



REVIEW ARTICLE

Fatigue loading of tendon

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SUMMARY

Tendon injuries, often called tendinopathies, are debilitating and painful conditions, generally considered to develop as a result of tendon overuse. The aetiology of tendinopathy remains poorly understood, and whilst tendon biopsies have provided some information concerning tendon appearance in late-stage disease, there is still little information concerning the mechanical and cellular events associated with disease initiation and progression. Investigating this *in situ* is challenging, and numerous models have been developed to investigate how overuse may generate tendon fatigue damage and how this may relate to tendinopathy conditions. This article aims to review these models and our current understanding of tendon fatigue damage. We review the strengths and limitations of different methodologies for characterizing tendon fatigue, considering *in vitro* methods that adopt both viable and non-viable samples, as well as the range of different *in vivo* approaches. By comparing data across model systems, we review the current understanding of fatigue damage development. Additionally, we compare these findings with data from tendinopathic tissue biopsies to provide some insights into how these models may relate to the aetiology of tendinopathy. Fatigue-induced damage consistently highlights the same microstructural, biological and mechanical changes to the tendon across all model systems and also correlates well with the findings from tendinopathic biopsy tissue. The multiple testing routes support matrix damage as an important contributor to tendinopathic conditions, but cellular responses to fatigue appear complex and often contradictory.

Keywords

fatigue, mechanics, rupture, tendinopathy, tendon

Introduction

Tendons are unidirectional fibre-reinforced composites, responsible for the transmission of load from muscles to bones. Collagen constitutes the building block of the fibrous phase reinforcing a matrix of hydrated proteoglycan-rich gel at a number of hierarchical levels as demonstrated in Figure 1. The principal role of tendon is to resist tension, but it must also allow for a certain degree of compliance within musculoskeletal mechanics. These apparently conflicting demands are resolved as a direct result of the hierarchical structure of tendon and the contrasting nature of the stiff collagen fibres and the viscous, highly hydrated and proteoglycan-rich matrix surrounding them (Benjamin *et al.* 2008). Tendon is stronger per unit area than muscle and has a tensile strength approximately equal to that of bone,

although with additional flexibility and a degree of elasticity and extensibility (James *et al.* 2008).

Like other unidirectional fibre-reinforced composites, the parallel arrangement of collagen fibres acts to resist tension, so that contractile energy is not lost during load transmission (James *et al.* 2008). In most everyday activities, the loads observed in tendons are comparatively low and certainly well below the tissue's ultimate tensile strength. However, in quick eccentric movements, movements where a limb must be rapidly decelerated, more significant stresses can be observed (Schechtman & Bader 1997). Stresses of between 42 and 110 MPa have been measured *in vivo* in the Achilles tendon, this maximum exceeding the often reported failure strength of the tendon (Komi *et al.* 1992; Schechtman & Bader 1997). As a result of these high *in vivo* loads, tendons such as the Achilles and patellar are

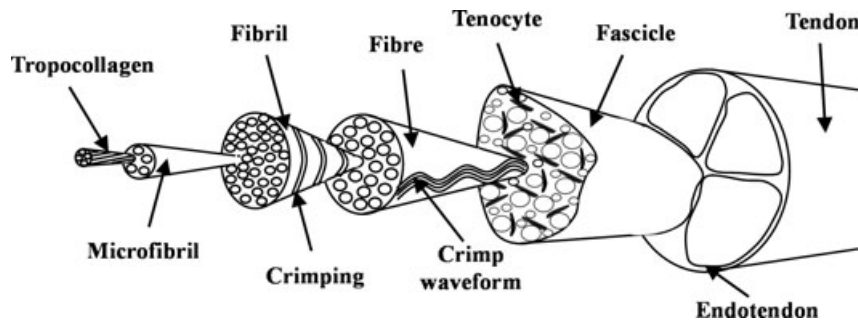


Figure 1 The hierarchical structure of tendon. Adapted from Screen *et al.* (2004a).

particularly prone to overuse injuries, often referred to as tendinopathies, an effect probably worsened by material and structural inhomogeneity as well as the poor healing response of the tissue (Fung *et al.* 2010).

Indeed, tendon injuries such as tendinopathy and tendon rupture are prevalent, debilitating and often painful clinical problems. Each year in the United States, there are 16.4 million tendon and ligament injuries, of which at least 100,000 involve the Achilles tendon (Wang *et al.* 2012), and as a greater proportion of the general population become involved in physical and recreational activities, the frequency of soft tissue injury is only likely to increase. However, characterizing tendon injuries and establishing how and why damage may occur can be complex.

Tendon cannot simply be considered a fibre composite, it is a living tissue, with a structure maintained by tenocytes, the resident cell population. As such, mechanical loading can create not only structural damage, but also a cell response to remodel the matrix (Wang 2006; Franchi *et al.* 2007; Kjær *et al.* 2009). An intimate interplay exists between mechanical signalling and biochemical changes within the tendon extracellular matrix, resulting in adaptations in tendon morphology, structure and material properties with use (Kjær *et al.* 2009).

The interplay between mechanically induced and cell-induced matrix changes is poorly understood and has led to considerable debate concerning the aetiology of tendon injuries. Data indicate that tendinopathy often occurs in the absence of any single traumatic event, implicating a gradual accumulation of micro-injuries (Wang 2006). This has led to the hypothesis that damage to tendon probably occurs every day as a result of all manner of normal activities and that damage accumulates only when equilibrium cannot be maintained between the rate of damage and the cellular-driven rate of repair (Archambault 2003). However, there is also considerable interest in the tenocytes and how they may contribute to, or perhaps even drive, the breakdown of tissue to tendinopathic states (Riley 2005).

