J Musculoskelet Neuronal Interact 2011; 11(2):94-105

Review Article

Hylonome

The interface of mechanical loading and biological variables as they pertain to the development of tendinosis

G.M. Thornton[1,2] and D.A. Hart[1,3]

[1]McCaig Institute for Bone and Joint Health, Department of Surgery, University of Calgary, Calgary, Alberta, Canada;
[2]Department of Orthopaedics and [3]Centre for Hip Health and Mobility, University of British Columbia, Vancouver, British Columbia, Canada

Abstract

Different tendons are designed to withstand different mechanical loads in their individual environments. Variable physiologic loading ranges and correspondingly different injury thresholds lead to tendon heterogeneity. Also, tendon heterogeneity is evident when examining how different tendons regulate their response to changes in mechanical loading (over- and under-loading). The response of tendons to changes in mechanical loading plays an important role in the induction and progression of tendinosis which is tendon degeneration without inflammation. Tendon overuse injury is likely related to abnormal mechanical loading that deviates from normal mechanical loading in magnitude, frequency, duration and/or direction. Mechanical loading that results in tendon overuse injury can initiate a repair process but, after failed initial repair, non-resolving chronic attempted repair appears to lead to a "smoldering" fibrogenesis. Contributions of regulatory components, including minor components in the "nerve-mast cell-myofibroblast axis", are key features in the development and progression of tendinosis. Hormonal and genetic factors may also influence risk for tendinosis. Further understanding of how tendinosis induction is related to mechanical use/overuse, how tendinosis progression is related to abnormal regulation of attempted repair, and how induction and/or progression are modulated by other risk factors may lead to interventions that mitigate risk and enhance functional repair.

Keywords: Mechanical Loading, Tendon Injury, Tendinosis, Tendinopathy

Introduction

Tendons are a heterogeneous group of structures which normally operate in active mechanical environments at around 30% to 40% of their ultimate tensile strength (UTS)[1]; however, different tendons experience different loads based on the muscles to which they are attached. Tendons are comprised of collagens (collagen I, III, and V) and other matrix molecules, as well as a subset of cells (tenocytes, mast cells, neural elements, and microvascular cells). The extracellular matrix (ECM) of tendons and their cellular complement varies along their length. Species variations occur in tendons. Thus, tendons are very heterogeneous, and are uniquely designed to operate within specific mechanical environments.

While tendons are diverse and heterogeneous, they can suffer overt injury with possible rupture, or overuse injury with chronically painful sub-acute loss of function termed tendinopathy. The overuse injury may be related to exposure to abnormal mechanical loading which deviates from normal mechanical loading by changes in magnitude, frequency, duration and/or direction. Tendinosis is tendon degeneration without clinical or histological signs of inflammation[2,3]. Degenerated tendons are more susceptible to partial or complete rupture than normal tendons, with one study reporting that 97% of ruptured tendons had degenerative changes[4]. Ruptured tendons had decreased crimp angle and collagen fibre diameters, suggesting that they were less resistant to tensile loading[5]. Histologically, tendinosis is collagen disarray, increased ground substance and cellularity, and possible neovascularization[6]. Tendinopathy describes clinical conditions in and around tendons arising from overuse[2]. The common sites of tendinopathy are tendons with high *in vivo* loading demands: Achilles tendon (AT) of the ankle, patellar tendon (PT) of the

The authors have no conflict of interest.

Corresponding author: Gail M. Thornton, Ph.D., P.Eng., McCaig Institute for Bone and Joint Health, Department of Surgery, University of Calgary, 3A18 Health Research Innovation Centre, 3280 Hospital Drive NW, Calgary, AB, Canada T2N 4Z6
E-mail: gail.thornton@ucalgary.ca

Edited by: S. Warden
Accepted 29 April 2011

knee, rotator cuff tendons of the shoulder, including the supraspinatus tendon (SST), and tendons of the elbow, including extensor carpi radialis brevis tendon[6]. Interestingly, tendinosis can arise in specific areas of tendons, but an analogous "ligamentosis" in ligaments has not been described. Ligaments are passive structures which usually operate in low-load mechanical environments at around 5% to 10% of their UTS[1].

Tendinosis is usually characterized by a lack of inflammatory cells, although abnormal concentrations of cells such as mast cells and neural elements and their associated neuropeptides, as well as tenocytes are present along with an abnormal ECM. Some derangements of the above are not associated with pain, and thus can precede development of overt symptoms. Tendinosis can develop in specific tendons of a subset of athletes engaged in specific sports (e.g. jumper's knee in volleyball players), is more common in male than female athletes[7], and can reproducibly occur in specific areas of tendons (e.g. fibrocartilagenous zone, insertions, etc). Whether the male-female differences persist during aging, particularly after menopause, is still an open question (e.g. [8]).

Purpose

The purpose of this review is to explore our understanding of the influence of mechanical loading on different tendons while considering the different loads they are designed to withstand in their individual environments; as well as, the biological variables (aging, sex, and genetics) which can modulate responsiveness to these individual environments. Having different tendons designed for different environments contributes to tendon heterogeneity, including differential regulation in response to mechanical loading. Response to mechanical loading is a key feature in the induction and progression of tendinosis which will be discussed in some detail, including the possible contributions of regulatory components in tendons to development and progression of tendinosis.

Normal mechanical loading (Different Tendons - Different Environments - Tendon Heterogeneity)

In general, tendons operate at 30% to 40% UTS compared to ligaments at 5% to 10% UTS[1], based on findings from goat PT[9] and anterior cruciate ligament[10]. *In vivo* loads have been determined for several human tendons[11] and the variation in these loads emphasizes the heterogeneity of tendons: the AT operates at 50% to 100% UTS in activities ranging from walking to running, the SST operates normally within 25% to 30% UTS; while finger flexor tendons operate around 10% to 20% UTS.

Tendons are designed to work in specific mechanical environments via developmental programs, growth and maturation cues, and to function optimally within boundary conditions. Tendon heterogeneity results, in part, from these different tendons being designed for different mechanical environments. For example, the AT in rabbits has almost three-fold greater collagen crimp amplitude than the corresponding PT[12]. The authors suggested that this increased wave height was designed to provide an increased margin of shock absorbance[12]. Other factors also contribute to tendon heterogeneity. Some tendons operate within a sheath (e.g. flexor, carpal tunnel) and not just the tendon, but also the integrity of the sheath likely has to be considered (i.e. a two component "system"). In addition, some tendons go around a bone which leads to tissue experiencing both compressive and tensile loads, as well as friction points.

Injuries from mechanical loading

Mechanical loading is required to maintain tendon homeostasis (Figure 1), but each tendon normally operates within a unique "physiologic window". Within this normal range or "window" of loading, increases or decreases in loading from the mechanical set point[13] evokes responses in cells at the molecular level: anabolism or catabolism (Figure 1). This leads to adaptation via growth factor expression and growth factor receptor function, cellular metabolism, matrix turnover, and cell-cell interactions[14].

Overt injury occurs to tendons when a single load exceeds a threshold for injury and, likely, leads to loss of function via integrity failure. Typically, the response to overt injury includes inflammation, initiation of healing, scar formation and tissue remodeling[14]. In this case, biomechanical overload initiates repair processes inherent in the tissue but also results in acute repair via wound healing pathways with influx of cells from systemic sources common to many tissues (Figure 1). The resulting scar tissue can partially restore tendon or ligament function, but the mechanical properties are compromised for years[15-17], partly due to composition of the ECM and its organization[18]. It is known that scar fibroblasts are different from normal tenocytes and their interactions via a cell network are also likely different[19]; (Rattner et al., manuscript in preparation). For instance, the crimp found in scar tissue is usually severely different from that of uninjured tissues[18]. This could alter functioning of the tendons.

