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Review

The pathogenesis of tendinopathy. A molecular perspective

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There are many publications that discuss the aetiology, diagnosis and treatment of the various forms of tendinopathy, but few are based on conclusive scientific evidence. The pathogenesis of tendinopathy is difficult to study because tendon biopsies are rarely obtained before a tendon has ruptured. There are interesting comparisons with animal tendinopathy, particularly in the equine athlete, although many animal models do not accurately reflect the human condition—the tendon lesions usually heal. However, the application of biochemical and molecular techniques to the study of both animal and human tendinopathy has led to a greater understanding of these common and disabling conditions. This article summarizes current knowledge of the pathogenesis of tendinopathy, with particular emphasis on the molecular pathology of the tendon matrix.

Classification and terminology

Tendons may be affected by a variety of different pathological conditions. Many systemic diseases are associated with general defects in matrix metabolism and structure that compromise tendon strength and elasticity, or result in inflammation of the tendon or its insertion [1–3] (Table 1). These conditions are not the subject of this review, although they must be considered as part of the differential diagnosis.

Tendonitis (or tendinitis) is the term traditionally used to describe a chronic painful tendon, and assumes that tendon injury is accompanied by an inflammatory response. This is contrary to the evidence from histopathological, biochemical and molecular studies, and the lesion is perhaps better described as a 'tendinosis' [4–9]. However, as we cannot exclude the possibility that inflammation is implicated at some stage in the condition, the term 'tendinopathy' is used throughout this article to describe disorders primarily affecting tendons, including tendon rupture and chronic pain. Unlike tendonitis and tendinosis, this term does not assume any knowledge about the underlying pathology.

Another term in frequent use is 'spontaneous tendon rupture', used to describe ruptures that occur without any preceding clinical symptoms [5, 10]. Because healthy tendons can withstand very high tensile loads—much higher than required for their normal function—these conditions are rarely truly spontaneous and are associated with at least some degree of matrix degeneration [10, 11]. The precise nature of the degenerative process is still the matter of debate. There are a variety of degenerative features associated with tendinopathy, including glycosaminoglycan (GAG) accumulation, calcification and lipid accumulation. However, many of these features are found in normal tendons and are not necessarily pathological [5, 6, 12–14].

Aetiology of tendinopathy

Tendinopathy can be associated with a variety of both intrinsic and extrinsic factors (Table 2). High body mass and constitutional factors such as leg-length discrepancies can place tendons under excessive or abnormal patterns of loading [15, 16]. A sedentary lifestyle allied with the particular physical demands of occupation or sporting activities may account for the increased incidence of tendon rupture in recent times. The influence of an individual's sex and genetic background is unknown, although some association with genetic factors has been postulated in a proportion of patients [15, 17]. Laxity of the joints may be a predisposing factor for patella tendinopathy and rotator cuff lesions [15]. Gene defects affecting tendon (collagen) fibre formation and metabolism may explain some of these conditions, although as yet no defect has been identified that is specifically associated with tendinopathy [1].

The literature is dominated by two hypotheses on the cause of tendon rupture—a mechanical theory and a vascular theory—but the two are not mutually exclusive. Many lesions are associated with low or reduced vascular perfusion in tendons such as the supraspinatus and Achilles [15, 18–21]. A decrease in circulation may occur as a result of ageing, vascular disease, physical disuse or trauma, leading to tissue hypoxia and reduced viability of the tendon cells. However, in apparent contradiction of this theory, chronic tendon lesions often show an increase in vascularity and increased cellularity [6, 13], a so-called angiofibroblastic response [22], and there is an increase in the tendon blood flow [23, 24]. Whether this represents evidence of a healing response, secondary to the initial lesion, is uncertain [24].

Many painful tendon lesions are thought to be the result of repetitive microtrauma, frequently described as 'overuse' pathologies [16, 25–28]. This is often explained with reference to the biomechanical properties of tendon and the stress–strain curve [29] (Fig. 1). Physiological loads usually cause less than a 4% increase in the length of the tendon [26, 30]. Strain above 4% results in damage to one or more of the tendon fibre bundles, and strains in excess of 8–12% result in complete tendon rupture. Most overuse tendinopathy is associated with repeated microstrain below the failure threshold, analogous to the fatigue failure that affects most materials placed under repetitive loading. It is generally assumed that tendon matrix damage is the primary event, overwhelming the ability of the resident cell population to repair structural defects.