To understand these processes further, a range of model systems have been developed to simulate tendon overuse, characterize the development of fatigue damage and investigate how this may relate to the aetiology of tendinopathy.

Monitoring fatigue *in situ* poses many challenges; hence, a number of *in vitro* and animal models have been developed to assist our understanding. This review outlines some of these methods and summarizes the current understanding of fatigue damage in tendon and the progression towards tendinopathy or rupture.

Fatigue loading

Fatigue can be considered as progressive and localized structural damage to a material as it is subjected to cyclic loading. Just as a turbine blade may suffer progressive damage during its many cycles of use, the repeated loading of tendons during normal use may well also generate microstructural damage. Fatigue testing of tendon can thus provide a means of investigating damage accumulation. There are two principal modes of fatigue testing: creep analysis, where samples are cycled to a constant peak load and increase in extension monitored (Figure 2a), and stress relaxation analysis, where loading is carried out to a constant peak displacement and the reduction in load considered (Figure 2b). With different boundary conditions, the two testing routes would be expected to elicit a different response from the loaded tendon, but are often used interchangeably. In ligaments (where the function is to connect bone to bone), it has been argued that the in-life loading is a simple creep situation, with the same loads repeatedly applied by the joint (Thornton *et al.* 1997). Tendons transfer the muscle force to the bone to facilitate locomotion, and as a result, load conditions may be more complex (Screen 2008).

Creep behaviour can be described by three stages of deformation, demonstrated in Figure 2(a). An initial primary stage associated with rapid extension is followed by a relatively stable secondary stage in which there is a steady increase in sample length, followed by a tertiary stage, as the sample rapidly extends to rupture. By contrast, stress relaxation curves tend to follow an exponential curve (Figure 2b), with stress steadily stabilizing after an initial rapid decrease. Whilst stress relaxation tests will not terminate in the failure of the sample, fatigue damage is generated; this has been confirmed by stopping tests and loading samples to failure, where significant decreases in quasi-static mechanical characteristics are

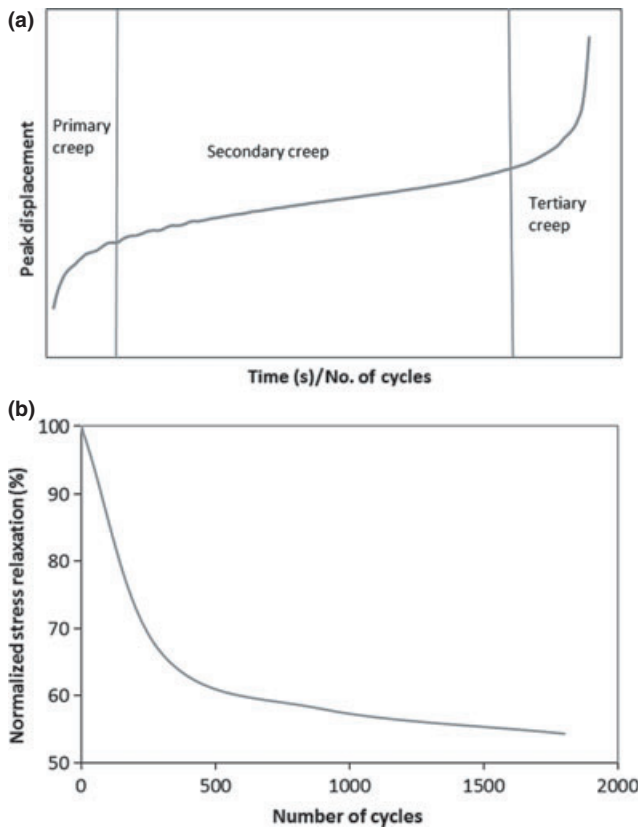


Figure 2 (a) Creep curve, labelled with the three stages of creep: an initial primary stage associated with rapid extension, the stable secondary stage where sample length increases steadily and tertiary creep as the sample rapidly extends to rupture; (b) typical exponential stress relaxation curve.

reported (Legerlotz *et al.* 2011). Typically, fatigue testing of tendon samples has considered creep loading so as to allow for progressive recruitment and stretching out of the collagen fibres and ultimately investigation of tendon rupture. However, there is also a need for investigating the damage generated through stress relaxation, particularly in the light of the frequent use of this modality when investigating cell response to loading and mechanotransduction (Li *et al.* 2004; Wang *et al.* 2004; Wall *et al.* 2007; Qi *et al.* 2011).

Methods for the fatigue testing of tendon

Fatigue testing of tendon can be broadly separated into: *in vitro* testing of non-viable samples; *ex vivo* testing of viable tendon samples, and *in vivo* models, which in turn can be subdivided into overuse models and controlled *in vivo* loading. All of these models have strengths and limitations and vary in terms of their applicability for characterizing the mechanical or cellular aspects of overuse. As such, to provide a complete picture concerning the progression of fatigue damage and the possible mechanisms of tendinopathy, the evidence collected from all of these various methods of fatigue testing of tendon should be considered.