Overuse injury can also occur in tendons when repeated loading deviates from normal mechanical loading by differences in magnitude, frequency, duration, and/or direction. In other words, the tendon is exposed to either excessive loads, loading outside the normal "window" of adaptation, or repetitive stimuli outside the "window" of normal inherent repair[14]. Fatigue loading is an example of overloading in tendons and ligaments where the peak load of each loading cycle is less than the load that would cause complete failure or overt injury; however, with repeated cyclic loading, mechanical damage accumulates and is marked by a reduction in modulus[20-22]. Overuse injury can involve microinjury to tendon components, accumulation of insults, activation of inherent inflammatory processes, and attempted repair[14]. In this case, biomechanical overload initiates inherent repair processes but the tissue experiences failed initial repair and this results in a chronic repair process (Figure 1). Non-resolving chronic attempted repair can lead to a "smoldering" fibrogenesis (Figure 2) with matrix

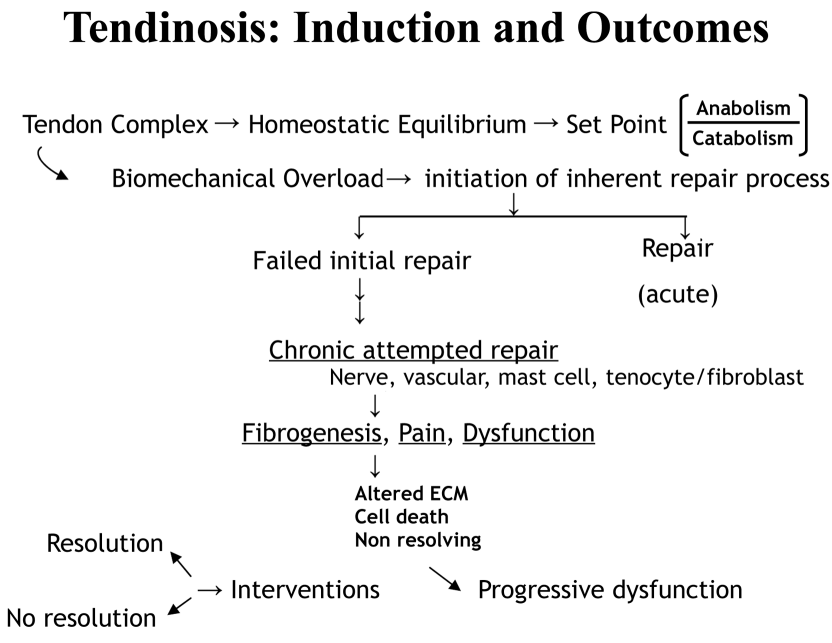

Figure 1. Tendinosis: Induction and outcomes.

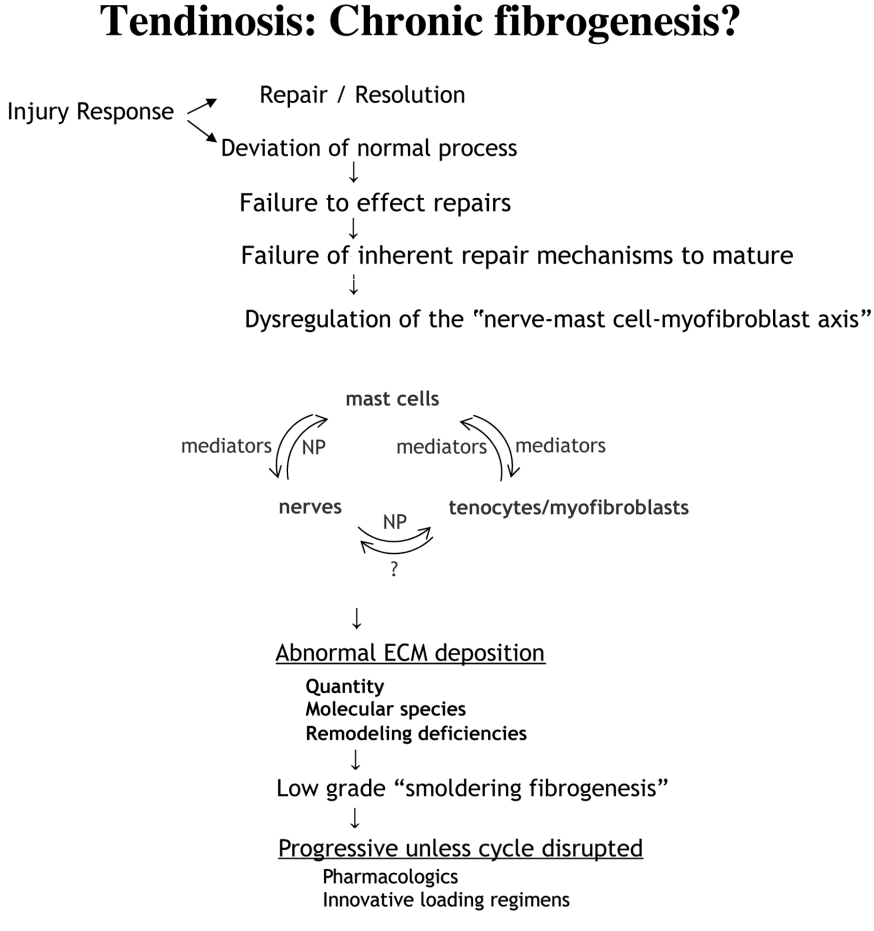

Figure 2. Tendinosis: Chronic fibrogenesis? (*NP = neuropeptides*).

turnover and cell activation without the normal maturation associated with the acute wound healing progression seen after overt injury with systemic cell contributions[23]. Over time, such chronic processes can become symptomatic (e.g. pain) and lead to altered functioning of the affected tissue. Without some intervention (mechanical, biological), it appears that such chronicity contributes to progressive decline in tendon function (Figure 2).

If there is rupture or damage of some, but not all, collagen fibres either due to overt or overuse injury, then some fibres in the tendon will experience less than normal load (underloaded or stress-deprived) and some fibres will experience more than normal load (over-loaded) even if the whole tendon is exposed to what would be considered normal mechanical loading if the tendon was intact. Thus, the partial subclinical injury plus a chronic fibrogenesis can contribute to the clinical definition (tendinopathy) and the features of tendon degeneration (tendinosis).

Interestingly, and somewhat paradoxically, specific loading protocols can contribute to restoration of tendon function after initiation of tendinopathy[24-27]. Thus, appropriate loading may facilitate repair which reinforces the idea that loading is essential for tendon function. Also relevant are reports that tendons from males and females differ in their adaptability to loading[28] as well as their responsiveness to loading[29]. This would support the concept of mechanical loading interfacing with biological variables, a concept discussed in more detail in later sections.

Abnormal mechanical loading

Abnormal mechanical loading can be less than normal loading when the tendon is stress-deprived or more than normal loading when the tendon is over-loaded. Abnormal mechanical loading may be different from normal mechanical loading in magnitude, frequency, duration, and/or direction. Direction refers to the exposure of a tensile load-bearing tendon to compressive or combined loading.

Injury may occur at different thresholds in different tendons because different tendons are designed for different *in vivo* loading demands. Likewise, injury may occur at different thresholds in ligaments and tendons because tendons are designed for greater loading demands than ligaments during normal daily activities. Like the difference in crimp amplitude between AT and PT[12], tendons and ligaments have structural and biological differences, reinforcing the concept that tendons function at higher loads than ligaments[30]. Collagen fibril diameters and crimp period are larger in tendon than ligament[12,30,31]. Total collagen is greater in tendon with a lower amount of collagen III than ligament[30]. Glycosaminoglycan (GAG) content and amount of reducible cross-links are lower in tendon than ligament[30]. The authors suggested that one possible reason for the differences between tendon and ligament was that these tissues mature or adapt to *in vivo* stresses, implying that the tendons were more stressed than the ligaments[30]. Because different individual tendons, and tendons and ligaments are designed to operate in specific mechanical environments, these tissues may also have unique responses to changes from their normal mechanical environments.

