The mechanobiological aetiopathogenesis of tendinopathy: is it the over-stimulation or the under-stimulation of tendon cells?

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Introduction
Mechanoreponsiveness is a fundamental feature of all living tissues and tendons are no exception (Banes et al. 1995a; Inger 1997; Lavagnino & Arnoczky 2005; Wang 2006). The ability of tendon cells to sense and respond to load is central to the concept of mechanotransduction and the subsequent maintenance of tissue homeostasis (Wang & Inger 1994; Banes et al. 1995a; Inger 1997; Ruoslahti 1997). Tendon cells sense load through a mechano-electrochemical sensory system(s) which detects mechanical load signals through the deformation of the cellular membrane and/or the cytoskeleton (Ben-Ze’ev 1991; Watson 1991; Adams 1992; Wang et al. 1993; Wang & Inger 1994; Banes et al.
1995a; Ingber 1997; Brown et al. 1998; Wang 2006). The cellular deformation which occurs with extracellular matrix strain produces tension in the cytoskeleton which can be sensed by the cell nucleus through a mechano-sensory tensegrity system to elicit a metabolic response (Ben-Ze’ev 1991; Watson 1991; Adams 1992; Wang et al. 1993; Wang & Ingber 1994; Banes et al. 1995a; Ingber 1997; Arnoczky et al. 2002; Wang 2006). Cellular deformation in response to tissue strain is thought to occur through the binding of the cell to extracellular matrix proteins such as collagen and fibronectin (Banes et al. 1995a; Rosales et al. 1995; Sung et al. 1996). These connections are mediated by the integrin family of cell surface receptors which link the extracellular matrix to the interior of the cell through the cytoskeleton (Ingber 1991; Wang et al. 1993; Banes et al. 1995; Janmey 1998). While the precise level (magnitude, frequency and duration) of mechanobiological stimulation required to maintain normal tendon homeostasis is not currently known, it is very likely that an abnormal level(s) of stimulation may play a role in the aetiopathogenesis of tendinopathy (Józsa & Kannus 1997; Arnoczky et al. 2002, 2007).

A proposed algorithm for the onset of overuse tendinopathy involves altered cell-matrix interactions in response to repetitive loading (Figure 1) (Archambault et al. 1995). In this scenario, repeated strains below the injury threshold of the tendon induce degenerative changes in the tendon-matrix composition and organization (Järvinen et al. 1997; Józsa & Kannus 1997; Jones et al. 2006). The degeneration of the extracellular matrix leads to a transient weakness of the tissue making it more susceptible to damage from continued loading. This damage then accumulates until the overt pathology of tendinopathy develops (Archambault et al. 1995). While this is a feasible algorithm for the development of overuse tendinopathy, the precise mechanism(s) which lead to altered cell-matrix interactions have not been described.

Several investigators have suggested that it is the tendon cells’ mechanobiologic response to over-stimulation secondary to repetitive loading that initiates the degenerative cascade that leads to tendinopathy (Archambault et al. 2001, 2002; Skutek et al. 2001; Tsuzaki et al. 2003; Wang et al. 2003). Over-stimulation of tendon cells in vitro has been shown to induce increases in inflammatory cytokines and degenerative enzymes (Almekinders et al. 1993; Banes et al. 1995a, 1999; Archambault et al. 2002; Tsuzaki et al. 2003; Wang et al. 2003). However, many of these investigations have utilized non-physiologic strain patterns (Almekinders et al. 1993; Wang et al. 2003) (high strain amplitudes and frequencies, as well as long durations) or the addition of external factors (Archambault et al. 2002; Tsuzaki et al. 2003) to elicit these cellular responses. Thus the clinical relevance of these studies must be called into question (Arnoczky et al. 2007).

