The pathogenesis of tendinopathy: balancing the response to loading

S. Peter Magnusson, Henning Langberg and Michael Kjaer

Abstract | Tendons are designed to withstand considerable loads. Mechanical loading of tendon tissue results in upregulation of collagen expression and increased synthesis of collagen protein, the extent of which is probably regulated by the strain experienced by the resident fibroblasts (tenocytes). This increase in collagen formation peaks around 24 h after exercise and remains elevated for about 3 days. The degradation of collagen proteins also rises after exercise, but seems to peak earlier than the synthesis. Despite the ability of tendons to adapt to loading, repetitive use often results in injuries, such as tendinopathy, which is characterized by pain during activity, localized tenderness upon palpation, swelling and impaired performance. Tendon histological changes include reduced numbers and rounding of fibroblasts, increased content of proteoglycans, glycosaminoglycans and water, hypervascularization and disorganized collagen fibrils. At the molecular level, the levels of messenger RNA for type I and III collagens, proteoglycans, angiogenic factors, stress and regenerative proteins and proteolytic enzymes are increased. Tendon microrupture and material fatigue have been suggested as possible injury mechanisms, thus implying that one or more 'weak links' are present in the structure. Understanding how tendon tissue adapts to mechanical loading will help to unravel the pathogenesis of tendinopathy.

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Introduction

Tendon tissue has an essential role in transmitting contractile forces to bone to generate movement, and is therefore uniquely designed to withstand considerable loads (up to ~8 times body weight) during human locomotion.[1–3] However, repetitive loading often results in overuse injuries, such as tendinopathy, which is a common clinical condition characterized by pain during activity, localized tenderness upon palpation, swelling of the tendon and impaired performance.[4,5] Tendinopathy is a problem in both elite and recreational athletes, as well as in the workplace.[6–8] In some elite athletes, the prevalence can be as high as 45%,[6,9–11] and the symptoms, as well as any reduction in performance, might be long lasting (many years, in some cases).[12] The injury mechanism is currently poorly understood. Human tendons have traditionally been considered largely inert structures, but are now known to be metabolically active in their response to mechanical loading.[13,14] Understanding how tendon tissue adapts to mechanical loading is key to understanding the pathogenesis of tendinopathy, and will thus provide the basis for preventing these overuse injuries. In this Review, we discuss current knowledge of how the various components of the human tendon respond to acute and chronic loading.

Force and the tendon

The average tensile stress (which relates to the force transmitted and the area over which it is transmitted) exerted on a tendon will depend on its cross-sectional area. Human tendons, including the commonly afflicted patellar and Achilles tendons, typically have a fracture stress of ~100 MPa. However, most tendons are only subjected to stresses of up to 30 MPa,[15] which gives tendons a reasonable safety margin, although the Achilles tendon might experience stresses of up to ~70 MPa.[1,16] Tendon microrupture, which is presumably associated with a lack of load in a local area along with its associated fibroblasts, has been suggested as a possible injury mechanism for tendinopathy.[6,17] It has also been suggested that fatigue, defined as the time-dependent damage that occurs in response to cyclic loading, might be an injury mechanism in tendon.[18] The precise mechanism of injury that leads to tendinopathy remains unknown, but the proposed mechanisms imply that there are one or more 'weak links' in the tendon structure that result in the pathological response of the fibroblast.