It should be noted that disruption of tendon integrity can also occur even when operating within a normal mechanical range (i.e. without abnormal mechanical loading) in a small subset of individuals exposed to environmental stimuli that are chemical in nature. The tendon that appears to be uniquely affected is the AT[32-35]. In a small subset (2-5%) of otherwise normal individuals exposed to fluoroquinolone antibiotics[32,33] or statins[34,35], they experience loss of function or overt rupture when on these drugs. Likely this outcome may be related to genetics, but how or why these drugs differentially influence the AT remains largely unknown. However, such observations raise the interesting point that these tissues are dynamic and can be influenced by both extrinsic and inherent variables.

Heterogeneous response to abnormal mechanical loading (Different Tendons - Different Environments - Different Regulation)

Less than normal load (stress-deprived)

As discussed above, tendons like other connective tissues that operate in a mechanically active environment subscribe to the "use it or lose it" paradigm when it comes to loading. That is, they require loading to maintain integrity, and, if the tissues are deprived of loading, they can undergo atrophy, just like other connective tissues such as menisci, ligaments, muscle, and bone[36]. While some principles are consistent in this regard, some variations have been noted when tissues from different species have been compared, or different tissues in the same general category have been compared (e.g. [37]).

In this regard, tendons with different *in vivo* loading demands exhibit tendon-specific responses to stress-deprivation. Before culturing for 4 hours, rat tissues were stress-deprived by separating tissues from their attachments. Rat SST had the greatest response to stress-deprivation with a 17-fold increase in matrix metalloproteinase-13 (MMP-13) expression compared to time-zero control tendons[37]. Rat AT had increased MMP-13 expression but this response was only 4-fold greater than time-zero control tendons[37]. The up-regulation of MMP-13 in the SST due to stress-deprivation was significantly greater than that in the AT, suggesting that a tendon designed for lower *in vivo* loading demands was more affected by the loss of loading. Rat medial collateral ligament (MCL) did not have a significant increase in MMP-13 expression as a result of stress-deprivation[38]. These results demonstrate the different response to stress-deprivation between two tendons with different *in vivo* loading demands, and between tendon and ligament.

Using stress-deprivation for 4 hours in culture, up-regulation of MMP-3 and TIMP-2 mRNA levels compared to time-zero controls was found in SST, AT and MCL[37,38]. The increased MMP-13 expression is similar to the increases in MMP-13 and MMP-1 observed in ruptured human SST[39,40] and AT[41,42]. These findings suggest that an up-regulation of MMP-13 following stress-deprivation may, in part, contribute to the progression of tendinopathy in the SST and AT. How-

ever, the MMP-3 expression was increased unlike the decreases observed in ruptured tendons[39,40,42]. This would suggest that the response of the cells in the stress-deprived portion of the tendon cannot completely account for the degenerative response and that the cells in the over-loaded portion of the tendon may have an important role in the progression of tendinosis in a partially-damaged tendon.

Up-regulation of MMP-13 with stress-deprivation in rat SST and AT is consistent with observations in rat tail tendon following stress-deprivation for 24 hours[43] and 7 days[44]. In tail tendon, MMP-13 staining was located in regions of damage following sub-rupture tensile loading which caused collagen fibrillar damage[43]. Also, tail tendons exposed to stress-deprivation had lower modulus and UTS compared to normal tendons[44]. If repetitive mechanical loading results in damage to portions of the tendon, up-regulation of MMP-13 due to stress-deprivation in these damaged portions of the tendon could contribute to matrix degradation and corresponding mechanical property deterioration in tendinosis.

Interestingly, in the rabbit MMP-1 appeared to be de-repressed by loss of loading to connective tissues but MMP-13 was not[36,45-47]. However, rats and mice do not have a classic MMP-1 in their genome and their equivalent has features of MMP-13[48]. Therefore, at the level of response to loss of loading, there are some consistent features regarding the mechanisms involved. Thus, the "use it or lose it" paradigm likely involves MMP-mediated atrophy[36,45,47].

More than normal load (over-loaded)

Tendons with different *in vivo* loading demands exhibit tendon-specific responses to similar overuse injury protocols. In a rat model, SST were injured by overuse activity associated with downhill treadmill running: 17m/min, 10 degree decline, 1 hour per day, 5 days per week[49]. The injured SST exhibited changes in geometry (cross-sectional area increased), mechanics (modulus and UTS decreased), and histology (cellularity increased and collagen fibre organization decreased). Using the same downhill treadmill running loading protocol, rat AT did not develop the same overuse injury as did the rat SST[50]. Although histology was not examined, the overuse injury protocol that affected the SST did not result in changes in cross-sectional area, modulus or UTS in the AT. Huang et al.[50] suggested that that the reason for the differences between the rat AT and SST may be due to differences in tendon anatomy, functional demands, loading levels, or injury mechanisms. When the running protocol was changed to uphill rather than downhill running, the rat AT still exhibited no changes in cross-sectional area and mechanical properties[51]. Unlike Huang et al.[50], Glazebrook et al.[51] examined histology and found increased cellularity and collagen staining, and decreased collagen fibre organization in the absence of changes in cross-sectional area and mechanics. The same overuse injury protocol[49,50] had different effects on different tendons with different *in vivo* loading demands, where the SST with the lower *in vivo* loading demand was injured when the AT was not. Altering the protocol to increase the load on the AT[51] still did not produce an injury similar to the SST[49] where there were changes in histology, geometry and mechanics.

Treadmill running protocols have different effects on different tendons from the same animals. Mice underwent a treadmill running protocol that included one week of training followed by running to exhaustion. Treadmill running increased prostaglandin E_2 levels in PT by 119% but only 51% in AT[52]. Prostaglandin E_2 is a mediator of pain and acute inflammation. Again, the tendon with lower *in vivo* loading demands (PT) appears more responsive than the tendon with higher *in vivo* loading demands (AT).

Tendons with different *in vivo* loading demands exhibit different responses to exposure to intermittent cyclic hydrostatic compression (ICHC). Exposing the attached rat SST to ICHC for 4 hours in culture significantly increased mRNA levels for MMP-13 compared to time-zero control tendon, while MMP-3 and TIMP-2 mRNA levels did not change[37]. For the attached rat AT, ICHC did not lead to alterations in mRNA levels of MMP-13, MMP-3 or TIMP-2 compared to the time-zero controls[37]. This type of "compressive" loading resulted in a 3-fold up-regulation of MMP-13 expression in the SST which was statistically greater than the levels in the AT which did not change. Unique up-regulation of MMP-13 due to ICHC in SST may be related to matrix turnover and could support the impingement injury theory for rotator cuff tears[53-55].

Taking these findings together demonstrates that different tendons designed for different mechanical environments are regulated differently in response to abnormal mechanical loading, whether the loading is less than normal (stress-deprived) or more than normal (over-loaded via magnitude, frequency, duration or direction). In addition, as mentioned earlier, different tendons are differentially affected by other external factors; for example, antibiotics, statins, and MMP inhibitors[56]. In this latter study, a subset of patients receiving a synthetic MMP inhibitor (Marimastat) for cancer developed frozen shoulder (~25%) which resolved after discontinuation of the drug[56]. Another 25% of the treated patients developed a Dupuytren's-like condition so the response to this MMP inhibitor differed from the experience with the antibiotics and statins where the AT was principally affected (discussed above). Thus, the findings with the MMP inhibitor may indicate that normal levels of MMPs in shoulder tendons are required for homeostatic regulation of tissue turnover. Therefore, tendons are heterogeneous at multiple levels and generalization from the study of one tendon cannot be made to another tendon. Also, tendons are dynamic, and not static as they appear to be, and are influenced by age, sex, loading history, and location.

