aging also induces aberrant changes in cellular expression of various genes and production of various types of matrix proteins in the tendon, and consequently lead to tendon degeneration and impaired healing in aging tendons; both could be improved by moderate physiological mechanical loading such as treadmill running. This article gives an overview on the mechanobiology research of young and aging animal tendons using *treadmill running* model. The challenges in such treadmill running studies are also discussed.

Keywords

Tendon; mechanical loading; treadmill running; aging; tendon cells; tendinopathy

1. Introduction

Tendons are a band of connective tissues that attach muscle to bone. They function to enable joint movements by transmitting muscular forces to bone. Normal tendon extracellular matrix (ECM) consists of mainly collagen, which forms a hierarchical structure[1]. Such a unique structure provides tendons the great tensile mechanical strength needed to bear mechanical loads placed on them[2]. Although collagen type I is the main component of the tendon, other types of collagens, including collagen type III, type V, and IX, and non-collagens such as fibronectin, tenascin-C, thrombospondin, and elastin, are also present in tendon ECM in small amounts[3]. Moreover, proteoglycans and glycosaminoglycans (GAG), which surround collagen, retain water and improve the elasticity of tendons[4]. The major cellular components of tendon are highly elongated tenocytes, or tendon fibroblasts, which plays a major role in tendon homeostasis, remodeling and repair of the tendon by producing matrix components such as collagens. A new type of tendon cells, which was discovered in

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Both authors contributed to concept, design and drafting of this review article, and both have read and approved the final submitted manuscript.

2007[5] is tendon stem/progenitor cells (TSCs). As adult stem cells, TSCs can self-renew and differentiate into tenocytes and non-tenocytes, depending on the environmental conditions (e.g., mechanical loading) acting on the tendon. Tendon stem cells differ from resident tenocytes in certain aspects such as shape, proliferation potential, and stem cell specific marker expression. In contrast to tenocytes that grow as highly elongated, fibroblast-like cells with smaller nuclei, TSCs are fast growing, cobblestone-shaped cells, with large nuclei in culture. Additionally, TSCs express stem cell markers such as Oct-4, SSEA-1/4 and nucleostemin (NS) in appropriate culture conditions[6].

Tendons, like patellar and Achilles tendons, are constantly subjected to mechanical loads and as “live structures,” they are highly mechano-responsive due to the presence of tendon cells. Appropriate mechanical loading is essential in maintaining the structural integrity and functional competence of the tendon. On the other hand, excessive mechanical loading causes tendon injury. Furthermore, aging alters tendon biology and leads to deterioration of structure and mechanical properties. These detrimental changes caused by either mechanical overloading or aging make the tendons weak in mechanical strength and consequently, susceptible to chronic injuries or tendinopathies, which are characterized by disordered arrangement of collagen fibers, increased vascularity, calcification, and mucoid degeneration among other degenerative changes[7]. Alterations in the profile of gene expression and ECM production in tendon cells are common consequences of the tendon to abnormal mechanical loading and aging. Rodent treadmill running is a commonly used model to study mechanobiology of young and aging tendons[8–11]. This article is to review mechanobiology research of the tendons, with a focus on animal treadmill running studies in the last decade. The challenges facing such mechanobiological studies of the tendon are discussed. Moreover, the recent findings on the beneficial effect of treadmill running in restoration of the deteriorated tendon in aging animals are highlighted.

2. Mechanobiological Responses in Young Tendons

a. Response to moderate mechanical loading

Treadmill running using rodents is one of the most common methods to apply mechanical loading to tendons, which is considered physiologic[12]. Rodents have distinct advantages over other animal species as models because they are less expensive, relatively easy to handle and control variability in genetic background, and possess homologous anatomy and physiology as humans. Both young and aging animals have been in use to study the mechanobiological responses of the tendons using moderate or excessive loading protocols. Young/adult rodents usually fall within an age range of 2 - 6 months and aging rodents from 9 - 30 months of age[13]. The running protocols, which vary widely in many studies, are selected based on pilot studies of individual researchers or previous similar studies from other groups. Treadmill running regimens with moderate running intensity is usually adopted to mimic physiological loading that lasts for a short duration, typically about an hour/day and not exceeding 4-6 weeks. As shown by many previous studies, such moderate treadmill running (MTR) does not induce catabolic responses and degenerative changes in tendons. However, to induce excessive mechanical loading on the tendon, intensive running regimens with extended duration, at least 1 h/day, over 10 - 12 weeks or longer, are widely

applied. Variations in selection of animal species, age, sex, and running speed and degree of incline/decline of treadmill in previous treadmill running model studies are common, which may explain variable mechanobiological responses of the tendon.