Structure of tendon tissue

The organization of tendon follows a strict hierarchical pattern (Figure 1).[19] Collagen molecules are organized precisely to give rise to the characteristic 67 nm D-periodization that forms fibrils. The collagen molecule is ~300 nm in length and 1.5 nm in diameter,[20] and aggregated molecules of the fibril are stabilized by covalent intermolecular crosslinks.[21,22] The crosslinks bind the collagen molecules to one another and thereby confer integrity on the fibril. Groups of fibrils then form fibers known as fascicle bundles, which finally comprise the tendon proper. There are at least 28 different collagen

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proteins, but tendon is predominantly made up of type I collagen.[23] The fibrillar collagen is embedded in a hydrophilic extracellular matrix consisting of proteoglycans, glycoproteins and glycosaminoglycans, which are involved in the development, organization and growth control of tendon.[24]

Force transmission within the tendon

The tendon might be functionally regarded as a single force-transmitting structure, but it remains unknown if force is transmitted evenly throughout the tendon, and therefore whether the stress–strain on tendons is homogeneous. Whether there is a 'weak link' in the force transmission and how it might adapt to loading conditions remain enigmatic issues.

Fascicles from the anterior and posterior portion of the human patellar tendon display substantially different mechanical properties.[25] Lateral force transmission between adjacent fascicles is relatively small, and therefore the fascicles might be considered to be functionally independent structures.[26] The fact that sliding can occur between fascicles might be advantageous as, for example, tendons wrap around bones. The interfascicular space contains fibroblasts, capillaries, nerves and small-diameter fibrils,[26] and it remains unknown if the structures in this space would be adversely affected by disproportionately large shear or possible focal adhesions, or both. It is, however, important to underline that mechanical stimulation of fibroblasts located between fascicles is important for the synthesis of collagen and the release of growth factors.

The collagen fibril is considered the fundamental force-transmitting unit of the tendon,[27] although the actual length of fibrils in mature tendon remains an unresolved issue, which precludes a detailed understanding of tendon force transmission. In fact, suggestions that fibrils are continuous[28,29] and discontinuous exist,[30–33] with currently no unequivocal proof of either proposal. Discontinuous fibrils would require force to be transferred between adjacent fibrils, and functionally continuous fibrils would mean that the fibrils assume most of the tensile load. Thus, it remains unknown whether individual fibrils might sustain microruptures, or whether other components of the extracellular matrix are damaged owing to large shear forces between fibrils.

The tropocollagen molecule comprises three polypeptides arranged as a triple-helical structure stabilized by hydrogen bonds.[34] The collagen molecules are organized in a precise pattern and an important contributor to the mechanical properties of the tendon is the intermolecular crosslinks.[21,22] During loading, the triple helix of the tropocollagen molecule might elongate, the gap between the longitudinally arranged molecules of the fibril might increase, or a relative slippage might occur between laterally adjacent molecules.[32,35–37] Individual collagen molecules have a fracture modulus that far exceeds that of the tendon fibril,[34,38] and are therefore unlikely to be the 'weak link'. However, it is unknown to what extent the proposed gliding mechanisms at the level of the molecule will affect the associated crosslinks. During tensile loading, fibroblasts and their cell nuclei, located between fibrils and in the interfascicular space, undergo deformation, which might be important in the mechanical signal transduction pathway of this tissue.[39,40] Loading can potentially place strain on several components of the tendon that might contribute to an 'injury' or material fatigue that requires repair. Such a repair process might comprise a fine balance

Key points

- Tendons are metabolically active and respond readily to both loading and unloading
- Mechanical loading results both in protein synthesis and degradation of collagen
- Without sufficient rest (24 h) after exercise, net loss of collagen might occur that leaves the tendon vulnerable to injury
- Tendinopathy is associated with neovascularization, but newly formed blood vessels (and nerves) disappear during healing
- The pathogenesis of tendinopathy can be accelerated by overloading