In vitro characterization of tendon fatigue properties

Whilst the quasi-static properties of tendons at various hierarchical levels have been investigated for many years (Rigby *et al.* 1959; Abrahams 1967; Lanir 1978), the first publications to describe fatigue analyses of tendon were not until the mid-1990s. In 1994, Wang *et al.* carried out cyclic creep tests on whole tendon samples and observed a series of linear relationships between the logarithm of fatigue life and the applied peak tensile stress across a wide range of loading frequencies (Wang *et al.* 1995). Stiffness was observed to reduce throughout the test, gradually at first, before accelerating rapidly immediately prior to failure, providing a measure of the accumulation of fatigue damage as previously observed in statically creep-loaded tendons (Wang & Ker 1995).

Other early studies of tendon fatigue considered the human extensor digitorum longus (Schechtman & Bader 1997), the tendons of the wallaby hind leg (Ker *et al.* 2000), the human Achilles tendon (Wren *et al.* 2003) and a comparison of the highly stressed plantaris and much less significantly loaded extensor digitorum lateralis of the sheep (Pike *et al.* 2000). An exponential decrease in cycles to failure with increasing applied peak stress was observed across all of these tendon types. Additionally, Schechtman and Bader (1997) compared the failure strain of tendon samples fatigue loaded to a variety of peak stresses, with their quasi-static failure strain, and found no significant variation, suggesting a failure mechanism determined by a limiting value of strain; in the case of the human extensor, approximately 15%. Wren *et al.* (2003) investigated the effects of static creep and cyclic creep loading on the mechanical properties of human Achilles tendons and in both instances found the initial strain to be the best predictor of time or cycles to failure. This again supports the hypothesis that strain is the primary mechanical parameter governing tendon damage accumulation and injury.

During cyclic creep tests to a consistent peak stress, it was observed that tendons subjected to low stresses in life failed at shorter times than those subjected to high stresses in life. However, when tests were normalized to take into account the expected stress in life, comparable fatigue lives were observed in all samples, pointing towards structural and mechanical specialization of tendons to suit their *in situ* load environment (Ker *et al.* 2000). In recent years, the specialization of tendons for specific functions has become more recognized. For example, the energy-storing bovine digital flexor tendon has been observed to exhibit significantly improved resistance to fatigue compared with the largely positional bovine digital extensor (Shepherd *et al.* 2012). There remains, however a need to more fully correlate response with tendon function and establish if susceptibility to fatigue damage is related to tendon injury risk.

Fatigue testing of *in vitro* tendon samples is complex. Because of the independent nature of the collagen fibres

within tendon, effective clamping has proved problematic, as has maintaining sample hydration during these long tests (Schechtman & Bader 1997; Ker *et al.* 2000). Methods that grip only the outer fibres, for example, gluing, can leave the inner fibres unloaded, and as such, the material will appear less strong and less stiff than it actually is (Ker *et al.* 2000). The transverse stiffness of tendon is low, so direct clamping between metal plates is not ideal. It can result in serious distortion, non-uniform loading, stress concentrations and ultimately premature failure (Ker 2007). Many tests now adopt hydrated samples with air-dried ends, particularly during longer-term tests.

However, even with appropriate test methods, *in vitro* tests have, without fail, provided values of fatigue life vastly unrealistic for the necessary lifetime of tendons *in situ*. Schechtman and Bader (1997), analysing the human extensor digitorum longus, estimated that *in vitro* data predicted *in vivo* failure after about 4 months of normal walking activity. They subsequently developed a cumulative damage model to try and take into account tendon healing, suggesting a healing rate of 1% per day would be sufficient to eliminate the damage induced in the tendon during normal locomotion (Schechtman & Bader 1997). Biochemical studies, however, imply a much slower rate of turnover in tendon (Birch *et al.* 2008), suggesting perhaps that such a model is an oversimplification.

To try and investigate this further, more recent studies have looked to correlate changes in tendon mechanics with morphological changes during fatigue loading, aiming to determine the sequential, microstructural events associated with the build-up of fatigue damage (Fung *et al.* 2009). Although tendon only shows significant changes in stiffness or hysteresis with high levels of fatigue, changes in the microstructure appear to occur rapidly. Under light microscopy, non-loaded control tendons exhibit highly aligned and parallel collagen fibres with their characteristic crimp and tenocytes in a columnar fashion between the fibres. Fatigue-loaded tendons displayed isolated regions of damage within an otherwise normal collagenous architecture. Even in minimally fatigued samples, showing no mechanical changes, fibres appeared kinked (ridge-like formations were observed) and the tenocyte arrangement distorted (Fung *et al.* 2009). At moderate levels of fatigue, where mechanical changes first become apparent, the number of local patterns of fibre disruption increases and discontinuities and a few instances of isolated fibre tears are observed. In highly fatigued samples, dissociation is observed among the fibres, along with transversely oriented fibre discontinuities and isolated fibre ruptures (Fung *et al.* 2009). We have recently observed a similar damage progression during the fatigue loading of bovine extensor tendon fascicles, as shown in Figure 3 (Shepherd *et al.* 2012).