Tendinosis induction and progression

The development and progression of tendinosis may be comprised of two central elements: excessive biomechanical use of the tendon (exceeding the threshold of subclinical disruption of tendon integrity; e.g. overuse syndrome); and a chronic disruption of the inherent biological repair process re-

quired to maintain such integrity leading to an abnormal repair phenotype (Figure 2). Why only a subset of people engaged in a specific activity develop tendinosis may, in part, be attributed to biomechanical factors related to intensity and duration of tendon use, style and appropriateness of the biomechanical stimulation, as well as the genetics of normal ECM composition and function (induction of damage to tendon integrity). As well, biological variation in initiation of acute and chronic aspects of the repair process plus genetic variation in such response phenotypes may contribute to the failure to repair and resolve the response to local injury (acute subclinical repair versus chronic fibrogenic response phenotype).

Regulation of Tendons by Mechanical Loading: Is it all about the ECM and the tenocytes when tendinosis is considered?

Tendons and ligaments consist primarily of a highly organized ECM populated mainly with cells called tenocytes. These are defined as mesenchymal cells of a tendon, but whether they are comprised of heterogenous subsets cannot be determined as good biomarkers are not yet available. Such cells are found dispersed throughout the matrix, but apparently are connected via a cell network[19]; (Rattner et al., manuscript in preparation) that develops during development and maturation. However, the tissue also contains a small number of other cells, some associated with the relatively low content of microvasculature, but others such as mast cells, tissue macrophages, possibly mesenchymal stem cells and nerve endings are also present. The nerve endings contain neuropeptides such as substance P (SP), calcitonin-gene related peptide (CGRP), and other related peptides[57,58]. Normal tenocytes and ligament cells appear to respond to such neuropeptides *in vitro*[59-62], and, if the nerves are compromised *in vivo*, it can lead to altered tendon and ligament healing[58,62-66]. Nerves and neuropeptides have also been implicated in tendinopathies[57] so abnormal regulation could contribute to development or progression of some forms of the condition in some tendons. Some of the nerve endings parallel the microvasculature and are believed to regulate vascular tone in these tissues. Other nerve endings appear to terminate associated with a mast cell, leading to the possibility that the nerve-mast cell complex also forms a regulatory axis (Figure 2). In addition to somewhat independent regulatory features, such an axis has also been postulated to interact with tenocytes and other fibroblast-like cells in tendons, ligaments and joint capsule leading to generation of myofibroblasts[7,14,23,60,61,67,68]. Thus, these putative regulatory systems in tendons and other connective tissues could also play important roles in maintenance of tendon function and tendinosis development and progression that are in excess of their relative paucity in the tissues. Thus, the tendency is to focus on the main cell types (e.g. tenocytes) and their interaction with the primary loading-bearing component (e.g. the ECM) and largely ignore the minor components and how they are affected by the matrix (over- or under-loading). This may be an oversight to not factor some of these "minor" components into consideration when associating loading with atrophy or tendinosis.

As mentioned previously, denervation can compromise the healing of both tendons[58] and ligaments[65] following a traumatic failure. However, the healing tissue is essentially unloaded during the initial inflammatory and matrix deposition phases of healing. More relevant is how these components respond to loading under normal and abnormal loading environments.

Interestingly, Butler[69,70] has provided some interesting speculations built on the knowledge that nerves in many locations are subjected to loading and have to stretch to adapt to the loading. He postulated that "the nervous system must have optimal mechanical function just like other structures in the body"[69]. Similarly, the microvasculature must also adapt to tensile loading by stretching to some extent. This concept has more recently been picked up by Webborn[71]. While still somewhat speculative, the concept for how some of the minor components could contribute to tendinosis development and progression is intriguing and deserves further investigation. While intriguing, such contributions likely cannot explain all features of tendinosis and tendon degeneration occurring in specific locations of tendons (e.g. fairly aneural and avascular areas of central tendons) or along their length (e.g. the fibrocartilage-like region of the SST). However, it could explain tendinosis in some tendons where there is extensive involvement of the paratendinous tissue (e.g. AT[72,73]); as well as some of the contralateral effects observed in tendinosis of some tendons which likely is based on nerve connections at the level of the spine and dorsal root ganglions[72].

A key distinction for the contribution of major and minor elements that are subjected to loading in the tendon may reside at the level of tendinosis induction/initiating events versus those involved in progression and chronic features of the process including symptoms such as pain. As shown by Scott et al.[74,75], many samples of patellar tendon from asymptomatic and symptomatic individuals express features consistent with early tendinosis. There were elevated numbers of nerves, mast cells, myofibroblasts and matrix alterations detected in a fair subset of the samples examined, so even in this "early" stage the putative "nerve-mast cell-myofibroblast axis" may be in play with the mechanical environment prior to overt symptom development. This "axis" consists of nerve endings containing neuropeptides such as SP and CGRP which when activated impact tissue mast cells to release mediators which in turn affect fibroblasts and their conversion to myofibroblasts[14,67,76]. It is clear from this literature that this is likely not a unidirectional "axis" and, in fact, is multidirectional. Dysregulation of this proposed axis would lead to altered matrix deposition and turnover contributing to the chronic fibrogenesis (Figure 2). Modifying this axis at the mast cell level using mast cell stabilizers approved for use in humans have been shown to influence tendon healing[77], and outcomes in other models of fibrogenesis[67,76], so potential interventions to provide new information on the role of this axis in human tendinopathy/tendinosis patients are available for clinical investigations.

In other preclinical models of tendinosis such as the Back-

man model of overuse in the rabbit[78,79], the alterations to the AT by "mechanical overuse" appears first in the lateral paratenon of the tissue complex and then later involves the tendon. This is interesting as it is not clear how direct loading of the paratenon occurs by the overuse protocol as most of the load should be transmitted via the tendon proper. As the paratenon is more vascularized and innervated than the tendon proper, perhaps the involvement of the nerve-mast cell axis[14] also involves the microcirculation and is bidirectional with the loading of the tendon leading to signals being transmitted to elements of the paratenon. Interestingly, this concept could explain the observed contralateral effect seen in the Backman model[72] with neural signals being transmitted to the spine and subsequently to the contralateral tendon inducing alterations. An additional unique feature of overuse loading and the mechanobiological alterations resulting from the protocols was the detection of non-neural expression of neuropeptides with progressive loading[73]. Whether these alterations were due to unique mechano-influenced differentiation of a subset of stem cells in the tendon which were induced by the environment to uniquely express neuropeptides remains to be determined, but it is an interesting finding which may have relevance to how mechanical loading leads to alterations in the axis with chronic stimulation to involve new elements.

Other Potential Modulating Influences on Development of Tendinosis/Tendinopathy by Mechanical Loading

Age / Sex-gender

Tendon and ligament injuries can happen to patients of all ages, with tendinopathy primarily affecting those over age 25[80]. Rotator cuff tendinopathies are increasingly frequent after age 55[8,80]. The incidence of patellar and Achilles tendinopathies increases between age 18 and 55 with clinical problems after age 30[80]; however, some patellar tendinopathies can onset before age 18 in athletes[81]. Tendinopathy may affect men and women differently. Sports-related overuse injuries affect more men than women[53,80] and work-related overuse injuries affect more women than men, particularly after age 30[7,80,82]. In the pre-menopausal years, women have lower risk than men for developing lower limb tendinopathies[83-85]. In the post-menopausal years, this risk in women appears to increase with aging for the AT based on tendon rupture[86]; and may be influenced by activity levels and use of hormone replacement therapy (e.g. endocrine estrogen)[83]. However, the mechanisms behind some of these observations are still not understood in detail[83,87,88].