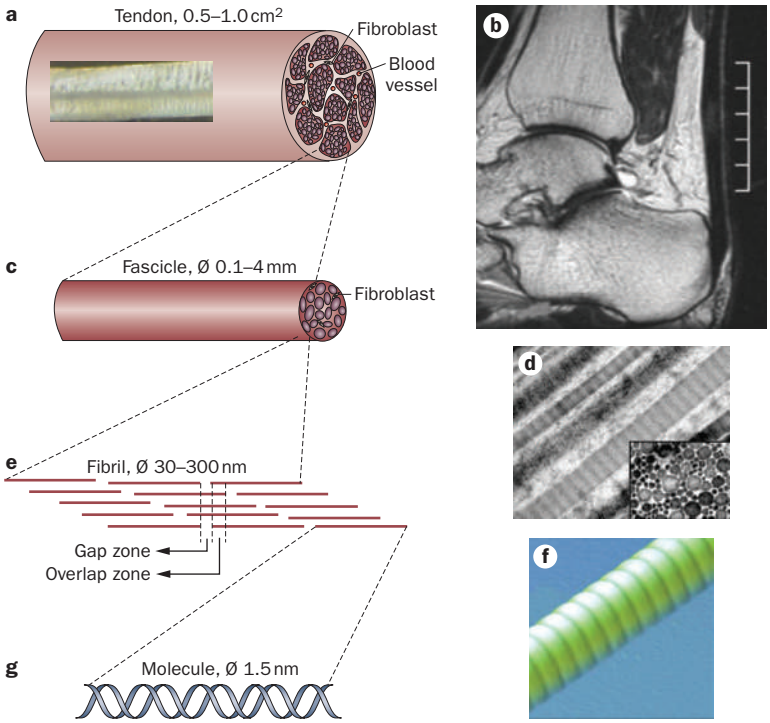

Figure 1 | The different hierarchical levels (tendon–molecule) of the human Achilles tendon. **a** | The whole human tendon comprises collagen fascicles. Inset: stereo micrograph of two adjacent human collagen fascicles with intact interfascicular loose connective tissue. The crimping pattern of the fascicle is visible. **b** | MRI of the human Achilles tendon, which can withstand stresses up to 100 MPa (scale in cm). **c** | The fascicle consists of collagen fibrils, fibroblasts, proteoglycans, glycoproteins and glycosaminoglycans. **d** | TEM of parallel aligned fibrils, which are the fundamental tensile-bearing units of tendon. Lower right corner inset: TEM cross-sectional area showing the fibril diameter distribution (30–300 nm). The interfibrillar space is the hydrophilic extracellular matrix, consisting of proteoglycans, glycoproteins and glycosaminoglycans that are involved in the development, organization and growth control of tendon. **e** | The collagen fibril has a quarter-stagger arrangement of collagen molecules. Crosslinking of the collagen molecules to one another confers integrity on the fibril. **f** | Atomic force microscopy image of an isolated single human fibril showing the characteristic (67 nm) D-band periodicity that represents the alignment of collagen molecules. **g** | The collagen molecule is made up of three polypeptide α-chains to form a triple helix. Abbreviation: TEM, transmission electron micrograph.

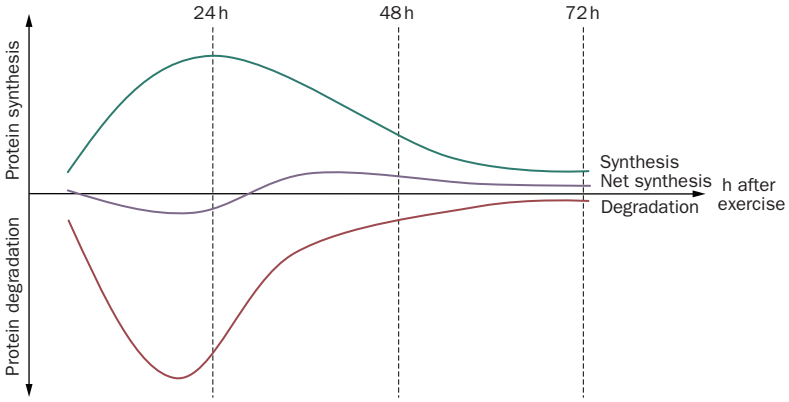

Figure 2 | Schematic representation of collagen synthesis and degradation. Acute exercise in humans is followed by an increase in both the synthesis and degradation of collagen. Over the first 24–36 h, this response results in a net loss of collagen, but is followed by a net synthesis 36–72 h after exercise. Repeated training with rest periods that are too short can result in a net degradation of the matrix and lead to overuse injury.[65,66,111]

between synthesis and degradation of the various components of the extracellular matrix. However, it should also be noted that too little stimulation (relative inactivity) might also offset such an anabolic–catabolic balance.[41]