Ex vivo testing – maintaining viable tendon samples

Whilst some of the studies described in the previous section utilized tendon samples very soon after sacrifice of the ani-

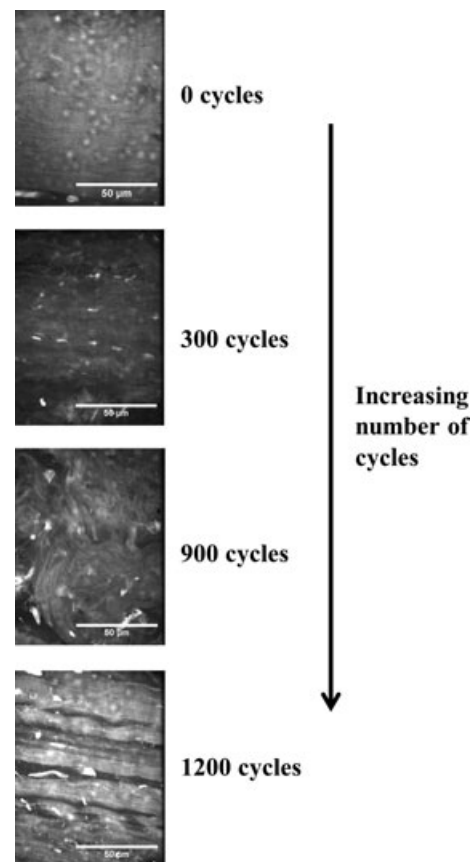


Figure 3 Progression of fatigue damage in bovine extensor tendon fascicles with increasing number of cycles of creep loading (25% of the ultimate tensile stress). Confocal images of fascicles stained with 5 mM acridine orange solution. Kinked fibres were observed after just 300 cycles with much more severe damage including widening of the interfibre space and fibre breakages at 900 and 1200 cycles.

mal, attempts were not made to maintain sample viability during testing; thus, no conclusions involving the cellular contributions to overuse changes could be drawn. By contrast, *ex vivo* studies, defined for the purpose of this review as studies carried out on viable tendon tissue, have intentionally maintained sample viability, with the aim of correlating mechanical fatigue with its biological effects. The literature concerning the effect of loading or displacement on viable tendon samples is significant, but much of it considers the application of static load; only tests directly considering fatigue loading will be mentioned here. In addition, the considerable literature concerning isolated cells and the mechanotransduction response to cyclic loading is also not discussed.

Structural comparisons between fatigue-loaded viable and non-viable samples indicate the same progression of microstructural fatigue damage (Parent *et al.* 2011). Perhaps this is not surprising, as the time frame of experiments and lack of systemic *in vivo* conditions mean that repair responses are unlikely to be representative. Nevertheless, the effect of

loading conditions on immediate cellular metabolism and early changes to matrix turnover can be investigated. Indeed, a study into the effect of stress deprivation and cyclic tensile loading on the mechanical and histological properties of the canine flexor digitorum profundus confirmed that cells can mediate tissue integrity and subsequently tissue mechanics in *ex vivo* settings. Differences between stress-deprived and stress-loaded samples were only observed in the presence of viable cells (Hannafin *et al.* 1995).

Banes *et al.* (1999a,b) carried out some of the early *ex vivo* work on viable tendon samples, concentrating particularly on cellular mechanotransduction responses. The cyclic creep loading of whole avian tendon samples was found to stimulate DNA and collagen synthesis, in a response highly dependent upon gap junctions and the interconnectivity and signalling of the cells.

Considerable controversy exists as to the possible role of systemic inflammatory reactions in overuse tendinopathy, and as such, the initiation of inflammatory pathways has been investigated in *ex vivo* fatigue studies. In one cyclic creep study, aggressively loaded samples were found to exhibit significantly reduced mechanical strengths as well as higher concentrations of the inflammatory mediator prostaglandin E₂ (PGE₂). It was suggested that the release of PGE₂ could contribute to the pathology of overuse tendinopathies by stimulating matrix metalloproteinase expression (Flick *et al.* 2006). Devkota *et al.* (2007) also hypothesized that PGE₂ was a key contributor to the induction of fatigue damage, showing that tendons undergoing loading over long periods of time became significantly weaker than control groups and that cellular turnover and collagenase production also increased. However, in contrast to these findings, a cyclic stress relaxation study found MMP-1 production to be inhibited for low-strain, low-frequency cyclic loading and eliminated entirely at high strains and high frequencies (Lavagnino *et al.* 2003).

Further controversy exists concerning the behaviour of the cells themselves in response to fatigue. In a light microscopy study of high-strain cyclic stress relaxation loading, loaded tendon samples were found to be associated with the presence of numerous apoptotic cells, whilst control tendons (unloaded) showed very few apoptotic cells (Scott *et al.* 2005). Another study, however, found no change in cell viability between unloaded and aggressively loaded avian tendon explants (Flick *et al.* 2006). These examples highlight the variability in cellular response and matrix metabolism during fatigue loading of viable tendon and the considerable challenges that exist in drawing firm conclusions. There is undoubtedly a need to correlate cellular response with tendon function and to consider more fully the significance, if any, of the choice of cyclic creep or stress relaxation testing.

Whilst the majority of the testing of viable tendon has considered the bulk tendon level, fascicular behaviour has also been investigated. The considerable issues associated with the gripping of whole tendon samples can be overcome by fascicle testing, and cross sections are generally much more consistent along the length than observed in the bulk

material. Furthermore, considering fatigue properties at smaller hierarchical levels than the bulk tendon can provide additional information about a structure, although careful validation is required and care must be taken not to assume that bulk tendon properties are defined at the fascicular level. Indeed, when considering quasi-static properties, Thorpe *et al.* (2011) presented work that showed the inter-fascicular matrix to have a significant effect on bulk tendon mechanics.