Experimental studies from our lab have shown that mechanobiological under-stimulation of tendon cells can also produce a pattern of catabolic gene expression that results in extracellular matrix degradation and subsequent loss of tendon material properties (Lavagnino et al. 2003, 2005a, 2006, 2006a; Arnoczky et al. 2004; Lavagnino & Arnoczky 2005; Egerbacher et al. 2006). We have also shown that at the extremes of physiologic loading, isolated fibril damage can occur in tendons which alters normal cell-matrix interactions in this damaged area (Lavagnino et al. 2006a). The inability of the damaged fibrils to transmit extracellular matrix loads to the tendon cells results in an under-stimulation of these cells which, in turn, initiates a catabolic response that can weaken the tendon making it more susceptible to damage from subsequent loading (Lavagnino et al. 2006a).

In this study, we will forward the hypothesis that it is a mechanobiological under-stimulation resulting from altered cell-matrix interaction and not a repetitive over-stimulation of tendon cells that is the aetiopathogenic stimulus for the degenerative cascade which may eventually lead to tendinopathy.

**Mechanobiology and the aetiopathogenesis of tendinopathy**

An increasing body of clinical material has suggested that tendons from tendinopathy patients exhibit an increase in degradative enzymes (matrix metalloproteinases) as well as...

Numerous studies have suggested that over-stimulation of tendon cells, secondary to repetitive loading, results in a pattern of gene expression that can lead to tendinopathy (Almekinders et al. 1993; Banes et al. 1995, 1999; Skutek et al. 2001; Archambault et al. 2002; Tsuzaki et al. 2003; Wang et al. 2003). The majority of these in vitro studies have been based on the response of tendon cells cultured on artificial substrates to various regimes of mechanical loading (Almekinders et al. 1993; Banes et al. 1995, 1999; Archambault et al. 2002; Arnoczky et al. 2002a; Tsuzaki et al. 2003; Wang et al. 2003). In these culture systems, large numbers of cells are subjected to a uniform loading regime. While this permits analysis of large amounts of cellular material and cellular products, it may not replicate the normal in situ environmental conditions of tendon cells within a three-dimensional collagenous matrix (Figure 2). As mechanotransduction signals are known to be mediated through the pericellular matrix to the nucleus via integrin-based cell-matrix connections (Sachs 1988; Ingber 1991; Watson 1991; Wang et al. 1993; Banes et al. 1995a; Ritty et al. 2003) it is not clear how, or even if, the complex cell-matrix interactions which occur in situ could be maintained or recreated in monolayer cell cultures.

In addition, the strain magnitudes and durations required to achieve an up-regulation in the expression of these inflammatory and catabolic genes may not be clinically relevant. Some of these studies have used a sustained (>20 h) application of cyclic strains in excess of 8% to elicit catabolic and inflammatory gene expression in tendon and ligament cells cultured on artificial substrates (Almekinders et al. 1993; Wang et al. 2003; Bhargava et al. 2004). Because tendon cell strain in situ has been shown to be appreciably less than whole tendon strain (Arnoczky et al. 2002), it is unlikely that such high levels of repetitive tendon cell strain could be reached and maintained in vivo without significant damage occurring within the extracellular matrix of the tendon (Woo et al. 1982). Also, as tendons are known to exhibit non-homogeneous strain patterns in response to tensile load (Kastelic et al. 1978), it would seem impossible to precisely recreate the complex and varied patterns of strain amplitudes experienced by a population of tendon cells in situ by uniformly straining a large population of isolated tenocytes in monolayer.

Finally, the large number of confluent, or near confluent, cells studied in these monolayer systems (Almekinders et al. 1993; Banes et al. 1995, 1999; Archambault et al. 2002; Arnoczky et al. 2002a; Tsuzaki et al. 2003; Wang et al. 2003) could produce a marked paracrine cellular stimulus that may not occur in the more limited cell populations seen in normal tendons. This could, in turn, provide an artificially enhanced cellular response to the repetitive loading stimulus. Thus, the in vitro application of high magnitudes of cyclic cellular strain for excessively long durations to tendon cells in monolayer may have little bearing on what is actually occurring to tendon cells in situ.
Therefore, to better understand how the mechanotransduction response of tendon cells under tensile load may contribute to the aetiopathogenesis of tendinopathy, our lab has utilized an in situ rat tail tendon model in an effort to maintain the tendon cells’ natural cell-matrix interactions as well as the naturally occurring strain fields that are developed in response to tensile loading (Lavagnino et al. 2003, 2006, 2006a; Arnoczky et al. 2004, 2007).