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Table 1. Systemic diseases affecting tendon

Disease	Structural defect or effect on tendon
Inherited disorders	
Ochronosis (homocystinuria)	Deficient collagen and elastin cross-linking
Aspartylglycosaminuria (AGU)	Abnormal collagen/deficient cross-linking?
Haemochromatosis	Accumulation of iron in matrix
Menkes kinky hair syndrome	Defect in collagen and elastin cross-linking
Mucopolysaccharidoses	Abnormal collagen fibrils, increased GAG
Marfan syndrome	Abnormal fibril structure
Ehlers–Danlos syndromes	Various defects in collagen processing and structure
Osteogenesis imperfecta	Genetic defects in type I collagen
Lipid storage diseases	Xanthomas: slow growing lipid deposits
Myopathies and dystrophies	Abnormal fibril structure
Endocrine and metabolic diseases	
Diabetes mellitus	Increased glycation and cross-linking of collagen
Adrenal disorders	Altered collagen metabolism
Thyroid disorders	Calcification and accumulation of deposits
Amyloidosis	Accumulation of deposits between fibrils
Renal disease	Elastosis, destruction of collagen fibres
Rheumatological diseases	
Rheumatoid arthritis	Destruction of collagen: inflammatory infiltrate
Spondylarthropathies	Inflammation at insertion, fibrosis and calcification
Reactive arthritis	Inflammation at insertion
Reiter's syndrome	Inflammation at insertion
Gout	Urate crystal deposits and inflammation
Pseudogout	Calcium pyrophosphate deposits and inflammation

Table 2. Factors implicated in chronic tendinopathy

Intrinsic factors	Extrinsic factors
Age	Occupation
Vascular perfusion	Sport
Nutrition	Physical load
Anatomical variants	Excessive force
Leg-length discrepancy	Repetitive loading
Mal-alignments	Abnormal/unusual movement
(e.g. genu valgum)	Training errors
Bony impingement	Poor technique
(e.g. acromion)	Fast progression
Joint laxity	High intensity
Muscle weakness/imbalance	Fatigue
Gender (?)	Shoes and equipment
Body weight	Environmental conditions
Systemic disease	Temperature
	Running surface

Because cell activity is required for the maintenance of connective tissues, it is equally possible that changes in cell metabolism—more specifically, the synthesis and degradation of the extracellular matrix—can influence the structural properties of the tendon. In other words, changes in cell activity in response to mechanical strain may be primary and not secondary to any physical lesion or 'micro-injury'.

It is now recognized that most tendinopathy is rarely associated with any single factor, and the degenerative process that precedes tendon rupture may result from a variety of different pathways and causative factors. Indeed, there is some evidence to suggest that the nature of the degenerative process varies among different sites [5]. Many questions remain about the role of tendon fibroblasts (tenocytes) and other cell types in the disease process. In the absence of an overt inflammatory process, there is no rational basis for the use of anti-inflammatories in chronic tendinopathy, and the source of tendon pain is not understood [31–33]. An objective approach to the treatment of tendinopathy requires a greater knowledge of the tendon matrix and its changes in health and disease.

Structure of tendon

The structure, composition and organization of the matrix are critically important for the physical properties of tendon. The smallest structural unit is the fibril, largely consisting of rod-like collagen molecules aligned end-to-end in a quarter-staggered array [34–36] (Fig. 1A). The fibrils range in diameter from 10 to 500 nm depending on the age, location and species from which the tendon is sampled [37–40]. One or more distinct fibril populations are normally present: a single population of small diameter fibrils in young animals, and a bimodal distribution of small and large diameter fibrils in mature animals [38, 39, 41].

Collagen fibrils aggregate together into fibres, and bundles of fibres ('fascicles') are bound together by a thin layer of loose connective tissue known as the endotenon [34] (Fig. 1B). The endotenon carries blood vessels, lymphatics and nerves which generally pass up and down throughout the body of the tendon [34, 42]. Bundles of fascicles are surrounded by the epitenon, a structure contiguous with the endotenon and also relatively rich in blood vessels, lymphatics and nerves [34]. Fibre bundles are predominantly aligned with the long axis of the tendon and these are responsible for the tensile strength of the tendon. A small proportion of fibres run transversely, and there are even spirals and plait-like formations [40, 43]. This complex ultrastructure provides resistance against transverse, shear and rotational forces acting on the tendon.