Mechanical stimulation of tendon fascicles has been shown to upregulate collagen production and enhance its retention within the matrix, as well as upregulating IL-6 production in a possible adaptive response to exercise (Screen *et al.* 2005; Maeda *et al.* 2007; Legerlotz *et al.* 2011). In an investigation into the mechanics of cyclically stress-relaxed fascicles, it was found that 5 h of cyclic loading at 30% failure strain was required to see a statistically significant decrease in the quasi-static ultimate tensile strength, yet when the strain was increased to 60% of the strain to failure, a statistically significant decrease was observed in just 15 min (Legerlotz *et al.* 2011). In the same study, it was observed that changes in cell metabolism in response to loading were observed well before any changes in the fascicle mechanics (Legerlotz *et al.* 2011).

The use of fascicles in the characterization of fatigue behaviour may also allow for more straightforward imaging of the effect of fatigue on cellular morphology and processes in combination with an understanding of matrix structure changes (Figure 4). Furthermore, postfatigue microstraining experiments, using the methods developed by Screen *et al.* (2004b), could be considered to investigate the effect of fatigue damage on mechanics at the fibre level.

In vivo studies

Overuse animal models

The *in vitro* testing methods discussed thus far can provide very controlled loading conditions, but cannot mirror the complexity of the native tissue environment. *In vivo*, so-called animal overuse models, overcome this by enabling consideration of cellular responses within the native tissue environment. However, the degree of reproducibility of loading can be harder to control.

Animal models of tendinopathy can be divided into two – chemically induced models and mechanically induced ones. Chemically induced models include the injection of collagenase to induce collagen breakdown (Foland *et al.* 1992; Soslowsky *et al.* 1996), cytokines to induce an inflammatory response (Stone *et al.* 1999) or the injection of prostaglandins (Sullo *et al.* 2001; Khan *et al.* 2005) or fluoroquinolone (Kato *et al.* 1995; Simonin *et al.* 2000) both believed to have an involvement in tendinopathy. These models allow a study of the interplay between inflammatory cells, mechanical loading and tissue healing (Lake *et al.* 2008) and are typically less labour intensive than mechanical means and generally provide more consistent tendon damage (Dirks & Warden

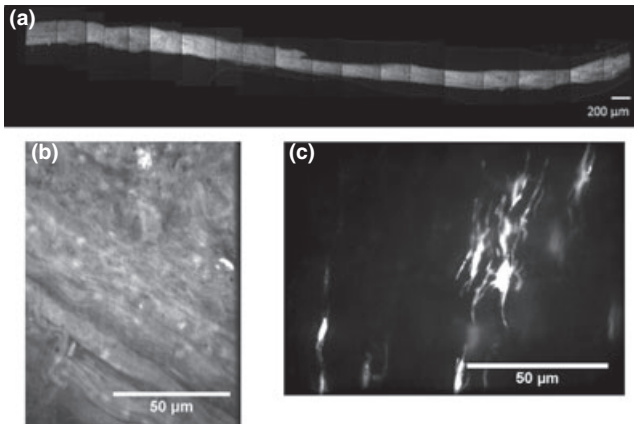


Figure 4 Characterization of fatigue damage in the tendon fascicle (bovine extensor tendon fascicle after 300 cycles of creep loading) using a combination of high-concentration acridine orange staining for collagen structure and calcein AM for cellular morphology: (a) low-magnification composite image of the fascicle length, (b) matrix structure after 300 cycles of creep loading and (c) calcein AM staining of tenocytes within the tendon fascicle after fatigue loading.

2011); however, they cannot be considered a route for fatigue testing of tendon and thus will not be considered further.

Mechanically induced models should be beneficial because they are designed to induce injury through repetitive loading, similar to how tendinopathy is believed to develop in the human condition. However, the process is typically sped up in the *in vivo* model, with a more rigorous loading protocol, and it should not be assumed that damage accumulation will be entirely consistent with chronic tendinopathy. Mechanical means of generating fatigue damage include electrical muscle stimulation, where the muscle is stimulated so as to produce contraction and loading of the tendon, repetitive stretching and grabbing activities, and treadmill running, which is typically either downhill or uphill, to ensure eccentric muscle contraction and the exertion of greater forces (Dirks & Warden 2011). Small animals are generally used for these mechanical models and with no clear evidence of tendinopathy in these animals during life, and it is difficult to establish how the aetiology of overuse may relate between species or how the pathophysiology may vary.

In 1999, Soslowsky *et al.* (2000) used a treadmill running model to investigate the histological and biomechanical effects of overuse activity on the supraspinatus of the rat. Tendons in the exercised animals exhibited larger diameters at all time points and significantly decreased modulus and ultimate tensile stress (UTS), in addition to increased cellularity and a reduction in the organization of the collagen structure. In a similar, more recent model, hypervascularity, glycosaminoglycan accumulation and collagen fragmentation have also been observed in response to fatigue (Scott *et al.* 2007). Scott *et al.* (2007) observed that in a downhill running rat model, early tendinosis was associated with local stimulation of tenocytes and not extrinsic inflammation or

apoptosis. Conversely, Millar *et al.* (2009) reported fatigue-induced expression of cytokines – IL-18, IL-15 and IL-64, increased expression of mediators of apoptosis and increased levels of heat shock proteins. They also reported increased expression of cartilage-associated genes, but reduced levels of typical tendon genes, with overuse (Millar *et al.* 2009).