Previous studies from our lab have demonstrated that mechanobiologic under-stimulation of tendon cells in situ through stress-deprivation results in an immediate and significant up-regulation of interstitial collagenase mRNA expression and protein synthesis in our rat tail tendon model (Lavagnino et al. 2003, 2005a, 2006a; Arnoczky et al. 2004). This has led to the theory that the destructive mechanism(s) that precedes overt pathological development of tendinopathy may, in fact, be a catabolic response of the tendon cells to the local loss of homeostatic strain as a result of isolated, microscopic, collagen fibre damage (Jones et al. 2006; Arnoczky et al. 2007).

Previous biomechanical studies have suggested that isolated collagen fibril damage occurs near the end of the linear portion of the load deformation curves of ligaments and tendons (Figure 3) (Viidik & Ekholm 1968; Viidik 1972, 1980; Woo et al. 1982; Józsa & Kannus 1997). The ability to produce isolated fibril failure within an otherwise intact tendon is likely attributable to the multicomposite structure of the tissue (Kastelic et al. 1978, 1980; Viidik 1980). The sequential straightening and loading of crimped collagen fibrils, as well as interfibrillar sliding and shear between fibres and/or fibrils, produce a non-linear, load-deformation behaviour of tendons that may put certain fibrils ‘at risk’ for damage before others (Viidik 1980). Using confocal laser microscopy and a previously described in vitro tensile loading apparatus (Arnoczky et al. 2002), our lab has been able to demonstrate that isolated collagen fibril damage does occur in tendons in response to increasing tensile loads (Figure 4). As previously predicted, this damage occurs well in advance of complete tendon rupture (Viidik & Ekholm 1968; Viidik 1972, 1980; Woo et al. 1982; Józsa & Kannus 1997). While such damage may not affect the ultimate tensile strength of the tissues (Panjabi et al. 1996) it could alter the cell-matrix interactions within the damaged portion of the tendon. A previous study has demonstrated that following isolated fibrillar damage in tendons the damaged fibrils relax (Knorzer et al. 1986). This would suggest an inability of these damaged fibrils to transmit load and therefore maintain a homeostatic mechanobiological stimulus to those cells associated with the damaged fibrils.

Based on the above findings, our laboratory has forwarded the hypothesis that an alteration of cell-matrix interaction secondary to isolated fibrillar damage could result in a mechanobiological under-stimulation of tendon cells which has been shown to result in an upregulation of collagenase mRNA expression and protein synthesis (Lavagnino et al. 2003, 2005a; Arnoczky et al. 2004; Lavagnino & Arnoczky 2005). This, in turn, causes an initial degeneration of the pericellular matrix that may further compromise cell-matrix interactions and mechanobiological signalling (Egerbacher et al. 2006). The degenerative process then progresses throughout the extracellular matrix resulting in a decrease in the material properties of the tendon (Figure 5). These changes could then put more of the extracellular matrix at risk for further damage with subsequent loading (Lavagnino & Arnoczky 2005). Then, when a critical level of damage has been reached the clinical and histological signs of tendinopathy may become evident.