Pathology of tendinopathy

Histological changes

Typical pathological changes that occur in tendinopathy include reduced numbers and rounding of fibroblasts, an increase in the content of proteoglycans, glycosaminoglycans and water, hypervascularization (with nerve ingrowth) and disorganized collagen fibrils. Immunohistochemical analysis of the affected tissue shows the presence of substance-P-positive nerve fibers and adrenergic receptors in injured but not healthy tissue.[42] Inflammatory cells have not been detected in tendinopathy tissue.[43] There are, however, a greater number of apoptotic cells in tendinopathy tissue,[44] which most likely arises through the activation of c-Jun N-terminal kinase and caspase-3 pathways occurring secondary to mechanical loading.[45–47]

Molecular changes

Changes in mRNA levels

Interestingly, the molecular 'blueprint' of tendinopathy is quite different from that of tendon rupture, implying that the pathogenesis of tendinopathy and tendon rupture differs.[48–50] Elevated levels of messenger RNA (mRNA) can be demonstrated for type I and III collagens, proteoglycans (for example, biglycan and fibromodulin),[51] angiogenic factors (for example, vascular endothelial growth factor [VEGF]), aggrecan, proteins that are required for a stress and regeneration response (for example, heat shock protein [HSP]), fibronectin and tenascin C, and proteolytic enzymes (for example, a disintegrin and metalloproteinase [ADAM]-12, ADAMTS2, ADAMTS3 and some matrix metalloproteinases [MMP1, MMP2, MMP9, MMP13 and MMP23]).[50] By contrast, levels of mRNA encoding MMP3, MMP10 and MMP12 and tissue inhibitor of metalloproteinase (TIMP)-3 are lower in tendinopathy tissue than in normal tissue,[50,52] whereas the mRNA expression levels of some proteoglycans (for example, decorin and versican) remain relatively unchanged.[51] MMPs are important for the normal turnover of tendon proteins during homeostasis and repair, but these enzymes and their inhibitors might also be involved in the pathology of tendon injuries.[53] Treatment of some types of cancer using MMP inhibitors results in a tendinopathy-like condition that disappears on cessation of treatment.[54,55] In insertional regions (regions at which the tendon interfaces with the bone) and areas of tendons that are subjected to compression, mRNA for cartilage-like molecules such as collagen II, aggrecan and sox9 are upregulated.[56]

Changes in protein levels

The protein levels of type I collagen decrease whereas those of type III collagen increase in tendinopathy tissue.[57,58] Although the expression and protein content of collagen type III are thereby correlated, the upregulation of collagen type I expression does not result in any net increase in collagen I content. The mechanism behind this apparent discrepancy is unknown, but it supports the view that in tendinopathic tendons the normal homeostasis of collagen I is disturbed. The amount of enzymatic crosslinking of collagen increases, whereas the level of nonenzymatic crosslinking is unchanged or reduced in tendinopathy.[59,60] Finally, the levels of tenascin C protein increase.[61]

Mechanobiology of fibroblasts

Fibroblasts (tenocytes) are the predominant cell type in tendon and are responsible for the production of collagen and other matrix proteins. Fibroblasts also release and respond to growth factors that regulate protein synthesis. It has been shown that tendon fibroblasts surrounded by biglycan and fibromodulin within the tendon not only respond to growth factors and can synthesize collagen, but that these 'niched' fibroblasts exibit stem-cell-like properties,[62] and that matrix proteins, such as biglycan and fibromodulin, are important for the expression of scleraxis, a transcription factor that is involved in tendon differentiation and collagen synthesis.[63]