When a similar downhill treadmill running model was used in order to induce injury in the rat Achilles, no significant variations were observed between control and exercise groups in either biomechanics or geometrics (Huang *et al.* 2004). The absence of damage was hypothesized to result from either the larger size of the Achilles compared with the supraspinatus or because Achilles damage only results from acute injury in rats (Huang *et al.* 2004). Another study that considered uphill running in rats found running and non-running animals to exhibit grossly similar Achilles tendon with cross-sectional areas that did not exhibit significant variation (Glazebrook *et al.* 2008). However, histology showed the tendons of the running rats to be significantly more disorganized with a higher cellular density (Glazebrook *et al.* 2008).

Voluntary forelimb repetitive reaching and grasping tasks have also been used to create overuse in the rat (Barbe *et al.* 2003). After 5–6 weeks, the repeated task was observed to result in significantly decreased motor performance and widespread tissue responses, with rats unable to maintain their baseline reach rate. Furthermore, at week 6, fraying of tendon fibrils was observed in the midforelimb. Electrical stimulation studies also report degenerative changes in the tendon at the 5–6-week time frame, including the presence of inflammatory cells, increased numbers of capillaries, oedema and fibrosis in the paratenon (Backman *et al.* 1990). Whilst not all overuse *in vivo* models report increased vascularity or inflammatory cells, microtears do appear to be consistently evident (Nakama *et al.* 2005).

An additional finding of electrical stimulation models highlights the complex interplay between mechanical and cellular response to overuse. It is commonplace in these models to use the contralateral limb as the control. However, Asundi *et al.* (2008) investigated the mRNA levels of many tendinopathy-associated proteins in both loaded and contralateral limbs and found no differences. In gene expression analysis, changes in contralateral limbs have also been observed during electrical stimulation, possibly as a result of central neuronal mechanisms (Andersson *et al.* 2011). Not only do these studies highlight that the contralateral limb should perhaps not be considered a suitable control but they also point to a clear cellular contribution to degenerative matrix changes with tendinopathy.

Controlled in vivo loading

Whilst overuse models overcome some of the limitations of *ex vivo* testing, they may not necessarily ensure measurable and standardized loading across the tendons due to natural variations in gait, anatomical size, strength or temperament (Fung *et al.* 2010; Andarawis-Puri & Flatow 2011). To

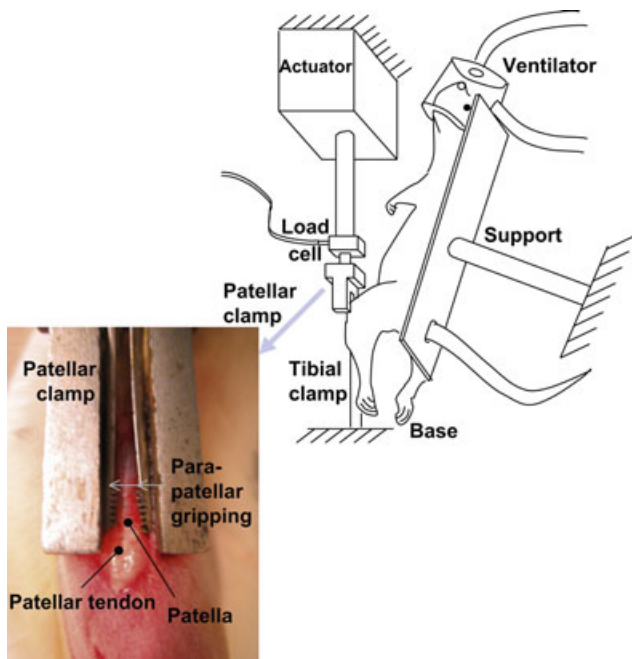


Figure 5 Experimental set-up of controlled *in vivo* loading in a rat model. (Reprinted from Fung *et al.* 2010, with permission from Elsevier).

address this limitation, novel, controlled, *in vivo* models have also been developed, for applying specified loading conditions (albeit acutely) to tendons in small animal models, initially in the rat (Fung *et al.* 2010). The limb of the anaesthetized animal to be fatigued is secured to an adjustable testing frame (Figure 5), and the required tendon fatigue loading protocol applied directly to the *in situ* tendon in order to explore the mechanisms of tendon degeneration. Following completion of the loading protocol, animals are allowed to resume normal cage activity, prior to euthanasia and tendon characterization at a later time point.

The biomechanics and microstructural progression of fatigue damage can be characterized using mechanical testing and second harmonic generation imaging (SHG) (Fung *et al.* 2010; Andarawis-Puri & Flatow 2011; Andarawis-Puri *et al.* 2012; Neviasser *et al.* 2012). However, gene expression changes (Fung *et al.* 2010) and the presence of MMPs and inflammatory cytokines can also be more appropriately assessed (Sun *et al.* 2008). Whilst there is concern that small animal models do not develop tendinopathy in the same manner as humans and large mammals, the breadth of these investigations has nevertheless facilitated a wider understanding of how fatigue damage may progress under controlled conditions *in vivo*.