The size of the fibre bundles is related to the macroscopic size and function of the tendon, with small fascicles generally found in digital tendons and large fascicles in big, weight-bearing tendons such as the Achilles [40]. The fascicular structure is thought to provide a fail-safe mechanism, so that failure of one or a few fibre bundles does not significantly reduce the strength of the whole tendon. Fibre bundles commonly exhibit a planar zigzag or 'crimp', best seen when longitudinal sections are viewed under polarized light [44] (Fig. 1C). The stretching out of the crimp accounts for the 'toe' region of the tendon stress–strain curve, which acts as a buffer against fibre damage [29] (Fig. 1D). The angle and length of crimp can vary in different tendons and at different sites within a tendon [45]. This has implications for the mechanical properties of the tissue, as fibres with a small crimp angle will fail before those with a larger crimp angle [45].

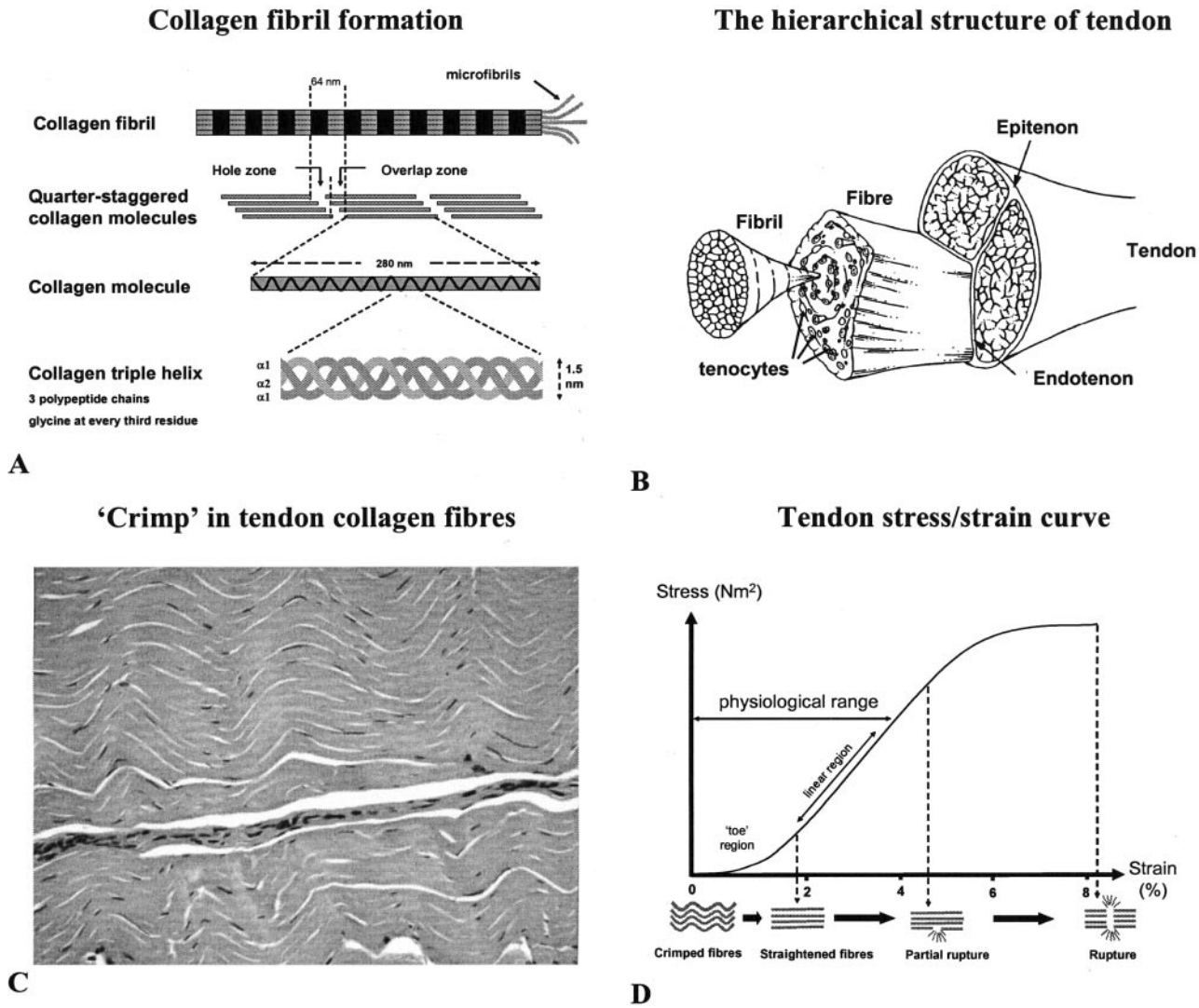


FIG. 1. The structure and physical properties of tendon. (A) Collagen (type I) forms rod-like molecules which spontaneously associate with other molecules to form a quarter-staggered array. The overlap and hole zones account for the characteristic banded fibril seen using the electron microscope, due to the differential uptake of electron-dense stains. (B) Bundles of fibrils form fibres, the fibres are bound together by a thin layer known as endotenon, and several fibre bundles are surrounded by an outer layer known as the epitenon. (C) Haemotoxylin and eosin-stained section of normal tendon showing the wave or crimp pattern of the collagen fibres. (D) When strain is applied to a tendon, there is first a straightening out of the crimp, resulting in the 'toe region' of the stress–strain curve. Strain within the physiological range causes elastic deformation of the tendon. Higher levels of strain result in partial or complete rupture of fibrils. Actual levels of strain required to induce tendon damage differ in different tendons and are affected by factors such as age and cross-linking of the matrix. This figure can be viewed in colour as supplementary material at *Rheumatology* Online.

Collagens in tendon

Although type I collagen constitutes around 60% of the dry mass of the tissue and approximately 95% of the total collagen [46–48], the fibrils are actually composites of several different collagens, proteoglycans and glycoproteins [49]. Type III collagen is the next most abundant collagen, constituting around 3% of the total in human supraspinatus and biceps brachii tendons [48]. In normal tendons, type III collagen tends to be restricted to the endotenon and epitenon [50]. However, it is also found intercalated into type I collagen fibrils, particularly in ageing tendons and at the insertion sites of highly stressed tendons such as the supraspinatus [51]. As type III collagen tends to produce