Strain and collagen synthesis

Mechanical loading of tendon results in an acute increase in collagen expression and increased collagen protein synthesis in animals and humans.[64,65] This elevated collagen expression is probably regulated by the strain imparted on the fibroblast, which can induce a 2–3-fold increase in collagen formation that peaks around 24 h after exercise and remains elevated for up to 70–80 h (Figure 2).[65,66] The degradation of collagen proteins also increases in response to exercise,[66] probably early on and to a greater extent than collagen synthesis (Figure 2). The levels of markers for proteolysis, such as MMPs or collagen degradation fragments, are elevated in response to exercise,[66,67] and this process represents part of the physiological response to loading. After cessation of exercise and up to 18–36 h thereafter (improved training status shortens this time frame) there is a negative net balance in collagen levels, whereas the balance is positive (anabolic

in relation to collagen) for up to 72 h after exercise (Figure 2). These data indicate that a net increase in collagen requires a certain restitution period, and that without sufficient rest a continuous loss of collagen is likely to occur, which might render the tendon vulnerable to injury. Tendinopathy arises perhaps, therefore, as a result of an imbalance between the synthesis and breakdown of matrix proteins, especially collagen.[41] Interestingly, the relationship between tendon loading and collagen synthesis increases up to a certain point, then levels off with increasing workload (Figure 3), which indicates that fibroblasts are unable to further synthesize collagen beyond this upper limit. The fact that procollagen expression is upregulated in the same manner in the tendon independent of muscle contraction mode (eccentric, isometric or concentric)[64] supports the notion that fibroblast strain regulates the collagen protein synthesis response. It is hypothesized that insufficient recovery time will tilt the balance between collagen synthesis and degradation, resulting in a net catabolic state.

Training/repetitive strain

Habitual loading (as occurs in response to training) will result in a higher rate of collagen synthesis in the basal state simply as a result of the constant effect of loading from the previous 24–48 h; this effect can be seen at the level of the whole tendon as tendon hypertrophy.[68] The rate of degradation also increases with training to ensure that the overall turnover is high, but not to the same extent as the increase in synthesis, which allows for a small—but consistent—positive net balance of collagen.[69] Habitual training thus results in a higher turnover of collagen, whereas inactivity lowers collagen synthesis and turnover.[70] This result illustrates why activity even in the presence of tendinopathy might be better for the regeneration of the tendon tissue than complete inactivity.

The fact that collagen and matrix proteins are important in the development of tendinopathy is supported by the fact that polymorphisms in the genes that encode collagen and tenascin C are associated with a higher than normal risk of developing tendinopathy (Box 1).[71,72] In addition to collagen, other matrix proteins also respond to loading. Several proteoglycans, such as decorin, versican, aggrecan, lumican, fibromodulin, keratocan and proteoglycan 4, increase their turnover in response to loading to maintain homeostasis in the tendon,[73–77] which further supports the use of loading activity in the treatment of tendinopathy. Finally, the expression of enzymes involved in protein crosslinking is also upregulated with exercise, which lends additional support to the notion that tendons respond readily to loading.[64]

Molecular response to exercise

Growth factors

Exercise results in an increase in the mRNA levels and in the tissue concentrations of growth factors such as insulin-like growth factor 1 (IGF-1), transforming growth factor β (TGF-β), connective tissue growth factor (CTGF) and interleukin (IL)-6. This response has been shown in animal tendons,[78] in human tendon homogenate,[79] and in microdialysis fluid representing the tendon interstitial concentration.[80] A key regulator of collagen synthesis is IGF-1, which has a stimulatory effect on collagen protein synthesis *in vitro* and *in vivo*.[79,81] TGF-β and CTGF stimulate fibroblasts within the patella tendon to synthesize collagen,[82] and exercise seems to enhance this effect.[83] Interestingly, the expression of both IGF-1 and TGF-β mRNA rises in response to exercise independent of muscular contraction type,[78] which suggests that both growth factors are important regulators of collagen synthesis in tendon. Surprisingly, inactivity by suspension of the hindlimb in rats or by lower limb casting in humans also resulted in an initial increase in the levels of IGF-1 mRNA,[84] which indicates an unloading IGF-1 response,[85] or a compensatory increase in the synthesis of growth factors to counteract the inactivity-induced drop in collagen synthesis. When activity is resumed after a period of rest, the expression of collagen is again normalized.[84] These findings demonstrate that inactivity does not follow a pattern opposite to that of the loading response, and that this pattern might reflect a protective mechanism towards the loss of tendon tissue during inactivity. In any case, these responses do not favor inactivity as a treatment for tendinopathy.