Animal model studies demonstrate many beneficial effects of MTR on young tendons (Table 1). These effects include an increase in the expression of tendon-related genes, including scleraxis, tenomodulin, and collagen type I in Achilles tendons of adult mice[14]. In particular, tendon cells robustly express scleraxis in response to treadmill running regimen. Scleraxis is a transcription factor that promotes fibroblast proliferation and matrix synthesis during the embryonic development of tendons[15]. The tendon cells that expressed scleraxis appeared to emerge from epitenon and migrated into the superficial regions of tendon fascicles[14]. In patellar and Achilles tendons of mice, MTR induces significantly higher expression of mechano-growth factor (MGF), an isoform of IGF-1, compared to the same tendons of non-running controls[16]. MGF is considered as a key factor that induces the expression of tenocyte-related genes including collagen type I and tenomodulin[16], and that promotes tissue growth in response to mechanical loading[17]. The overall effects of moderate loading suggest tendon matrix remodeling under normal physiological loading conditions. A rat treadmill running model used concentric (15° incline similar to uphill running) and eccentric loading (15° decline similar to downhill running) at a moderate level[18]. The tricipital, patellar, and Achilles tendons had higher number of blood vessels and accumulated larger quantity of collagen in the eccentric group compared to sedentary controls and concentric loading group. The results suggest that eccentric loading is more beneficial than concentric loading especially in terms of promoting the healing of injured tendon. Overall, treadmill walking at 9 m/min for 30 min by rats results in differential expression of numerous genes in healing rat Achilles tendons[19]. About 150 genes were up- or downregulated with the strongest response in gene expression seen 3 h after mechanical stimulation. However, after 24 or 48 h fewer than seven genes were regulated. Genes involved in inflammatory response (iNOS, PGE_1, IL-1β) and wound healing were upregulated, and genes for proteoglycans and angiogenesis were downregulated after 3 h and 12 h. This study suggests that optimal stimulation of healing requires a daily mechanical loading at low levels. Additional study to investigate the immediate effect of treadmill walking on gene regulation shows that the primary genes regulated immediately after a short walk (5 min as opposed to 30 min in the previous study) include four transcription factors (c-Fos, FosB, Egr1, and Egr2) and one negative regulator of G-protein signaling (Rgs1)[20]. Although non-specific for tendons, Egr1 and Egr2 are important during tendon development, healing, and differentiation[21]. In TGF-β1- induced murine Achilles tendinopathy model, MTR exercise exerts beneficial effects[22]. In this study, 12 weeks old mice began uphill treadmill running 24 h after TGF-β1 injection into the mid portion of Achilles tendon. Tendons from the mice exercised for 4 weeks showed essentially no chondroid cells, and the expression of aggrecan, Col1a1, Col2a1, Col3a1, and MMP3 was significantly reduced relative to the 4-week cage group. Therefore, MTR may eliminate chondroid deposits and restore tensile mechanical properties of injured Achilles tendons. Since ADAMTSs play a major role in a range of repair and regeneration processes[23], the follow-up study used the same treadmill running model with ADAMTS5$^{-/-}$ mice. In contrast to the wild type mice, the knockout mice subjected to same treadmill running regimen were unable to reverse the biochemical deficit, and thus mechanical loading was ineffective in the

healing of tendinopathic tendons[22]. Collectively, their results indicate that healing of mouse tendons with tendinopathy induced by TGF-β injections requires both mechanical loading and ADAMTS5.