smaller, less organized fibrils [52], this has implications for the mechanical strength of the tendon. Type V collagen is intercalated into the core of the type I collagen fibril, where it forms a template for fibrillogenesis and modulates fibril growth [53, 54]. Type IV collagen, which forms a meshwork structure, is restricted to the basement membrane of the tendon blood vessels [47]. Type VI collagen forms sheet-like structures and is usually found co-distributed with type I collagen fibres in normal tendon [47, 55]. There is a different distribution in tendon fibrocartilage at the insertion, where type VI collagen is predominantly cell-associated, as it is in cartilage [55]. Type XII and type XIV collagens are associated with the surface of the type I collagen fibrils, particularly at the insertion [56]. These collagens are FACITs (fibril-associated collagens with interrupted triple helix), with relatively large non-helical domains that are thought to mediate interactions with other matrix components. Other collagens found in small quantities in tendon include types II, IX, X and XI [57]. Once thought to be restricted to cartilage, these collagens are found in the fibrocartilage at the bone insertion, where they are may function to dissipate stress concentration at the hard tissue interface [55, 57].

Collagens in the matrix are stabilized by the formation of cross-links [58, 59]. Some cross-links are formed between adjacent amino acids after modification by the enzyme lysyl oxidase. Initially, reducible cross-links are formed between two amino acids [59]. As the tissue ages, these combine with another adjacent amino acid to form mature trifunctional cross-links. Not all of the mature cross-links have been identified, although the best-characterized are hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP). LP, otherwise known as deoxypyridinoline, is essentially restricted to bone and is present only in small quantities in soft connective tissues [59–61].

The amount of HP in a given connective tissue is related to its mechanical function, with the highest concentration in hyaline cartilage and intervertebral discs, which contain approximately two HP cross-links per collagen molecule [58]. Tendons have a high HP content compared with other soft tissues, although there are substantial differences between tendons. For example, human supraspinatus tendons had over three times the HP content of biceps brachii tendons (0.81 and 0.25 HP cross-links per collagen molecule respectively), presumably reflecting the different mechanical demands placed on these tendons [61]. A greater concentration of HP is generally found in compressed tendons, associated with the fibrocartilaginous composition found at these sites [62–64]. However, the HP content does not change significantly after skeletal maturity, and these cross-links probably do not contribute to the altered physical properties of ageing tendons [61].