As mentioned above, there appears to be some sex differences in the development of tendinosis/tendinopathy in younger athletes, and this may also be evident in older populations (e.g. post-menopausal females) for some tendons (e.g. the AT). Therefore, the hormonal environment may influence how the mechanobiological signals from overloading result in development of symptoms and tendon degeneration. As all connective tissues appear to express sex hormone receptors, it is clear that such tissues should be responsive to hormones but some sex differences have been noted for the response of tendons to neuropeptides in experimental models[60]. Intrinsic differences were also noted for male versus female tendons, as well as pregnant versus non-pregnant young rabbits when the influences of neuropeptides were assessed. Pregnancy leads to alterations in endocrine sex hormone levels so is relevant to this discussion. Some joints become more lax during pregnancy[89-91], so ligaments and tendons are targets for such influences but the mechanisms are not clear[92] and may involve a genetic component. Interestingly, the responsiveness to neuropeptides was abolished in some ligaments and tendons during pregnancy, likely indicating that interplay exists between different biological regulatory systems. Such a conclusion is also supported by a case report where a woman with long standing bilateral AT tendinopathy experienced an extended remission of symptoms when pregnant[93], symptoms that reappeared in the post-partum period. While it is unknown whether there were changes to the tendons directly during pregnancy, the reappearance after pregnancy would suggest not, so one can likely separate symptoms from pathology in such cases. Therefore, under non-overuse conditions some sex-associated factors are apparently operative. The details of the mechanisms underlying the pregnancy-associated changes in ligaments and tendons are not known, but certainly in preclinical rabbit models, the tissues appear to become resistant to influence by neuropeptides at multiple levels[94,95].

Much of the literature would indicate that menstrual cycle variations do not influence the function of a variety of human tendons *in vivo* in otherwise healthy young females[96-98]. While relevant to tendons operating within a normal physiologic window, the real test would be to assess the influence of fluctuating hormone levels on development and progression of mechanically-induced overuse/tendinosis. This has not been done for obvious reasons as yet, but could be simulated in some preclinical models such as the Backman model[78,79] in the future.

In contrast to menstrual cycle variations, menopause leads to a permanent alteration via the loss of systemic estrogen and progesterone production by the major producing tissues. Except for local production by some cells, this leads to an alteration in the hormonal "set point" for tendons and other connective tissues, in particular, the balance between estrogens/progestins and androgens. While the literature in this area (e.g. the interplay between mechanical loading and menopause) is not complete or well developed presently, it is clear that older females do have a high level of degenerative changes in some tendons[8], so it is possible that the "physiologic window" for females regarding tendon loading is dynamic, depending in part on hormonal status. While speculation at this point, it is clear that in the postmenopausal state some females have a higher incidence of osteoporosis, osteoarthritis, and other conditions, observations that perhaps may include tendons as well[83,99]. Some literature has investigated potential hormonal and loading influences on pre- and post-menopausal women which support a role in normal tendon function[29,100,101].

Mechanical Loading, Tendinosis/Tendinopathy and Genetics/Genomics – the final frontier?

It is clear that not all athletes or participants in an activity develop tendinosis/tendinopathy. While some of this variability may be related to non-genetic factors (e.g. style, frequency of training, nutrition, age, etc.), the role of genetic factors in the development and progression of tendinosis/tendinopathy remains unclear. It is clear that single gene mutations such as Marfan's syndrome and the Ehlers-Danlos syndromes can influence a variety of connective tissues, including tendons[102-105]. Similarly, there is some evidence that conditions like Joint Hypermobility Syndromes have a genetic component[106,107].

However, it is possible that the genome may also harbor many genetic variations that remain undetected under normal usage circumstances, but in aggregate contribute to development of tendinopathies and/or their progression. Thus, the influence of some genetic variation may only become evident when the tendons are stressed mechanically in some manner, either through variations in the actual ECM components and/or their organization, or at the level of repair processes to maintain integrity of the tissue. As discussed previously, tendons appear to differ in their loading environments and respond to a lack of loading in a tendon-specific manner. Therefore, some genetic variation may become evident when a specific tendon is subjected to mechanical stresses, but not others. Recent evidence[108-111] has started to elucidate the association of single nucleotide polymorphisms and RFLP sequences with tendon injuries and tendinopathies, so it is likely that extending such approaches may provide further insights into the complex relationship(s) between mechanobiology and maintenance of tissue integrity with regard to risk for tendinopathy/tendinosis development and progression in specific tendons. With the availability of the human genome sequence and the emerging number of animal model sequences, an effort to gain a more complete understanding in this area is not only becoming more feasible, but is also one which could impact athletes and their training as well as occupational health for those engaged in a variety of jobs which may put them at risk.

Summary

Mechanical loading likely plays a critical and central role in the development and progression of tendinopathies and tendinosis. Specific tendons appear to have unique and distinct features contributing to their success in a variety of loading environments. How loading via mechanobiological response patterns uniquely affects the integrity of a tendon and relates to sex, genetics and activity is starting to become elucidated. Continued development of new understanding of how different tendons are regulated normally, particularly at the loading and genetic levels, may in the future lead to identification of those at risk for developing tendon dysfunctions as a result of athletic and occupational activities. Specifically, how:

1. Induction of tendinosis may be related to biomechanical factors regarding use/overuse.
2. Progression of chronic tendinosis in at least some patients may be related to abnormal regulation of attempted repair processes (e.g. non-resolving fibrogenic responses).
3. Both 1 and 2, or either component may be influenced by hormonal variables and genetic risk factors (e.g. risk for injury and/or dysfunctional repair).

As very active structures working in complex loading environments, tendons have risk for developing dysfunctions (e.g. tendinosis/tendinopathies) that ligaments appear not to harbor. Further understanding of processes leading to non-function restoring chronic fibrogenic responses (e.g. at the loading, genetic, molecular and cellular levels) should lead to improved interventions to enhance functional repair and restore pain-free mobility.

Acknowledgements

The authors thank the Canadian Institutes for Health Research (Institute for Gender and Health, Institute for Musculoskeletal Health and Arthritis), the Natural Science and Engineering Research Council of Canada, The Arthritis Society, the Canadian Arthritis Network and the Canadian Space Agency for support of many of the studies cited from the authors' laboratories. DAH is the Calgary Foundation-Grace Glaum Professor in the McCaig Institute for Bone and Joint Health.

References

1. Butler DL, Dressler M, Awad H. Functional tissue engineering: Assessment of function in tendon and ligament repair. In: Guilak F, Butler DL, Goldstein SA, David M, editors. Functional Tissue Engineering, New York: Springer; 2003. p. 213-26.
2. Maffulli N, Khan K, Puddu G. Overuse tendon conditions: Time to change a confusing terminology. Arthroscopy 1998;14(8):840-3.
3. Khan KM, Cook JL, Bonar F, Harcourt P, Astrom M. Histopathology of common tendinopathies: Update and implications for clinical management. Sports Med 1999; 27(6):393-408.
4. Kannus P, Jozsa L. Histopathological changes preceding spontaneous rupture of a tendon. A controlled study of 891 patients. J Bone Joint Surg Am 1991;73(10):1507-25.
5. Jarvinen TA, Jarvinen TL, Kannus P, Jozsa L, Jarvinen M. Collagen fibres of the spontaneously ruptured human tendons display decreased thickness and crimp angle. J Orthop Res 2004;22(6):1303-9.
6. Khan K, Cook J. The painful nonruptured tendon: Clinical aspects. Clin Sports Med 2003;22(4):711-25.
7. Hart DA, Archambault JM, Kydd A, Reno C, Frank CB, Herzog W. Gender and neurogenic variables in tendon biology and repetitive motion disorders. Clin Orthop Relat Res 1998;351:44-56.
8. Oag H, Daines M, Nicholls A, Kiran A, Spector T, Hart D, et al. Relationship of rotator cuff tears, shoulder pain and functional loss in a normal population. The International Scientific Tendinopathy Symposium 2010, p. 29,