b. Response to excessive mechanical loading

It is now well recognized that moderate mechanical loading is beneficial in terms of promoting tendon remodeling or repair, and excessive mechanical loading causes detrimental effects that may lead to tendinopathy. Tendinopathic tendons display major changes at the structural, cellular, and molecular levels. The major histological features of tendinopathy are increased vascularity, lipid deposits, proteoglycan accumulation, and calcification[24]. The mechanobiological responses of tendons due to excessive mechanical loading are generally investigated in rodents using intensive treadmill running (ITR). However, the challenge is that rodents are habitual runners, and it is difficult to induce overuse injuries. Despite the challenge, several investigators have devised various treadmill running regimens, which are effective in inducing overuse injuries on the animal tendons (Table 2). Rat supraspinatus tendon responds to overuse treadmill running regimens by increased expression of cartilage-related genes such as Col2a1, aggrecan, and Sox-9 suggesting that the tendon may be transforming into a cartilage phenotype as a result of mechanical overload[25]. Intense treadmill running on rat Achilles tendon produces histological features including more intense collagen staining, decreased collagen fiber organization, and increased number of cell nuclei similar to those observed in human Achilles tendinopathy[26]. A limitation of this study is that histological evaluation was performed at a single time point. Ng et al. (2011) did a modified and more detailed study and showed that enforced bipedal downhill running by rats resulted in tenocyte proliferation, change in tenocyte appearance, collagen bundle disintegration, and decrease in stiffness and ultimate tensile strength in Achilles tendons[27]. Their results agree with that of Glazebrook's model. This study adopted a modified downhill running by suspending the upper body of the rats to force them to use their hind limbs as both shock absorbers and propellers. Therefore, this method may subject the Achilles tendons of hind limbs to much eccentric loading as opposed to downhill running method used in a previous study[26]. Similar to the results from the bipedal running study[27], another uphill running study shows abnormal tenocyte morphology and morphological changes in the fibrillar collagen matrix of mechanically loaded Achilles tendons[28]. Using highly refined second harmonic generation (SHG) and multi-photon excitation fluorescence (MPEF) microscopy analysis, they observed significantly reduced collagen density and organization in focal micro-regions of mechanically loaded tendons. They argue that the increased collagen staining observed in the previous treadmill-run rat tendons[26] may be due to the misleading evaluations by haemotoxylin and eosin (H&E) staining. A limitation of these studies is that their outcomes measures were based on a single time point. An overuse rat model in Achilles tendons, however, presents a quantitative analysis of time-dependent histological changes[29]. The histological analyses show significant cellularity, microtearing, collagen deposition, and GAG at three different intervals of 4, 8, and 16 weeks after protocol start. These results are in accordance with the histological patterns of tendinosis (tendon degeneration) in humans[30–32]. Moreover, the study by Silva et al. (2011) also showed that in the time intervals, cell density did not change significantly in treadmill running and non-treadmill

running groups, and that inflammatory cells were absent in mechanically loaded groups. These results support the hypothesis that tendons attempt to adapt to persistent overuse. Intensive treadmill running induces MGF and tenocyte-related genes, collagen type I and tenomodulin as well as non-tenocyte-related genes, LPL, Sox-9, Runx-2 and osterix in mouse patellar and Achilles tendons[16]. Although MGF and tenocyte-related gene upregulation are important for tendon repair and/or remodeling as observed with MTR, ITR may also cause degenerative changes in tendons by inducing aberrant differentiation into non-tenocyte phenotypes such as adipocytes, chondrocytes, and osteocytes. Another recent study in a rat model of overuse injury to assess Achilles tendinopathy shows some histological changes characteristic of overuse tendinopathy (higher cell density, more cell clusters, and disorganized collagen) as well as decreased mechanical properties, increased substance P, and dynorphin A peptides but without pressure pain sensitivity[33]. While the above-described studies demonstrated changes characteristic of overuse tendinopathy, some recent studies show contrasting results. For example, using the same model as that of Glazebrook, another study observed that uphill running improved rat Achilles tendon tissue mechanical properties, and altered gene expression without inducing pathological changes even after the running speed was progressively increased[34]. It is possible that non-tenocyte related genes (e.g. Sox-9 and Runx2), which were not measured in this study might have been upregulated. In addition, the rats might have adapted to the persistent overuse at the time of evaluation, and repair process may have already begun. Also, an uphill treadmill running by rats selectively bred for high-capacity running does not generate Achilles tendon changes, including collagen arrangement, tenocyte morphology, cellularity, vascularity, and calcification[35]. This study relied on these outcome measures with a single time-point evaluation. Additionally, selective breeding of their rats for aerobic capacity may have led to the development of a tendon phenotype that enhanced tendon resistance to degeneration. Moreover, it is possible that a prolonged treadmill acclimation period, which lasted two weeks, and subsequent relatively short period (7 weeks) of running at full speed and duration, might have potentiated tendon adaptation to running, which may cause the difficulty to detect pathological changes in the tendon. The same group performed a similar study in which they injected collagenase into Achilles tendons of treadmill running rats selectively bred for high capacity running[36]. In cage control rats, collagenase induced molecular, histopathological, and mechanical changes within the Achilles tendon at 4 weeks. Although the mechanical changes persisted at 10 weeks, histopathological and most of the molecular changes were absent in collagenase-injected control rats. Treadmill running neither induced any significant changes nor exacerbated the collagenase-induced changes. They concluded that a combined collagenase injection and treadmill running did not create Achilles tendon pathology in rats selectively bred for high capacity running. Again, the selective breeding may have increased the resistance to tendon degeneration. The cumulative findings from these studies indicate that treadmill running protocols have to be adapted to the running capacity of rats in order to create a valid rat tendinopathy model.