Recently, our laboratory has demonstrated that creation of isolated tendon fibrillar damage within an otherwise intact tendon fascicle results in an up-regulation of collagenase mRNA expression and protein synthesis by only those tendon cells associated with the damaged fibrils (Figures 6 and 7) (Lavagnino et al. 2006a). This would suggest a loss of load-transmitting function in the damaged fibril(s) and a subsequent altered cell-matrix interaction within the affected area. The presence of increased levels of collagenase protein in these injured tendons is similar to what has been reported in clinical cases of tendinopathy (Fu et al. 2002; Riley et al. 2002; Lo et al. 2004; Magra & Maffulli 2005; Sharma & Maffulli 2005).
A clinical study examining matrix metalloproteinase (MMP) activity in ruptured human tendons demonstrated an altered expression and activity of several members of the MMP family (Riley et al. 2002). MMP-1 levels were significantly higher in ruptured tendons compared with normal controls, whereas MMP-2 and 3 levels were reduced, possibly representing a failure in the normal remodeling process (Riley et al. 2002; Riley 2004). This increase in collagenase activity was associated with a deterioration in the quantity of the collagen network. Increased expression of MMP-1 was also found in human patellar tendinosis tissue (Fu et al. 2002).

The result of this MMP-mediated degradation of the extracellular matrix is reflected in the histopathological findings in tendinosis that reveal irregular orientation of collagen, fibre disruption, change in fibre diameter, a decrease in the overall density of collagen and an upregulation of collagen type III production (Józsa et al. 1990; Kannus & Józsa 1991; Järvinen et al. 1997). Another study that examined the histopathology of ruptured and tendinopathic Achilles tendons suggested that while the ruptured tendons were significantly more degenerated than the tendinopathic tendons, the general pattern of degeneration was common to both groups (Tallon et al. 2001).

In addition to the documented increase in collagenase activity seen in clinical cases of tendinopathy, other studies have suggested that apoptosis may play a role in the pathogenesis of tendinopathy (Yuan et al. 2002, 2003; Hosaka et al. 2005). Studies on the pathogenesis of rotator cuff disorders demonstrate a significant increase in the number of apoptotic cells detected in degenerative supraspinatus tendons compared with normal control tendons (Yuan et al. 2002; Tuoheti et al. 2005). It is theorized that the increased number of apoptotic cells seen in the degenerative tissues of
tendinopathy patients could adversely affect the rate of collagen synthesis and the potential for repair (Yuan et al. 2003). Apoptosis was also detected in samples of inflamed superficial digital flexor tendon in the horse possibly resulting in tendon weakness and increased risk of tendinopathy (Hosaka et al. 2005). However, at present, it is still unknown whether apoptosis is the result or the cause of tendon degeneration.

Experimental studies from our laboratory have documented an increase in Caspase-3 mRNA expression and protein synthesis as well as an increase in the number of apoptotic cells (demonstrated by detection of single stranded DNA) following 24 h of stress-deprivation in our rat tail tendon model (Egerbacher et al. 2007). While another ex vivo study was able to induce apoptosis in tendon cells following exposure to high strains (20% strain for 6 h at the rate of 1 Hz), it is probable that the high strains utilized in the experiment likely damaged tendon fibres or fibrils (Scott et al. 2005). This could result in the under-stimulation of the tendon cells associated with the damaged fibres or fibrils and the subsequent induction of apoptosis secondary to the release of cellular tension (Grinnell et al. 1999). Thus, these experimental and clinical studies point to a possible effect of mechanical stimulus, or lack thereof, on the induction of apoptosis in tendon cells. However, the precise mechanism(s) that trigger programmed cell death under these conditions must still be defined.

In addition to the increase in MMP expression and apoptosis reported in clinical cases of tendinopathy, classic histological changes have been described. These include collagen

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**Figure 6** Images of a rat tail tendon fascicle at various points throughout the testing protocol: (a) prior to loading (the crimp pattern is clearly visible), (b) during loading in the linear portion of the stress–strain curve demonstrating the elimination of the crimp pattern, (c) onset of fibrillar damage as manifested by a change in the reflectivity of the damaged fibrils (arrows), and (d) unloading of the tendon to 100 g and the reoccurrence of the crimp pattern within the damaged fibrils (arrows). (bar = 200 µm). (Reprinted from Lavagnino et al. (2006a) Isolated fibrillar damage in tendons stimulates local collagenase mRNA expression and protein synthesis. J. Biomech. 39, 2355–2362, with permission from Elsevier.)