Umea, Sweden, September 30-October 1, 2010.
9. Korvick DL, Cummings JF, Grood ES, Holden JP, Feder SM, Butler DL. The use of an implantable force transducer to measure patellar tendon forces in goats. J Biomech 1996;29(4):557-61.
10. Holden JP, Grood ES, Korvick DL, Cummings JF, Butler DL, Bylski-Austrow DI. *In vivo* forces in the anterior cruciate ligament: Direct measurements during walking and trotting in a quadruped. J Biomech 1994;27(5):517-26.
11. An K. *In vivo* force and strain of tendon, ligament, and capsule. In: Guilak F, Butler DL, Goldstein SA, David M, editors. Functional Tissue Engineering, New York: Springer; 2003. p. 96-105.
12. Amiel D, Kleiner JB. Biochemistry of tendon and ligament. In: Nimni ME, Olsen BR, editors. Collagen. Cleveland: CRC Press; 1988. p. 223-51.
13. Arnoczky S, Lavagnino M, Egerbacher M, Caballero O, Gardner K, Shender M. Loss of homeostatic strain alters mechanostat "Set point" of tendon cells *in vitro*. Clin Orthop Relat Res 2008;466(7):1583-91.
14. Hart DA, Frank CB, Bray RC. Inflammatory processes in repetitve motion and overuse syndromes: Potential role of neurogenic mechanisms in tendons and ligaments. In: Gordon SL, Blair SJ, Fine LJ, editors. Repetive Motion Disorders of the Upper Extremity. Rosemont: American Academy of Orthopaedic Surgeons; 1995. p. 247-62.
15. Thornton GM, Leask GP, Shrive NG, Frank CB. Early medial collateral ligament scars have inferior creep behaviour. J Orthop Res 2000;18(2):238-46.
16. Thornton GM, Shrive NG, Frank CB. Healing ligaments have decreased cyclic modulus compared to normal ligaments and immobilization further compromises healing ligament response to cyclic loading. J Orthop Res 2003; 21(4):716-22.
17. Chimich D, Frank C, Shrive N, Dougall H, Bray R. Effects of initial end contact on medial collateral ligament healing. A morphological and biomechanical study in a rabbit model. J Orthop Res 1991; 9(1):37-47.
18. Frank CB, Shrive NG, Lo IKY, Hart DA. Form and function of tendon and ligament. In: Einhorn TA, O'Keefe RJ, and Buckwalter JA, editors. Orthopaedic Basic Science Foundation of Clinical Practice. Third ed. Rosemont: American Academy of Orthopaedic Surgeons: 2007. p. 191-222.
19. Chi SS, Rattner JB, Sciore P, Boorman R, Lo IK. Gap junctions of the medial collateral ligament: Structure, distribution, associations and function. J Anat 2005; 207(2):145-54.
20. Thornton GM, Schwab TD, Oxland TR. Fatigue is more damaging than creep in ligament revealed by modulus reduction and residual strength. Ann Biomed Eng 2007; 35(10):1713-21.
21. Wren TA, Lindsey DP, Beaupre GS, Carter DR. Effects of creep and cyclic loading on the mechanical properties and failure of human Achilles tendons. Ann Biomed Eng 2003;31(6):710-7.
22. Wang XT, Ker RF, Alexander RM. Fatigue rupture of wallaby tail tendons. J Exp Biol 1995;198(Pt 3):847-52.
23. Hildebrand KA, Zhang M, Salo PT, Hart DA. Joint capsule mast cells and neuropeptides are increased within four weeks of injury and remain elevated in chronic stages of posttraumatic contractures. J Orthop Res 2008; 26(10):1313-9.
24. Khan KM, Scott A. Mechanotherapy: How physical therapists' prescription of exercise promotes tissue repair. Br J Sports Med 2009;43(4):247-52.
25. Jonsson P, Alfredson H, Sunding K, Fahlström M, Cook J. New regimen for eccentric calf-muscle training in patients with chronic insertional Achilles tendinopathy: Results of a pilot study. Br J Sports Med 2008; 42(9):746-9.
26. Young MA, Cook JL, Purdam CR, Kiss ZS, Alfredson H. Eccentric decline squat protocol offers superior results at 12 months compared with traditional eccentric protocol for patellar tendinopathy in volleyball players. Br J Sports Med 2005;39(2):102-5.
27. Norregaard J, Larsen CC, Bieler T, Langberg H. Eccentric exercise in treatment of Achilles tendinopathy. Scand J Med Sci Sports 2007;17(2):133-8.
28. Magnusson SP, Hansen M, Langberg H, Miller B, Haraldsson B, Westh EK, et al. The adaptability of tendon to loading differs in men and women. Int J Exp Pathol 2007; 88(4):237-40.
29. Miller BF, Hansen M, Olesen JL, Schwarz P, Babraj JA, Smith K, et al. Tendon collagen synthesis at rest and after exercise in women. J Appl Physiol 2007;102(2):541-6.
30. Amiel D, Frank C, Harwood F, Fronek J, Akeson W. Tendons and ligaments: A morphological and biochemical comparison. J Orthop Res 1984;1(3):257-65.
31. Lo IK, Marchuk LL, Leatherbarrow KE, Frank CB, Hart DA. Collagen fibrillogenesis and mRNA levels in the maturing rabbit medial collateral ligament and patellar tendon. Connect Tissue Res 2004;45(1):11-22.
32. Melhus A. Fluoroquinolones and tendon disorders. Expert Opin Drug Saf 2005;4(2):299-309.
33. McGarvey WC, Singh D, Trevino SG. Partial Achilles tendon ruptures associated with fluoroquinolone antibiotics: A case report and literature review. Foot Ankle Int 1996;17(8):496-8.
34. Carmont MR, Highland AM, Blundell CM, Davies MB. Simultaneous bilateral Achilles tendon ruptures associated with statin medication despite regular rock climbing exercise. Phys Ther Sport 2009;10(4):150-2.
35. Marie I, Delafenetre H, Massy N, Thuillez C, Noblet C. Tendinous disorders attributed to statins: A study on ninety-six spontaneous reports in the period 1990-2005 and review of the literature. Arthritis Rheum 2008; 59(3):367-72.
36. Hart DA, Natsuume T, Sciore P, Tasveski V, Frank CB, Shrive NG. Mechanobiology: Similarities and differences between *in vivo* and *in vitro* analysis at the functional and molecular levels. Recent Res Develop Biophys Biochem 2002;2:153-77.