Another form of collagen cross-linking occurs by the process of non-enzymic glycation. Reducing sugars such as pentose derived from the circulation bind irreversibly to long-lived proteins in the matrix [59, 65]. There are no enzymes involved, and sugars attach throughout the length of the collagen molecule. Over time, Amadori rearrangement results in the formation of irreversible cross-links between adjacent sugars, creating Maillard browning products, perhaps better known as advanced glycation end-products (AGEs). AGEs are responsible for many of the altered physical and chemical properties of ageing tissues, such as the reduction in elasticity and decreased solubility. They also have an effect on cell–matrix interactions in the tissue, a possible cause of some of the altered cell activities seen in diseases of ageing, such as osteoarthritis [66, 67]. Although there are a variety of AGEs, the best characterized is the naturally fluorescent cross-link known as pentosidine [68]. Because the turnover of matrix proteins such as collagen is generally very low, AGEs such as pentosidine accumulate gradually on collagen molecules with age. Consequently, the pentosidine content serves as a marker of the molecular age of the tissue, and this property has been exploited in recent studies of the tendon matrix [61] (see below).

Proteoglycans in tendon

Proteoglycans are a heterogeneous group of proteins with many different functions in the matrix, although all usually carry at least one side-chain of GAG, a complex sugar moiety of repeating disaccharides [69]. In the tension-bearing regions of a bovine flexor tendon, proteoglycans constitute between 0.2 and 0.5% of the tendon dry weight [70]. The most abundant proteoglycan is decorin, and there are small amounts of biglycan [71]. Small proteoglycans represented almost 90% of the total, and the remainder was a large proteoglycan, thought to be a processed form of aggrecan (lacking the G1 domain) rather than versican [72]. In weight-bearing regions of bovine flexor digitorum profundus tendons, the proteoglycan content was around 3.5% of the tendon dry weight, with a high content of aggrecan and biglycan [62, 70]. A similar proteoglycan composition has been described in fibrocartilaginous regions of rabbit and dog tendons [73, 74]. The accumulation of aggrecan occurs after weight-bearing in the neonate, and the maintenance of synthesis is dependent on compressive load [75]. The fibrocartilage at these sites is a protective adaptation, the aggrecan holding water within the tissue and acting to resist compression [62].

Regional differences in tendon morphology and composition have been identified in human tendons [55, 63, 76, 77]. In Achilles tendons, decorin, biglycan, lumican and fibromodulin were identified in both fibrocartilaginous and tension-bearing regions, at both the mRNA and the protein level [55]. Versican was the major large proteoglycan in the tendon mid-substance, with lesser amounts of aggrecan. In contrast, the fibrocartilage of the Achilles insertion contained mainly aggrecan with lesser amounts of versican.

Site-specific variations in proteoglycan content are related to the mechanical history and function of the tendon. Tendons of the short head of biceps brachii contain around 0.2% proteoglycan, the majority carrying dermatan sulphate GAG side chains (80%) and the remainder chondroitin sulphate, consistent with a predominance of decorin in flexor tendons, which experience mainly tensile loads [63]. Substantially higher levels of proteoglycan are found in supraspinatus tendons, mainly chondroitin sulphate with lesser amounts of dermatan sulphate and keratan sulphate [63]. These proteoglycans have since been characterized, confirming that aggrecan is the major large proteoglycan, together with significant amounts of biglycan [64]. This fibrocartilaginous composition is thought to be a result of adaptive metaplasia to the compressive load experienced by the supraspinatus tendon in the rotator cuff, which wraps around the head of humerus and may experience impingement from the overlying bone and ligament.

Similar fibrocartilaginous regions have also been described in human peroneus, tibialis and extensor digitorum tendons [76, 77]. Although thought to be a protective adaptation, the formation of fibrocartilage may have pathological significance, modifying the structural properties and affecting the tendon response to injury, for example. Autoimmune reactions against cartilaginous constituents of fibrocartilage may also explain why tendon insertions are prone to inflammation in the spondylarthropathies.