In mice, a bout of rigorous treadmill running produced higher levels of PGE_2 in patellar and Achilles tendons compared to control tendons[37]. Because *in vitro* treatment of isolated TSCs with PGE_2 decreased cell proliferation, and induced adipogenic and osteogenic differentiation[37], the findings of this study suggest that chronic mechanical loading of

tendons, such as in the athletic setting, may cause lipid accumulation and calcification that are often present in lesions of tendinopathic patients.

Mechanical overloading in rotator cuff tendons before and after inducing injury shows altered transcriptional regulation of several chondrogenic genes[38]. Returning to overuse after injury, significantly upregulated aggrecan and Sox-9 genes (10.8 and 9.4-fold) with a trend towards upregulation of collagen type II and downregulation of VEGF in subscapularis tendon compared to cage control animals. In healing supraspinatus tendon, 4 weeks of treadmill running prior to injury did not change cell shape, cellularity, collagen organization, and mechanical properties 1 and 4 weeks post surgery. However, overuse groups had significantly enhanced expression of IL-1β, a potent inflammatory cytokine, and CD45 (a marker for leukocyte) 1-week post injury compared to control[39].

In brief, the animal models of mechanical loading demonstrate that tendons of young animals are highly mechano-responsive, and they display differential responses to moderate and excessive loading conditions. Mechanical loading through MTR brings many beneficial changes to help maintain tendon homeostasis, repair and/or remodeling by upregulated expression of tenocyte-related genes such as collagen type I and tenomodulin, suggesting that repetitive loading at physiological level is essential for maintaining healthy tendons in young. On the contrary, excessive mechanical loading by ITR may cause numerous detrimental effects typical of tendinopathic tendons by disrupting collagen structure through dysregulation of collagens, TIMPs, and MMPs, and also by inducing non-tenocyte (adipogenic, chondrogenic, and osteogenic) related gene expression.

3. Mechanobiological Responses in Aging Tendons

Aging progressively causes degeneration in tendons with substantial changes in the tendon matrix and cells. Gross changes in structural and cellular properties are evident due to aging in tendons such as loss of tensile strength and stiffness, reduction in the collagen fibril diameter and cross-links, reduced number of fibroblasts, and cellular senescence[40;41]. Although degenerative changes in the tendon mechanical properties due to aging and their restoration have been studied widely[42], the mechanobiological studies in aging tendons are rather limited.

The first study to determine the effects of mechanical loading on aging tendons used both *in vitro* and *in vivo* models of aging mouse tendons and their isolated TSCs. In aging mice, MTR decreases lipid deposition, proteoglycan accumulation and calcification, and increases the expression of stem cell marker, NS, in patellar tendons[10]. While TSCs derived from aging mice proliferate much slower compared to those isolated from young mice, moderate mechanical stretching of mouse TSCs in culture increases cell proliferation, and enhances cellular expression of stem cell markers, NS and Nanog, while excessive mechanical stretching suppresses their expression. Furthermore, moderate stretching increases the expression of tenocyte-related genes including collagen type I and tenomodulin, whereas excessive stretching increases the expression of non-tenocyte related genes such as LPL, Sox-9, and Runx-2 that are marker genes of adipocytes, chondrocytes, and osteocytes,

respectively. Therefore, moderate mechanical loading reduces aging-induced detrimental effects on aging tendons by enhancing TSC “quantity” and “quality.”