The advent of SHG imaging has additionally provided a unique insight into collagen fibril architecture in minimally processed tendon allowing a clear description of the progression of fatigue damage (Figure 6) as summarized in the paper by Neviasser *et al.* (2012). By correlating this with mechanical data (Fung *et al.* 2010), a more complete picture

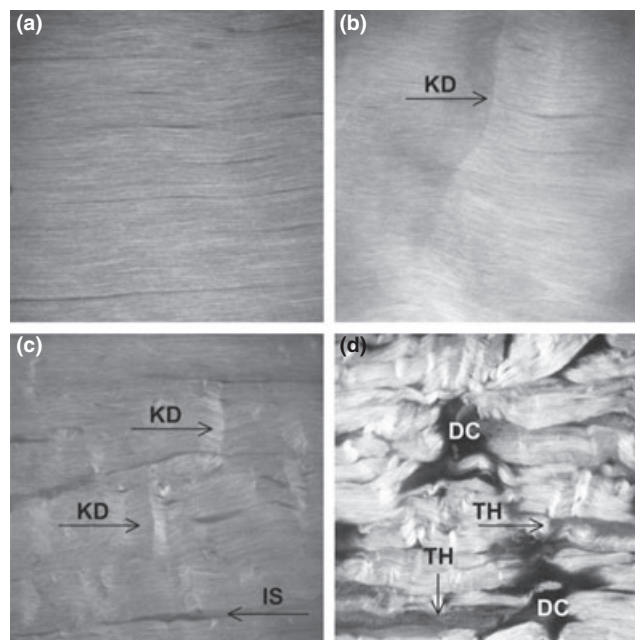


Figure 6 Second harmonic generation imaging of the progression of fatigue damage during controlled *in vivo* loading. (A) Control tendons without fatigue, (B) Low fatigue demonstrating isolated *kinked* fibre deformations (KD), (C) Moderate fatigue showing increased kinking and widening of the interfibre space (IS), (D) High fatigue showing severe matrix disruption, poor fibre alignment, and greater widening of interface space. Regions of low signal intensity suggest fibre thinning (TH) and more severely, matrix discontinuities (DC). Field of view = 400 microns (Reprinted from Fung *et al.* (2010), with permission from Elsevier).

of fatigue accumulation can be developed (Table 1). It is notable that the progression of structural and mechanical changes with fatigue damage appears to be broadly consistent across all of the reviewed methods of fatigue damage generation.

The particular advantage of controlled *in vivo* testing lies in the assessment of systemically controlled molecular changes at all stages of fatigue damage generation (Andarawis-Puri & Flatow 2011). In one study, the temporal profiling of gene expression with loading indicated that the molecular profile after 100 cycles (representative of exercise) is anabolic, whilst after 7200 cycles (moderate-level fatigue loading), this had become catabolic (Sun *et al.* 2010). Studies have also shown that significant variation in the expression of collagen 1 and 3 as well as interleukin-1 β and MMP-13 is only observed in moderate and high levels of fatigue (Andarawis-Puri & Flatow 2011). This suggests a threshold of loading that once overcome changes the tendon response from beneficial to degenerative. It may be that a certain amount of accumulated damage is necessary to induce molecular inflammation, and once induced, this may play a role in altering the response of the tendon to further loading (Andarawis-Puri & Flatow 2011).

Table 1 The progression of fatigue damage during controlled *in vivo* loading; the effect on collagen fibril architecture and mechanics. Data collated from Neviaser *et al.* (2012) to Fung *et al.* (2010)

Level of fatigue	Collagen fibril architecture	Mechanics
No fatigue	Aligned collagen fibrils, exhibiting typical collagen crimp	
Low-level fatigue	Kinked fibre deformations	Increased stiffness and decreased hysteresis
Moderate fatigue	Kinked fibre deformation with widening of the interfibre space	Mechanics did not differ from low-level fatigue samples
High-level fatigue	Severe matrix disruption, fibre thinning, angulations and fibre discontinuities	Reduction in stiffness and increase in energy dissipation alongside steep increase in tendon strain

Controlled *in vivo* loading rigs have more recently been developed to investigate fatigue damage generation in mice, mainly to take advantage of the abundance of well-characterized in-bred genetic strains of mice and the established technologies for knocking out their specific genes (Sereysky *et al.* 2012). Collagen architecture and damage accumulation have been shown to be similar to that of the rat, with damage initially leading to changes in structure (increase in damage area fraction), which subsequently result in change in function. Indeed, one outcome of these studies is to concur with the earlier *in vitro* studies, suggesting that damage area fraction should be considered an early indicator of fatigue damage (Sereysky *et al.* 2012).

However, mouse and rat models may not be ideal for characterizing tendinopathy, and there remains an incomplete understanding of the behaviour and physiology of these animals. As previously outlined, there is no evidence of the development of tendinopathy in small rodents during normal life and it is suggested that they may also use different mechanisms for balancing, locomotion and pain perception compared with humans, and as such, fatigue response could also differ (Lui *et al.* 2011). Furthermore, with ethical limitations to the amount of time for which animals can be anaesthetized, damage can only be generated acutely. Tendinopathy is not believed to be an acute condition, but instead occurs over months or possibly years. Nevertheless, *in vivo* models such as this can provide valuable insights into the cumulative damage process as the amount of induced fatigue damage can be carefully controlled.

Tendinopathy – correlating tissue biopsies with fatigue-damaged samples

Although tissue biopsies can provide information on molecular changes, microstructural variation and mechanics in late-stage tendinopathy, they do not tell us about the progression of the disease. However, comparison of biopsy data with that achieved from the fatigue loading of tendon is critical in order to ascertain the value of various fatigue loading protocols.