Ageing of the tendon matrix

Age affects the tendon matrix in a variety of ways. During maturation, the cell density decreases dramatically and there is a corresponding increase in collagen content and a decrease in glycosaminoglycan content [30, 78]. The collagen cross-link profile changes from immature, difunctional cross-links to mature, trifunctional cross-links. After maturity, there is no significant change in the total collagen concentration, which remains predominantly type I collagen [48]. However, the distribution of minor collagens, such as type III, may change, with a greater proportion incorporated into the type I fibres [48]. Collagen fibre bundles tend to become larger, the thermal stability increases and the tendon becomes stiffer and less elastic [39, 79, 80]. There is a substantial decrease in the solubility of collagen, thought to be associated with the accumulation of AGE cross-links, such as pentosidine [81]. Some of these changes may be counteracted by exercise, although there is some evidence that mature tendons have little capacity for adaptation and are damaged by exercise [82]. There is evidence of accumulated physical damage in ageing tendons, with increases in the amount of denatured collagen and increased proteolytic cleavage of matrix components [86]; these changes are all associated with deterioration in the physical properties of the tendon. Age is commonly associated with increased prevalence of degenerative changes, such as lack of fibre organization, decreased cellularity and increased GAG content [6, 12]. However, degeneration is not an inevitable consequence of ageing, because other tendons may be unaffected [14]. Tendons from particular sites, such as the shoulder, elbow, knee and ankle, are most likely to show degenerative changes, which increase in severity with age and are associated with the high physical demands placed upon the tendons at these joints [20, 83].

Matrix turnover in tendon

Matrix turnover, involving both the synthesis and degradation of matrix components, is important for the maintenance and repair of all connective tissues, and tendon is no exception. Once thought of as inert and incapable of participating in repair, tenocytes have been shown to be active throughout the lifespan, expressing a variety of matrix proteins and matrix-degrading enzymes [9, 84–86]. However, differences between tendons from different sites and regional variations within tendons may be important in tendon disease, as discussed in more detail below.

Some studies have investigated the effects of exercise on Achilles tendon collagen turnover, using microdialysis catheters inserted into the peritendinous space [87–89]. Vigorous exercise induced increased formation of type I collagen, measured by procollagen peptide analysis of the dialysate 72 h after exercise [87]. There was a decrease in the rate of collagen degradation during the early recovery phase, but no significant difference from controls at 72 h. Thus, exercise was shown to induce anabolic, adaptive processes in the human Achilles tendon, at least in trained individuals. The training effect was further demonstrated in a later study, which showed an increased rate of turnover of collagen (both synthesis and degradation) after 4 weeks of training, but predominantly anabolic effects after 11 weeks of training [89].

Similar studies have shown that exercise increases the peritendinous levels of various mediators of vasodilation and inflammation, such as bradykinin, adenosine, interleukin (IL) 6, thromboxane and prostaglandin E_2 [87, 90–93]. There was also a two-fold increase in lactate and glycerol, demonstrating changes in lipid and carbohydrate metabolism as well as inflammatory activity after short bouts of exercise [90]. These activities are presumably implicated in the adaptive response of tendon, although excessive stimulation of some or all of these factors may conceivably have a role in the onset of tendinopathy as a result of overuse (see below).

Collagen degradation in tendon

Collagen type I is relatively resistant to enzymatic degradation, and once laid down and cross-linked into the matrix it has a long half-life [94]. Consequently, with age the collagen becomes increasingly glycated, and the AGE cross-link pentosidine accumulates, forming a useful marker of the protein residence time [94, 95]. In the biceps brachii tendon, pentosidine accumulates in a linear fashion with age, consistent with low levels of collagen turnover in this tendon [61] (Fig. 2). Pentosidine does not accumulate at the same rate in ageing supraspinatus tendons, indicating relatively high levels of collagen turnover [61, 86]. The increased rate of turnover compared with the biceps tendon is thought to be associated with the high levels of strain normally experienced by supraspinatus tendons in everyday use. The enzymes responsible for this activity are consequently particularly important, as inappropriate or excessive matrix turnover is a likely cause of the matrix damage in tendinopathy.

Some collagen in tendon is probably degraded intracellularly after phagocytosis, with fibroblasts and macrophages engulfing collagen molecules which are then digested by lysosomal enzymes [96, 97]. This is a major activity in the rapidly remodelling peridontal ligament, although few studies have investigated the relative importance of this route in tendon. Most studies have focused on collagen degradation occurring in the extracellular environment and mediated by secreted proteases.

Collagenases, members of the matrix metalloproteinase (MMP) superfamily, are some of the few enzymes capable of cleaving the intact type I collagen molecule in the extracellular environment [98–100]. Cleavage occurs at a specific locus in the collagen triple helix, between residues 775 and 776 [99], and is the rate-limiting step in (fibrillar) collagen turnover, generating three-quarter- and one-quarter-length fragments that are in turn susceptible to other