37. Thornton GM, Shao X, Chung M, Sciore P, Boorman RS, Hart DA, et al. Changes in mechanical loading lead to tendon-specific alterations in MMP and TIMP expression: Influence of stress deprivation and intermittent cyclic hydrostatic compression on rat supraspinatus and Achilles tendons. Br J Sports Med 2010;44(10):698-703.
38. Thornton GM, Kuchison ME, Chung M, Shao X, Sciore P, Boorman RS, et al. Matrix metalloproteinases and a related factor in ligament stress-deprivation. International Symposium on Ligaments and Tendons - VII, p. 35, San Diego, CA, February 10, 2007.
39. Lo IK, Marchuk LL, Hollinshead R, Hart DA, Frank CB. Matrix metalloproteinase and tissue inhibitor of matrix metalloproteinase mRNA levels are specifically altered in torn rotator cuff tendons. Am J Sports Med 2004; 32(5):1223-9.
40. Riley GP, Curry V, DeGroot J, van El B, Verzijl N, Hazleman BL, et al. Matrix metalloproteinase activities and their relationship with collagen remodelling in tendon pathology. Matrix Biol 2002;21(2):185-95.
41. Ireland D, Harrall R, Curry V, Holloway G, Hackney R, Hazleman B, et al. Multiple changes in gene expression in chronic human Achilles tendinopathy. Matrix Biol 2001;20(3):159-69.
42. Jones GC, Corps AN, Pennington CJ, Clark IM, Edwards DR, Bradley MM, et al. Expression profiling of metalloproteinases and tissue inhibitors of metalloproteinases in normal and degenerate human Achilles tendon. Arthritis Rheum 2006;54(3):832-42.
43. Lavagnino M, Arnoczky SP, Egerbacher M, Gardner KL, Burns ME. Isolated fibrillar damage in tendons stimulates local collagenase mRNA expression and protein synthesis. J Biomech 2006;39(13):2355-62.
44. Arnoczky SP, Lavagnino M, Egerbacher M, Caballero O, Gardner K. Matrix metalloproteinase inhibitors prevent a decrease in the mechanical properties of stress-deprived tendons: An *in vitro* experimental study. Am J Sports Med 2007;35(5):763-9.
45. Natsu-ume T, Majima T, Reno C, Shrive NG, Frank CB, Hart DA. Menisci of the rabbit knee require mechanical loading to maintain homeostasis: Cyclic hydrostatic compression *in vitro* prevents derepression of catabolic genes. J Orthop Sci 2005;10(4):396-405.
46. Majima T, Marchuk LL, Sciore P, Shrive NG, Frank CB, Hart DA. Compressive compared with tensile loading of medial collateral ligament scar *in vitro* uniquely influences mRNA levels for aggrecan, collagen type II, and collagenase. J Orthop Res 2000;18(4):524-31.
47. Majima T, Marchuk LL, Shrive NG, Frank CB, Hart DA. *In vitro* cyclic tensile loading of an immobilized and mobilized ligament autograft selectively inhibits mRNA levels for collagenase (MMP-1). J Orthop Sci 2000; 5(5):503-10.
48. Balbín M, Fueyo A, Knäuper V, López JM, Álvarez J, Sánchez LM, et al. Identification and enzymatic characterization of two diverging murine counterparts of human interstitial collagenase (MMP-1) expressed at sites of embryo implantation. J Biol Chem 2001; 276(13):10253-62.
49. Soslowsky LJ, Thomopoulos S, Tun S, Flanagan CL, Keefer CC, Mastaw J, et al. Neer award 1999. Overuse activity injures the supraspinatus tendon in an animal model: A histologic and biomechanical study. J Shoulder Elbow Surg 2000;9(2):79-84.
50. Huang TF, Perry SM, Soslowsky LJ. The effect of overuse activity on Achilles tendon in an animal model: A biomechanical study. Ann Biomed Eng 2004;32(3):336-41.
51. Glazebrook MA, Wright JR,Jr, Langman M, Stanish WD, Lee JM. Histological analysis of Achilles tendons in an overuse rat model. J Orthop Res 2008;26(6):840-6.
52. Zhang J, Wang JH. Production of PGE(2) increases in tendons subjected to repetitive mechanical loading and induces differentiation of tendon stem cells into non-tenocytes. J Orthop Res 2010;28(2):198-203.
53. Jarvinen M. Epidemiology of tendon injuries in sports. Clin Sports Med 1992;11(3):493-504.
54. Burke WS, Vangsness CT, Powers CM. Strengthening the supraspinatus: A clinical and biomechanical review. J Orthop Res 2002;402:292-8.
55. Mehta S, Gimbel JA, Soslowsky LJ. Etiologic and pathogenetic factors for rotator cuff tendinopathy. Clin Sports Med 2003;22(4):791-812.
56. Hutchinson JW, Tierney GM, Parsons SL, Davis TR. Dupuytren's disease and frozen shoulder induced by treatment with a matrix metalloproteinase inhibitor. J Bone Joint Surg Br 1998;80(5):907-8.
57. Scott A, Bahr R. Neuropeptides in tendinopathy. Front Biosci 2009;14:2203-11.
58. Ackermann PW, Salo PT, Hart DA. Neuronal pathways in tendon healing. Front Biosci 2009;14:5165-87.
59. Murphy PG, Hart DA. Plasminogen activators and plasminogen activator inhibitors in connective tissues and connective tissue cells: Influence of the neuropeptide substance P on expression. Biochim Biophys Acta 1993; 1182(2):205-14.
60. Hart DA, Kydd A, Reno C. Gender and pregnancy affect neuropeptide responses of the rabbit Achilles tendon. Clin Orthop Relat Res 1999;365:237-46.
61. Hart DA, Frank CB, Kydd AS, Ivie T, Sciore P, Reno C. Neurogenic, mast cell, and gender variables in tendon biology: Potential role in chronic tendinopathy. In: Maffulli N, Renstrom P, Leadbetter WB, editors. Tendon Injuries: Basic Science and Clinical Medicine. London: Springer-Verlag; 2005. p. 40-8.
62. Salo P, Bray R, Seerattan R, Reno C, McDougall J, Hart DA. Neuropeptides regulate expression of matrix molecule, growth factor and inflammatory mediator mRNA in explants of normal and healing medial collateral ligament. Regul Pept 2007;142(1-2):1-6.
63. Bring DK, Reno C, Renstrom P, Salo P, Hart DA, Ackermann PW. Joint immobilization reduces the expression of sensory neuropeptide receptors and impairs healing after tendon rupture in a rat model. J Orthop Res 2009;27(2):274-80.