Additional studies show that MTR enhances wound healing in rats by improving organization of collagen fibers and decreasing senescent cells in the wounded tendons[11]. In aging rats, MTR for 4 weeks before creating window defect lowers vascularization and increases the number and proliferation of TSCs than the non-running control. In addition, MTR significantly increases the expression of stem cell markers, tenocyte genes, and down regulates non-tenocyte related genes. A recent study subjected 28-months old mice to 10 weeks of MTR (13 m/min, 6° incline, 30 min/day, 5 days/week); sedentary 8- and 28-month-old mice served as controls[43]. The gene expression analysis from Achilles tendons of trained aging rats shows that Col1a1 and MMP8, which were decreased due to aging are restored to the same levels as that of adult mice after training. Advanced glycation end-products (AGEs) adduct concentration increases 60% with aging in tibialis tendons whereas treadmill running decreases AGE adducts to a value similar to adult controls. AGEs are implicated as contributing factors in aging and the development of degenerative diseases[44]. Calcification, which is minimal in adult tendons, increases significantly with age. However, mechanical loading significantly reduces Achilles tendon calcification in old mice following exercise[43]. The findings of this study indicate that age-related changes in tendon can be modified with mechanical loading; they also reaffirm that even tendons from old animals are capable of replacing damaged and dysfunctional components of ECM to match with that of adult. Thus, moderate mechanical loading can help “rejuvenate” aging tendons and enhance healing in aging tendons likely through a stem cell-based mechanism.

4. Discussion

Tendons are subjected to large mechanical loading in vivo and as such, tendon injuries such as tendinopathy are common and cost billions of healthcare dollars each year in America alone. However, current treatments are mostly palliative because the pathogenic mechanisms of tendinopathy remain elusive. Moreover, with ever increasing older population, aging-induced tendon degeneration becomes a major problem. Thus, it is necessary to have a better understanding of mechanobiological responses of tendon so that new strategies can be devised to prevent tendinopathy development and treat tendinopathy more effectively.

Years of research has established that moderate mechanical loading is beneficial to normal young tendons and aging tendons, because it promotes anabolic responses of the tendon. On the other hand, mechanical overloading is detrimental to both young and aging tendons since it induces tendon inflammation and degeneration in young tendons and worsens the conditions in aging tendons. At the cellular level, this implies that mechanical loading at physiological levels is essential for normal functioning of tendon cells for young and aging tendons, while excessive mechanical loading induces dysfunction of tendon cells. However, the precise molecular mechanisms that regulate tendon cell function in the normal and excessive mechanical loading conditions are not well understood, and require further studies.

Treadmill running models have been widely used in many previous tendon mechanobiology studies. However, in these studies, the age and sex of animals, type of tendons, and protocols

varied among the studies, and this may account for inconsistent results among some studies. Also, ITR is used to induce overuse tendinopathy, but it is noted from review of the literature that consistency is a problem in using treadmill running to create tendinopathy model. Such reproducibility issues may arise from the variations in the rat/mouse strain and age, differences in the running protocols including running speed, and duration as well as inclination/declination of the treadmill. So in future studies, all these parameters need to be considered closely in order to create consistent animal tendinopathy models using treadmill running approach. Moreover, appropriate methods after treadmill running have to be used in detecting changes in the tendon at the molecular, cellular and tissue levels. For example, H&E staining has been used to characterize structural changes in animal tendons. While this method can reveal overall changes in the integrity of the tendon's structure, it cannot detect more subtle changes in tendon cell differentiation and accumulation of non-tendinous tissues inside the tendon due to treadmill running. Other methods such as immunohistochemical analysis may be required to accomplish this task. Finally, the data from animal treadmill running study may not correspond to human data. Their discrepancies may be due to several factors, such as variations in the loading levels and the greater potential for growth and adaptation of animal tendons compared to human tendons, since they may still be in the growth phase although mature[45].

A remarkable event in the field of tendon mechanobiology research is the discovery of TSCs, which may play a central role in tendon physiology. These tendon-specific stem cells may also play an important role in tendon pathology, such as the development of degenerative tendinopathy by undergoing aberrant differentiation into non-tenocyte lineages of cells in response to excessive mechanical loading placed on the tendons. This possibility needs to be more closely investigated in future research using in vivo models like treadmill running with rodents.