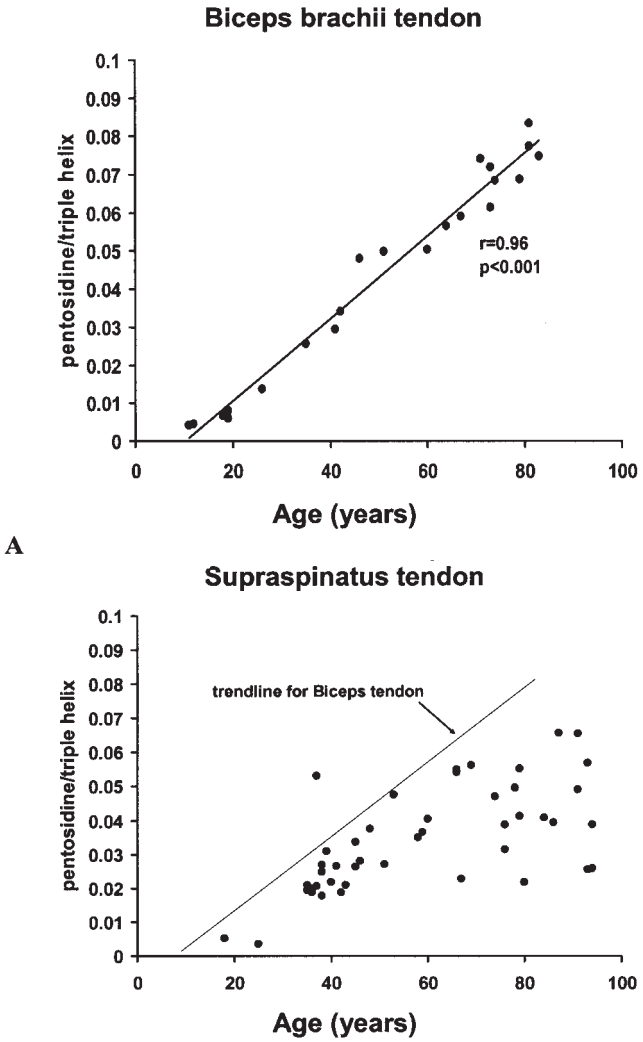


FIG. 2. Tendon pentosidine content, a marker of matrix age. (A) The AGE product pentosidine accumulates in a linear fashion with increasing age in the human biceps brachii tendon. These tendons were histologically normal, and the data are consistent with very low levels of matrix (collagen) turnover throughout the lifespan. (B) There is a very poor relationship of pentosidine content with age in the human supraspinatus tendon. Although there was no history of shoulder pathology, some evidence of degenerative change in the tendon was common. The pentosidine content demonstrates that much of the mature glycated collagen network has been replaced during an individual's life. Data from Bank *et al.* [61].

proteinases, such as the gelatinases. MMPs with collagenase activity include MMP-1 (collagenase-1, EC 3.4.24.7), MMP-8 (neutrophil collagenase, EC 3.4.24.34) and MMP-13 (collagenase-3, MEROPS ID M10.013) as well as the gelatinase MMP-2 (gelatinase A, EC 3.4.24.24) and the membrane-type MMP-14 (MT1-MMP, MEROPS ID M10.014). The collagenases differ in their activities against the various fibrillar collagens [101], although precisely which enzymes are implicated in the physiological and pathological turnover of connective tissue is still the subject of extensive research.

Studies of tendon in explant culture have shown that MMP-1 is released into the culture medium and associated with the collagen breakdown that occurs usually after 14–21 days in culture [102, 103]. An extended culture period is required, possibly because

removal of the proteoglycan component from around the collagen fibril is a prerequisite for collagen degradation. Breakdown can be stimulated by the inflammatory cytokine IL-1 and potentiated by the cytokine oncostatin M, and is associated with increased MMP-1 activity and reduced levels of TIMP-1 (tissue inhibitor of metalloproteinases) in the media [103]. Factors that regulate enzyme activities in tendon are consequently likely to have a role in tendinopathy, as discussed in more detail below.

Proteoglycan degradation in tendon

Proteoglycans are turned over much more rapidly than the fibrillar collagens. Although some members of the MMP family, such as MMP-3 (stromelysin 1), can degrade proteoglycans such as aggrecan *in vitro*, most activity *in vivo* is associated with a related but distinct group of metallo-endopeptidases commonly known as 'aggrecanases'.