Generally, biopsies from tendinopathic tissue have demonstrated an abnormal fibril and fibre morphology compared

with normal controls (Józsa & Kannus 1997; Movin *et al.* 1997; Tallon *et al.* 2001; Kongsgaard *et al.* 2010). For example, Movin *et al.* (1997) considered biopsy specimens from patients with long-standing achillodynia and found that slight and moderate cases exhibited a separation of the fibres with increased waviness, whilst extreme cases showed a total loss of fine fibre structure and hyalinization. However, whilst Järvinen *et al.* found spontaneously ruptured tendons to display focal regions with decreased collagen fibre thickness, decreased crimp angle and disrupted crimp continuity (Järvinen *et al.* 2004), Magnusson *et al.* (2002) did not observe any change in crimp morphology although a decrease in the number of larger diameter fibrils in the core of ruptured tendons was reported. In agreement with observations of damage within fatigue-loaded samples, Józsa and Kannus (1997) reported only patches where fragmentation and fraying of collagen fibres were seen, along with loss of the normal wavy alignment.

Not only are the microstructural changes in tissue biopsies generally consistent with those in fatigue models, but there is also evidence they may precede cellular, molecular and mechanical changes. Kongsgaard *et al.* (2010) observed that whilst fibril morphology was abnormal in tendinopathic biopsy specimens, *in vivo* tendon mechanical properties did not appear altered. In another study, only once, cases of tendinopathy had developed to a moderate stage were decreases in tenocyte number observed, and as pathologic changes progressed (largely in spontaneously ruptured specimens), the nuclei became progressively more rounded (Tallon *et al.* 2001). An increase in the glycosaminoglycan and non-collagenous extracellular matrix has also been observed in late-stage tendinopathy (Movin *et al.* 1997).

Another key area of interest is the role of inflammation in tendinopathy and rupture. Just as in many of the fatigue models discussed, an absence of inflammatory cells is most commonly reported in tissue biopsies from both tendinopathic and ruptured tendon (Jozsa *et al.* 1990; Kannus & Jozsa 1991; Fredberg & Stengaard-Pedersen 2008). However, in a study from 2003, immunohistochemical staining identified acute inflammation in all of 60 ruptured tendons (Cetti *et al.* 2003). Biochemically, the presence of inflammatory mediators such as COX-2 and PGE-2 is reported in some studies (Fu *et al.* 2002; Fredberg & Stengaard-Peder-

sen 2008), but others found no upregulation of mRNA for the investigated cytokines and cytokine receptors in chronic Achilles tendinopathy, suggesting no chemical inflammation (Alfredson *et al.* 2003). It is important to note that tendinopathy and spontaneous tendon rupture may have different aetiologies, but there is little clear evidence of how they may differ or if inflammation may be related to just a subset of tendon conditions.

Conclusion

Whilst tissue biopsies may provide some information concerning late-stage tendinopathy *in situ*, there is still little information about the early-stage disease. This means that despite the prevalence of tendinopathy, its clinical management is limited. The correlation between the data from fatigue models and the microstructural, biological and mechanical changes observed in tendinopathic tissue biopsies does suggest that tendinopathy is associated with the fatigue damage of tendon. Nevertheless, data from the contralateral limbs in *in vivo* models highlight the importance of the cells, and whilst interactions between cell response and structurally induced damage remain unclear, they are undoubtedly critical.

There is unfortunately no one method that can provide a complete understanding of the development of tendinopathy. Testing of non-viable bulk tendon samples is comparatively straightforward and provides the clearest information concerning the mechanics of fatigue damage, for example, the importance of strain as the primary mechanical parameter governing tendon damage accumulation and injury. It also provides evidence concerning the nature of failure, but it cannot take into account *in vivo* processes of healing and remodelling or provide any information concerning cellular response to fatigue damage. *Ex vivo* testing of viable tendon samples provides information concerning the initial cellular and matrix response to loading but still cannot take into account *in vivo* healing, and considerable complications exist in maintaining tissue viability. The *ex vivo* testing of fascicles may provide the opportunity of understanding the effect of fatigue on tendon's various hierarchical levels but care should be taken not to assume bulk tendon properties are defined at the fascicular level. Overuse *in vivo* models enable the consideration of cellular responses within the native tissue environment and can allow consideration of mechanics and tissue degradation after a considerable fatigue period, but the degree of reproducibility of loading and thus its effects can be harder to control. *In vivo* fatigue testing allows the application of very controlled levels of fatigue to natural tissue, but with ethical consideration regarding animals under anaesthesia, one can only ever use small animals and generate acute damage.

Multiple testing routes support the hypothesis that matrix damage is the primary indicator of fatigue damage and that this is observed before any decrease in mechanical properties. The nature and progression of fatigue damage show real similarity between the various fatigue testing routes,

both with and without the presence of viable cells, and also correlate strongly with biopsy samples. Observed microstructural changes supported the changes in mechanical properties, whilst cellular responses to fatigue, including the expression of inflammatory mediators, were more complex and contradictions existed between the various studies. It appears likely, however, that there is a threshold of loading that once overcome, changes the response of loading of the tendon from beneficial to degenerative. The role of inflammation in tendinopathy still remains controversial but it appears likely that inflammatory factors will only ever occur with quite a significant degree of matrix damage. Whilst close similarities exist between matrix damage in fatigue loading and in tendinopathic specimens, the tendinopathic situation should not be oversimplified. The exact *in vivo* loading conditions of human tendon remain unknown and the healing of some cases of tendinopathy poorly investigated. Care must also be taken with the assumption that tendinopathy and spontaneous tendon rupture have the same aetiology and follow the same progression of micro-damage accumulation. Further research is essential to better understand the loading environment *in situ* so as to improve further the models of fatigue damage generation.

Conflict of interest

The authors have no conflict of interest.

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