**Figure 7** Representative images of a rat tail tendon fascicle following fibrillar damage. (a) Presence of the crimp pattern on the bottom of the tendon fascicle (arrows) indicates the site of isolated fibrillar damage. (b) In situ hybridization of the tendon fascicle reveals interstitial collagenase mRNA expression in those cells associated with the damaged fibril(s). The borders of the tendon fascicle are delineated by lines. (bar = 100 µm). Reprinted from Lavagnino et al. (2006a). Isolated fibrillar damage in tendons stimulates local collagenase mRNA expression and protein synthesis. J. Biomech. 39, 2355–2362, with permission from Elsevier.)
disruption and disarray along with an alteration in the crimping pattern (Jarvinen et al. 1997). There can also be great variation in the density and appearance of the tendon cells ranging from hypercellularity to hypocellularity (Jarvinen et al. 1997). In the latter case, many of the remaining cells may appear necrotic, or as noted above, apoptotic. This loss of metabolically active cells can lead to the classically described changes in the extracellular matrix of the tendons such as mucoid degeneration, hyaline degeneration and fibrocartilagenous metaplasia (Galliani et al. 2002; Jozsa & Kannus 1997). In this study, stress-deprived tendons underwent a progressive, cell-mediated degeneration of their extracellular matrix (Hannafin et al. 1995). These changes were manifested by widespread hypocellularity, alterations (rounding up) in the morphology of the tenocytes, and a loss of collagen orientation and packing (Figure 8). The histological changes induced by stress-deprivation provide further support for the theory that mechanobiological under-stimulation of tendons cells is responsible for initiating the degenerative cascade that is associated with tendinopathy.

Clinical relevance and significance

The aetiology of tendinopathy remains unclear, and many causes have been theorized (Jozsa & Kannus 1997; Sharma & Maffulli 2005). Central to these theories is the concept that excessive loading of tendons during vigorous physical activity is the main pathological stimulation for degeneration of the extracellular matrix (Selvanetti et al. 1997). Some investigators have suggested that it is the tendon cells’ mechanobiologic response to excessive loading that initiates the degenerative cascade of events that leads to tendinopathy (Skutek et al. 2001; Archambault et al. 2002; Tsuzaki et al. 2003; Wang et al. 2003). The idea being that prolonged mechanical stimuli of tendon cells induce production of degradative cytokines and inflammatory prostaglandins which are thought to be mediators of tendinopathy (Sharma & Maffulli 2005). However, as noted above, the level of stimuli required to elicit these cellular responses are not clinically relevant and have, to date, only been demonstrated in cultured cells on artificial substrates (Almekinders et al. 1993; Wang et al. 2003; Bhargava et al. 2004).

We contend that it is actually an absence of mechanical stimuli, secondary to microtrauma that is the mechanobiological stimulus for the degradative cascade that leads to tendinopathy. Numerous investigators have postulated that during excessive, repetitive loading, microtrauma occurs within the tendon matrix (Archambault et al. 1995; Jozsa & Kannus 1997; Sharma & Maffulli 2005; Wang et al. 2006). If this microtrauma is not balanced by an active repair response from the tenocytes, it will result in cumulative damage and ultimately, degradation of the tendon (Ker 2002). Our research supports the concept that isolated fibril damage can occur during the extremes of physiologic loading (Lavagnino et al. 2006a). This damage alters cell-matrix interactions (mechanobiologic signalling) in the area causing an upregulation in collagenase and a weakening of the collagen structure (Lavagnino & Arnoczky 2005; Lavagnino et al. 2006a). This could make the tendon more susceptible to damage from additional loading at lower strains. We have also shown that mechanobiological under-stimulation can induce apoptosis in tendon cells (Egerbacher et al. 2007). The loss of cells could further compromise the tendon’s ability to repair itself or even maintain its local extracellular matrix.