64. Bring D, Paulson K, Renstrom P, Salo P, Hart D, Ackermann P. Decreased substance P levels after chemical denervation correlate with impaired tendon repair. Wound Repair Regen, In Review.
65. Ivie TJ, Bray RC, Salo PT. Denervation impairs healing of the rabbit medial collateral ligament. J Orthop Res 2002;20(5):990-5.
66. Beye JA, Hart DA, Bray RC, Seerattan RA, McDougall JJ, Leonard CA, et al. Denervation alters mRNA levels of repair-associated genes in a rabbit medial collateral ligament injury model. J Orthop Res 2006;24(9):1842-53.
67. Monument MJ, Hart DA, Befus AD, Salo PT, Zhang M, Hildebrand KA. The mast cell stabilizer ketotifen fumarate lessens contracture severity and myofibroblast hyperplasia: A study of a rabbit model of posttraumatic joint contractures. J Bone Joint Surg Am 2010;92(6):1468-77.
68. Berglund ME, Hildebrand KA, Zhang M, Hart DA, Wiig ME. Neuropeptide, mast cell, and myofibroblast expression after rabbit deep flexor tendon repair. J Hand Surg 2010;35(11):1842-9.
69. Butler DS. Mobilization of the nervous system. Melbourne: Churchill-Livingstone; 1991.
70. Butler D, Gifford L. The concept of adverse mechanical tension in the nervous system part 1: Testing for "dural tension". Physiotherapy 1989;75(11):622-9.
71. Webborn AD. Novel approaches to tendinopathy. Disabil Rehabil 2008;30(20-22):1572-7.
72. Andersson G, Forsgren S, Scott A, Gaida JE, Stjernfeldt JE, Lorentzon R, et al. Tenocyte hypercellularity and vascular proliferation in a rabbit model of tendinopathy: Contralateral effects suggest the involvement of central neuronal mechanisms. Br J Sports Med 2011;45(5):399-406.
73. Andersson G. Influences of paratendinous innervation and non-neuronal substance P in tendinopathy: Studies on human tendon tissue and an experimental model of Achilles tendinopathy [dissertation]. Umea, Sweden: Umea University; 2010.
74. Scott A, Lian Ø, Bahr R, Hart DA, Duronio V, Khan KM. Increased mast cell numbers in human patellar tendinosis: Correlation with symptom duration and vascular hyperplasia. Br J Sports Med 2008;42(9):753-7.
75. Scott A, Lian Ø, Bahr R, Hart D, Duronio V. VEGF expression in patellar tendinopathy: A preliminary study. Clin Orthop Relat Res 2008;466(7):1598-604.
76. Gallant-Behm CL, Hildebrand KA, Hart DA. The mast cell stabilizer ketotifen prevents development of excessive skin wound contraction and fibrosis in red duroc pigs. Wound Repair Regen 2008;16(2):226-33.
77. Sharma A, Abraham T, Sampaio A, Cowan M, Underhill M, Scott A. Sodium cromolyn reduces expression of CTGF, ADAMTS1, and TIMP3 and modulates post-injury patellar tendon morphology. J Orthop Res 2011; 29(5):678-83.
78. Backman C, Boquist L, Friden J, Lorentzon R, Toolanen G. Chronic Achilles paratenonitis with tendinosis: An experimental model in the rabbit. J Orthop Res 1990; 8(4):541-7.
79. Backman C, Friden J, Widmark A. Blood flow in chronic Achilles tendinosis. Radioactive microsphere study in rabbits. Acta Orthop Scand 1991;62(4):386-7.
80. Renstrom P, Woo S. Tendinopathy: A major medical problem. In: Woo S, Renstrom P, editors. Tendinopathy In Athletes, Malden: Blackwell Publishing; 2007. p. 1-8.
81. Cook JL, Khan KM, Kiss ZS, Griffiths L. Patellar tendinopathy in junior basketball players: A controlled clinical and ultrasonographic study of 268 patellar tendons in players aged 14-18 years. Scand J Med Sci Sports 2000;10(4):216-20.
82. Kivi P. Rheumatic disorders of the upper limbs associated with repetitive occupational tasks in Finland in 1975-1979. Scand J Rheumatol 1984; 13(2):101-7.
83. Cook JL, Bass SL, Black JE. Hormone therapy is associated with smaller Achilles tendon diameter in active postmenopausal women. Scand J Med Sci Sports 2007; 17(2):128-32.
84. Cook JL, Khan KM, Harcourt PR, Kiss ZS, Fehrmann MW, Griffiths L, et al. Patellar tendon ultrasonography in asymptomatic active athletes reveals hypoechoic regions: A study of 320 tendons. Victorian institute of sport tendon study group. Clin J Sport Med 1998;8(2):73-7.
85. Gibbon WW, Cooper JR, Radcliffe GS. Distribution of sonographically detected tendon abnormalities in patients with a clinical diagnosis of chronic Achilles tendinosis. J Clin Ultrasound 2000;28(2):61-6.
86. Maffulli N, Waterston SW, Squair J, Reaper J, Douglas AS. Changing incidence of Achilles tendon rupture in Scotland: A 15-year study. Clin J Sport Med 1999; 9(3):157-60.
87. Knobloch K, Kraemer R, Vogt PM. Letter to the editor. Scand J Med Sci Sports 2007;17(4):457-8.
88. Cook JL, Bass SL, Black JE. Letter to the editor. Scand J Med Sci Sports 2007;17(4):459-60.
89. Charlton WPH, Coslett-Charlton LM, Ciccotti MG. Correlation of estradiol in pregnancy and anterior cruciate ligament laxity. Clin Orthop Relat Res 2001;387:165-70.
90. Dumas GA, Reid JG. Laxity of knee cruciate ligaments during pregnancy. J Orthop Sports Phys Ther 1997; 26(1):2-6.
91. Schauberger CW, Rooney BL, Goldsmith L, Shenton D, Silva PD, Schaper A. Peripheral joint laxity increases in pregnancy but does not correlate with serum relaxin levels. Am J Obstet Gynecol 1996; 174(2):667-71.
92. Marnach ML, Ramin KD, Ramsey PS, Song SS, Jacqueline J, An K. Characterization of the relationship between joint laxity and maternal hormones in pregnancy. Obstet Gynecol 2003;101(2):331-5.
93. Tumia N, Kader D, Arena B, Maffulli N. Achilles tendinopathy during pregnancy. Clin J Sport Med 2002; 12(1):43-5.
94. Hart DA, Reno C. Pregnancy alters the *in vitro* responsiveness of the rabbit medial collateral ligament to neuropeptides: Effect on mRNA levels for growth factors,

cytokines, iNOS, COX-2, metalloproteinases and TIMPs. Biochim Biophys Acta 1998;1408(1):35-43.
95. McDougall JJ, Bray RC, Hart DA. Late gestational changes in sympathomimetic sensitivity in primagravid rabbit ligaments. Can J Physiol Pharmacol 2000; 78(7):528-34.
96. Burgess KE, Pearson SJ, Onambele GL. Menstrual cycle variations in oestradiol and progesterone have no impact on *in vivo* medial gastrocnemius tendon mechanical properties. Clin Biomech 2009;24(6):504-9.
97. Burgess KE, Pearson SJ, Onambele GL. Patellar tendon properties with fluctuating menstrual cycle hormones. J Strength Cond Res 2010; 24(8):2088-95.
98. Kubo K, Miyamoto M, Tanaka S, Maki A, Tsunoda N, Kanehisa H. Muscle and tendon properties during menstrual cycle. Int J Sports Med 2009;30(2):139-43.
99. Hart DA, Achari Y. Alterations to cell metabolism in connective tissues of the knee after ovariohysterectomy in a rabbit model: Are there implications for the postmenopausal athlete? Br J Sports Med 2010;44(12):867-71.
100. Hansen M, Kongsgaard M, Holm L, Skovgaard D, Magnusson SP, Qvortrup K, et al. Effect of estrogen on tendon collagen synthesis, tendon structural characteristics, and biomechanical properties in postmenopausal women. J Appl Physiol 2009;106(4):1385-93.
101. Hansen M, Koskinen SO, Petersen SG, Doessing S, Frystyk J, Flyvbjerg A, et al. Ethinyl oestradiol administration in women suppresses synthesis of collagen in tendon in response to exercise. J Physiol (Lond) 2008; 586(12):3005-16.
102. Gao L, Luo F, Hui R, Zhou X. Recent molecular biological progress in marfan syndrome and marfan-associated disorders. Ageing Res Rev 2010;9(3):363-8.
103. Shirley ED, Sponseller PD. Marfan syndrome. J Am Acad Orthop Surg 2009;17(9):572-81.
104. Moretti B, Notarnicola A, Moretti L, Garofalo R, Patella V. Spontaneous bilateral patellar tendon rupture: A case report and review of the literature. Chir Organi Mov 2008;91(1):51-5.
105. Jarvinen M, Jozsa L, Kannus P, Jarvinen TLN, Kvist M, Leadbetter W. Histopathological findings in chronic tendon disorders. Scand J Med Sci Sports 1997;7(2):86-95.
106. Zweers MC, Hakim AJ, Grahame R, Schalkwijk J. Joint hypermobility syndromes: The pathophysiologic role of tenascin-X gene defects. Arthritis Rheum 2004; 50(9):2742-9.
107. Hakim AJ, Cherkas LF, Grahame R, Spector TD, MacGregor AJ. The genetic epidemiology of joint hypermobility: A population study of female twins. Arthritis Rheum 2004;50(8):2640-4.
108. September AV, Schwellnus MP, Collins M. Tendon and ligament injuries: The genetic component. Br J Sports Med 2007;41(4):241-6.
109. September AV, Cook J, Handley CJ, van der Merwe L, Schwellnus MP, Collins M. Variants within the COL5A1 gene are associated with Achilles tendinopathy in two populations. Br J Sports Med 2009;43(5):357-65.
110. Raleigh SM, van der Merwe L, Ribbans WJ, Smith RK, Schwellnus MP, Collins M. Variants within the MMP3 gene are associated with Achilles tendinopathy: Possible interaction with the COL5A1 gene. Br J Sports Med 2009;43(7):514-20.
111. Collins M, Raleigh SM. Genetic risk factors for musculoskeletal soft tissue injuries. Med Sport Sci 2009;54:136-49.