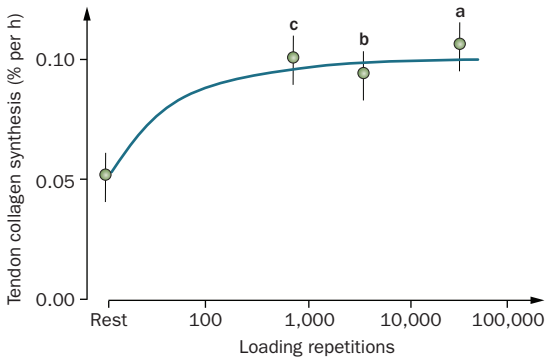

Figure 3 | Response of collagen to loading. The synthesis of collagen, based on the number of loading repetitions from human studies, to **a** | 36 km running,[111] **b** | 1 h of maximal knee kicking[65] or **c** | 10 times 10 repetitions of knee extension (70% 1 repetition maximum). The graph indicates a similar increase in collagen synthesis independent of exercise volume (repetitions), which suggests that there is a ceiling effect in collagen synthesis. It also indicates that adding exercise repetitions (cumulative load) will not increase collagen synthesis further, but potentially increase degradation and further amplify a negative net balance in collagen.

Cytokines, prostaglandins and inflammation

A potent response to exercise is a rise in the levels of IL-6 in the peritendinous tissue;[80] this rise seems to parallel that of collagen synthesis. Infusion of IL-6 in the vicinity of the tendon tissue has been found to induce collagen protein synthesis similar to that evoked during exercise (M. B. Andersen, J. Pingel, M. Kjaer, H. Langberg, unpublished work). This result supports the view that cytokines are potent stimulators of collagen synthesis in tendon. Estrogen might have an inhibitory role in the adaptation response of collagen and matrix tendon to loading.[86]

Box 1 | The genetic component of tendinopathy

Genetic variations have been implicated in the development of tendinopathies. The collagen, type V, alpha 1 (*COL5A1*) and *TNC* genes encode the collagen alpha-1(V) chain and tenascin C, both of which are important structural components in tendons and ligaments; variations within these genes, along with variations in the gene encoding matrix metalloproteinase 3, have been shown to cosegregate with chronic Achilles tendinopathy.[71,72] The collagen alpha-1(V) chain is involved in the assembly of collagen fibers and influences fiber diameter, and variations in this component might alter collagen strength. Similarly, tenascin C is known to be involved in the response of collagen to mechanical loading in a dose-dependent manner. The genes encoding these proteins have also been shown to be associated with anterior cruciate ligament ruptures.[109,110] These data indicate that genetic variations might be involved in the development of certain tendon pathologies.

Young women—especially if taking estrogen-containing oral contraceptives—demonstrate lower basal levels of collagen and a lower increase in collagen synthesis in response to loading compared with males.[87–89] This result might explain why women show an attenuated adaptive tendon response with habitual loading,[90] and why they might need longer for tendon adaptation to loading. The lower increase in collagen synthesis in response to loading might also explain why women are more susceptible to certain soft tissue overload injuries.[91] The mechanism remains unknown, but as estrogen levels are inversely related to the levels of IGF-1 in tissues, estrogen might exert its inhibiting effect indirectly via attenuating the response to IGF-1.