Figure 8 Photomicrographs of the histological changes seen in canine flexor digitorum profundus tendons following stress-deprivation for 2, 4, 6 and 8 weeks. Note how the tendon cells change morphology and ‘round up’ in response to the loss of normal homeostatic tension. Also note the progressive decrease in cell number and the progressive disruption of the collagen architecture with time of stress-deprivation. (H & E x100)
While mechanobiological under-stimulation of tendon cells is a feasible explanation for the increase in apoptosis and collagenase expression reported in clinical cases of tendinopathy (Ireland et al. 2001; Fu et al. 2002; Riley et al. 2002; Yuan et al. 2002, 2003; Alfredson et al. 2003; Lo et al. 2004; Riley 2004; Hosaka et al. 2005; Magra & Maffulli 2005; Sharma & Maffulli 2005; Tuoheti et al. 2005), the question remains as to the role of repetitive strain in the aetio-pathogenesis of tendinopathy. As we have demonstrated in our model system, a single high load event was able to cause sufficient fibril damage to initiate a cell-mediated response as a result of mechanobiological under-stimulation (Lavagnino et al. 2006a). Tendon microtrauma can also result from a non-uniform stress occurring within a tendon producing abnormal loading concentrations and localized fibre damage (Ker 2002). Therefore, it is possible that during a series of repetitive loading cycles a single abnormal loading cycle could produce strains sufficient enough to induce isolated fibril damage but not cause clinical injury. This abnormal loading cycle could be a result of muscle fatigue and/or altered kinematics that can occur with the performance of repetitive activities (Józsa & Kannus 1997). It has long been suggested that mental fatigue (and altered neuromuscular responses) may also play a role in tendinopathy (Darling 1899a, 1899b).

Thus, while repetitive loading, per se, may not be responsible for initiating the cascade of events that lead to tendinopathy, it is likely that continued loading of the compromised tissue plays a significant role in the progression of the pathological process. Additional research is needed to determine the magnitude of tendon forces experienced in activities that are often associated with the development of tendinopathy (jumping, running and throwing). In addition, the effect of muscle fatigue and/or altered kinematics on these tendon forces must be determined to gain insight into the mechanobiological mechanism(s) which may play a role in the aetio-pathogenesis of tendinopathy.

The response of tendon cells to changing loading conditions has significant implications in unraveling the aetio-pathogenesis of tendinopathy. While the knowledge base regarding the potential role(s) of tendon cell mechanobiology in tendon health, injury, and repair is continuing to expand (Wang 2006), additional research is required to determine how changes (mechanical, chemical and structural) in the in situ extracellular environment affect the mechanotransduction response(s) of tendon cells. In addition, we must determine how (or if) tendon cells can adapt to these changing loading conditions and/or changes in extracellular matrix composition.

Finally, we must assure that these in vitro investigations into tendon cell mechanobiology are clinically relevant so that the basic science data gleaned from these studies can be appropriately translated into the clinical situation. To do this, we must have a comprehensive understanding of what is happening to tendons (on both a structural and material level) during actual in vivo activities.

**Summary**

The role of the mechanobiological response of tendon cells in the aetio-pathogenesis of tendinopathy remains a point of controversy and debate. In this study, we have presented an argument for mechanobiological under-stimulation of tendon cells, secondary to microtrauma and isolated collagen fibril damage, as a predisposing factor for the pathological changes (collagen disruption, increased MMP levels and apoptosis) reported in clinical cases of tendinopathy. While our basic science data appears to support this hypothesis, additional translational research is needed to determine how, or even if, these proposed mechanobiological mechanisms are involved in the aetio-pathogenesis of clinical tendinopathy.

**References**


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