It has been shown that use of exercise through MTR may revive the aging TSCs to some extent in terms of enhanced stemness and proliferation[10]. This finding is significant because it indicates that MTR may be used to modify altered function of aging TSCs to improve the structure and function of aging tendon. Moreover, it is known that aging tendons contain senescent TSCs[46], and previous studies suggest that mechanical loading increases TSC numbers[16]. It is possible that MTR will slow TSC aging by "awakening" or reactivating senescent cells from their cell cycle arrest. The molecular mechanisms responsible for such a "reactivation" warrant future study.

Acknowledgments

The authors wish to acknowledge the funding support from NIH AR061395, AR065949, and AR070340 (JHW).

Abbreviations

ADAMTSs	A disintegrin and metalloproteinase with thrombospondin motifs
AGEs	Advanced glycation end-products
CTGF	Connective tissue growth factor

ECM	Extracellular matrix
Egr	Early growth response
GAG	Glycosaminoglycans
H&E	Haemotoxylin and eosin
IGF-1	Insulin growth factor-1
IL-1β	Interleukin1-β
iNOS	Inducible nitric oxide synthase
ITR	Intensive treadmill running
LHB	Long head of the biceps
LPL	Lipoprotein lipase
MGF	Mechano-growth factor
MMP	Matrix metalloproteinases
MPEF	Multi-photon excitation fluorescence
MTR	Moderate treadmill running
NS	Nucleostemin
Oct-4	Octamer-binding transcription factor4
PGE_1	Prostaglandin E_1
PGE_2	Prostaglandin E_2
Rgs1	Regulator of G-protein signaling1
SHG	Second harmonic generation
Sox-9	Sex determining region Y (Sry)-box9
SSEA	Stage-specific embryonic antigen
TGF-β1	Transforming growth factor-β1
TNF-α	Tumor necrosis-α
TIMPs	Tissue inhibitors of metalloproteinases
TSCs	Tendon stem/progenitor cells
VEGF	Vascular endothelial growth factor

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Table 1

Moderate Mechanical Loading Responses in Young Animal Tendons

Author, year	Animal/tendon type	Age (months)/total no. of animals	Protocol of treadmill running	Responses
Bell et al., 2013a	C57BL/6 mice/Achilles	3 M/10	19.2 m/min, 17° uphill, 20 min/day, 5 days/week for 2 to 4 weeks	No chondroid cells, significant reduction of expression of aggrecan, Col1a1, Col2a1, Col3a1, and MMP3 in 4-week loaded tendons relative to 4-week cage group
Bell et al., 2013b	C57BL/6 TS5$^{-/-}$ mice/Achilles	3 M/10	19.2 m/min, 17° uphill, 20min/day, 5 days/week for 2 to 4 weeks	Inability to reverse the biochemical deficit and healing of tendinopathy in knockout mice compared to wild type after loading
Eliasson et al., 2012	SD female rats/Healing Achilles	Not mentioned/100	A single loading of 9 m/min uphill walking, 30 min	Upregulation of inflammatory response genes and wound healing, and down-regulation of genes for proteoglycans and angiogenesis after 3 h and 12h
Eliasson et al., 2013	SD female rats/Healing Achilles	Not mentioned/54	A single loading of 9 m/min uphill walking, 5 min	Increased expression of 4 genes, egr-2, c-Fos, Fos-B, Rgs1 after a short duration loading
Kaux et al., 2013	SD rats/Tricipital, Patellar&Achilles	2 M/18	Concentric (+15° incline similar to uphill running) and eccentric loading (−15° incline similar to downhill running) 17 m/min, 1h, 3 times/week for 5 weeks	Higher number of blood vessels and accumulation of larger quantity of collagen in the eccentric group compared to sedentary controls and concentric loading group
Mendias et al., 2012	Transgenic ScxGFP mice/Achilles	4 M/10	8-14 m/min, 0-15° incline over the course, 30 min/day, 5 days/ week, 6 weeks	Increased expression of scleraxis, tenomodulin, and type 1 collagen genes
Zhang et al., 2013	Mice/Patellar &Achilles	2.5 M/18	13 m/min, 5° incline, 50 min/day, 5 days/week, 3 weeks	Higher expression of MGF, tenocyte -related genes, collagen type I and tenomodulin

Table 2

Mechanobiological Response of Young Animal Tendons to Excessive Mechanical Loading