A rise in the concentration of prostaglandins in tendon tissue is part of the physiological response to loading, and blocking this response has been shown to inhibit the synthesis of collagen protein (S. G. Petersen, L. Holm, M. Kjaer, unpublished work). Such inhibition is in line with what is known to occur in skeletal muscle contractile protein[92] and for skeletal muscle stem cells (satellite cells).[93] Levels of inflammatory mediators in tendinopathy are not elevated in the resting state,[94] which further supports the notion that tendinopathy is not an inflammatory condition. However, the inflammatory response might increase immediately after exercise, despite the absence of inflammatory cells in the chronically overloaded tendons, indicating a susceptibility of the overloaded tissue towards an increase in inflammation with loading. This result would explain the difference between the lack of inflammatory observations during surgery and the documented positive short-term effect of anti-inflammatory medication (for example, glucocorticoid injection) in tendinopathy.

Vascular and neural regulation

Poor blood supply has been implicated as a factor contributing to tendon injuries, but tendon vascularization appears ample both around and inside the tendon in patients with tendinopathy.[95,96] During exercise, the blood flow of tendon can increase by up to seven-fold, and it is mainly regulated by the release of prostaglandins. This response only represents 20% of the maximal capacity of the tendon during ischemic reperfusion,[97] and therefore blood flow is remarkably low during rest. In individuals who undergo extensive physical training, resting blood flow is not elevated.

Tendinopathy itself is often, but not always,[98] associated with neovascularization and elevated intratendinous blood flow[99–101] that seems to normalize during the course of exercise-based conservative treatment.[100] Increased flow during exercise probably represents a physiologically important response, whereas an elevated flow in the resting state accompanies tendinopathy. However, rather than being important in the pathogenesis of tendinopathy, the latter response represents a secondary regenerative phenomenon. Indeed, the elevated flow during loading might be advantageous for tendinopathy. In patients with clinical signs of tendinopathy and hypervascularization, it has been suggested that the primary source of pain is the result of nerves growing intimately with the new vessels into the tendons.[102] It has been demonstrated that tendon tissue injury (partial rupture model) leads to both an angiogenic response[103] and marked nerve ingrowth, as well as the presence of substance P and calcitonin-gene-related peptide, both of which are involved in pain transmission.[104,105] These newly formed nerves and blood vessels disappear during healing, a process that is accelerated with physical activity and delayed with prolonged inactivity in the recovery phase.[106,107] How nerve ingrowth occurs in tendinopathy is unclear, but the process seems to occur subsequently to alterations in protein synthesis, and might therefore explain why clinical pain occurs when the tendinopathy is already quite advanced.

Conclusions and perspectives

Understanding how tendon tissue adapts to mechanical loading, and how and when this process is attenuated during tendinopathy, will contribute to our understanding of the pathogenesis of this condition. In part, the limited knowledge of the pathogenesis of tendinopathy resides in the fact that the actual injury mechanisms are quite advanced before symptoms are experienced by the patient. New methods, such as tissue biopsy sampling, infusion of growth factors and determining local tissue reactions to acute loading or overloading by use of microdialysis, have yielded promising new information on the turnover of the connective tissue. An intervention model that combines immobilization with acute loading might also unveil the pathways of overloading in fragile (immobilized) tissue. To study the actual injury mechanism and the early stages of the injury, it might be useful to turn to animal models, the use of which has already been demonstrated.[108] The definitive establishment of knowledge about the injury mechanism is an important step in the development of more effective treatment and prevention strategies.

Review criteria

We searched for original articles focusing on tendinopathy in MEDLINE and PubMed, published between 1970 and 2009. The search terms we used were "tendinosis", "tendinitis", "tendinopathy", "collagen" and "fibroblast". All papers identified were English-language, full text papers. We also searched the reference lists of identified articles for further papers.

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