Author, year	Animal/tendon type	Age (months)/ total no. of animals	Protocol of treadmill running	Responses
Abraham et al., 2011	SD male rats/Achilles	4 M/12	16.7 m/min, 10° uphill, 1 h/day, 5-7 days/week for 12 weeks	Pathological changes associated with altered tenocyte morphology, decrease in collagen density and organization
Archambault et al., 2007	SD male rats/Supraspinatus	Adults (age not mentioned)/20	17 m/min, 10° downhill, 1 h/day, 5 days/week for 4 weeks	Increased expression of cartilage genes, col12a1,aggrecan,and Sox-9
Dirks et al., 2013a	High capacity running (HCR) rats/Achilles	6-7 M/26	20-30 m/min, 15° uphill, 1 h/day, 5 days/week for 7 weeks	No significant changes in cellularity, vascularity, collagen organization, calcification, adipocytes and tenocyte morphology in running rats compared to control
Dirks et al., 2013b	HCR rats/Achilles	6-7 M/88	30 m/min, 15° uphill, 1 h/day 5 days/week for 4 or 10 weeks	No hismiddleathological and molecular changes after the run
Glazebrook et al., 2008	SD rats/Achilles	2 M/10	17 m/min, 10° uphill, 1 h/day, 5 days/week for 12 weeks	Decreased collagen fiber organization, more intense collagen staining, increased cell nuclei compared to non-running rats
Heineimeir et al., 2012	SD male rats/Achilles	Adults (age not mentioned)/20	17-20 m/min, 10° uphill, 1 h/day, 5 days/week for 12 weeks	Increased gene expression of collagen III, IGF-1, collagen I unchanged, and decreased gene expression of fibromodulin, biglycan, MMPs, TGF-β1, and CTGF
Jafari et al., 2015	SD rats/Achilles	3-4 M/16	~17.75 m/min to 18.55 m/min, 10° uphill, 1 h/day, 5 days/week for 6 weeks	Higher cell density, more cell clusters, more disorganized collagen, increased Substance P and dynorphin after loading
Ng et al., 2011	SD female rats/Achilles	3 M/14	17 m/min, 20° downhill, 1 h/day for 8 weeks	Collagen bundle disintegration, tenocyte shape change, and increased cellularity
Reuther et al., 2013	SD male rats/Rotator cuff	Adults (age not mentioned)/40	17 m/min, 10° downhill, 1 h/day, 5 days/week, 4 weeks prior to injury and 7 weeks after injury with 1 week cage activity after injury	Significant upregulation of chondrogenic genes, ACAN and Sox-9 in the overuse group (OV), a trend towards upregulation of Col II (15.6-fold) and downregulation of VEGF (1.6-fold) with OV, and LHB a trend toward downregulation of the chondrogenic genes, Sox-9 and Col II
Silva et al., 2011	Wistar rats/Achilles	4 M/30	26.8 m/min, 10° uphill, 80 min/day, 5 days/week for 4, 8, and 16 weeks	Increased cellularity, collagen III, GAG, apoptotic cells, and transcription activity. At all time points, increased cellular turnover and matrix deposition as mechanical loading becomes chronic
Tucker et al., 2016	SD male rats/Rotator cuff	Adults (age not mentioned)/31	17 m/min, 10° downhill, 1 h/day, 5 days/week, for 4 weeks with 2 weeks training period before inducing injury.	No changes in cell shape, cellularity, collagen fiber organization, mechanical properties post 1 and 4 weeks surgery. Significant increase in IL-1β protein expression 1 week post surgery at both injury site and mid-substance, significant increase in CD45 leukocyte staining at the mid-substance but not at the injury site, no difference in TNF-α staining at both sites
Zhang et al., 2010	C57BL/6 female mice/ Patellar &Achilles	2.5 M/10	13 m/min, 5° uphill until exhaustion, mean running time 212±50 min	Significant increase in PGE_2 in both tendon tissues

Author, year	Animal/tendon type	Age (months)/ total no. of animals	Protocol of treadmill running	Responses
Zhang et al., 2013	C57BL/6 female mice/ Patellar &Achilles	2.5 M/18	13 m/min, 3 h/day, 4 h/ day, and 5 h/day for 5 days in the second, third, and fourth weeks	Induction of higher expression of MGF, tenocyte -related genes, collagen type I and tenomodulin, as well as non-tenocyte-related genes, LPL, Sox-9, Runx-2, and Osterix