Aggrecanases were recently identified as members of the ADAMTS family, a subgroup of ADAM (a disintegrin and metalloproteinase) with thrombospondin (TS) type I motifs [104]. The TS domains bind to GAG and may function to sequester aggrecanases in the matrix. ADAMTS-4 (aggrecanase 1) and ADAMTS-5 (aggrecanase 2) both cleave aggrecan at a specific locus, between residues Glu[373] and Ala[374] in the interglobular domain of the core protein [104–106]. ADAMTS-1 can also degrade aggrecan at the same locus *in vitro*, and may be a third aggrecanase [107]. ADAMTS-4 also has activity against the brain-specific proteoglycan brevican [108], although little is known about the enzymes responsible for the degradation of other proteoglycans.

Recent studies have demonstrated rapid catabolism and loss of proteoglycans from tendon explants maintained in culture [109, 110]. Studies with antibodies to aggrecan, biglycan and decorin showed that catabolites of aggrecan and decorin were present in both young and mature tendon, consistent with constitutively high levels of proteoglycan turnover [110]. There was no evidence of MMP-mediated proteoglycan turnover, although aggrecan turnover did not correlate with levels of mRNA expression for ADAMTS-4 or ADAMTS-5. Currently little is known about the factors that regulate aggrecanase activities in connective tissues.

The matrix in chronic tendinopathy

There have been relatively few biochemical studies of chronic tendinopathy, and most have been of material collected at the end-stage of the condition after tendon rupture. Studies of degenerate supraspinatus tendons found a small but significant decrease in the total collagen content, and an increased proportion of type III collagen relative to type I collagen [48]. Post-translational modification of the collagen network was also different compared with age-matched normal tendons. Hydroxylysine and the mature collagen cross-links (HP and LP) were significantly increased, to a much greater extent than expected given the increased collagen type III content, which normally contains half the hydroxylysine and HP content of collagen type I [61]. Similar changes have been found in animal tendons in chronic tendinopathy [111] and after acute injury induced by surgical trauma or collagenase injection [112–115]. Although levels of hydroxylation and the HP cross-link tend to diminish as healing proceeds, incomplete remodelling commonly results in persistently high levels in scar tissue [116].

Other biochemical studies have shown an increase in hyaluronan and various proteoglycans in degenerate tendons, as yet not fully characterized [63, 111]. There are differences in the sugar moieties expressed in ruptured Achilles tendons, as shown by changes in lectin staining properties [117]. Glycoproteins such as tenascin-C were increased in ruptured supraspinatus, with differences in the isoforms expressed as well as small peptide fragments; this is evidence for enzyme-mediated cleavage in the tissue [118]. Fibronectin immunostaining was significantly increased in ruptured tendon, and there was accumulation of necrotic tissue and fibrin [119]. These matrix changes are consistent with a wound healing process occurring in the degenerate tendon, albeit with impaired or incomplete remodelling, rather than any functional adaptation. The available evidence generally supports the hypothesis that accumulated micro-injuries result in a gradual deterioration in the quality of the tendon matrix. There is a gradual transformation of the matrix, from organized type I collagen fibrils to a tissue consisting of randomly organized, small-diameter fibrils containing type I and type III collagen.

Matrix turnover in tendinopathy

Although some of the changes found in ruptured tendons may be the result of rupture rather than the cause, there are reasons to suspect that a change in collagen turnover precedes and predisposes to tendon rupture. A study of the molecular age of the collagen network by an analysis of pentosidine content demonstrated greater levels of matrix turnover in supraspinatus tendons compared with age-matched biceps brachii tendons [61] (Fig. 2). Pentosidine did not accumulate in a linear fashion with age, and it was estimated that up to 50% of the collagen in the supraspinatus had been replaced over the individuals' lifetime [61] (Fig. 2B). Levels of pentosidine were substantially lower in ruptured tendons, demonstrating that a greater proportion of the matrix collagen, up to 90% of the total, was replaced in ruptured supraspinatus tendons [61]. These data have since been supported by an analysis of the racemization of the amino acid aspartate, another indicator of the molecular age of the protein network [86]. Thus it appears that a relatively high level of matrix remodelling is common in tendons such as the supraspinatus, and that this process is linked to the onset of degenerative pathology.

Increased collagen turnover in supraspinatus tendons was associated with the increased expression and activity of several members of the MMP family. In control supraspinatus tendons, MMP-1, MMP-2 and MMP-3 activities were significantly higher compared with normal biceps brachii tendons, which showed little or no collagen turnover [86]. In ruptured supraspinatus tendons, there were increased levels of MMP-1 activity, reduced levels of MMP-2 and MMP-3, and evidence of increased collagen denaturation and turnover [86]. MMP-3 (stromelysin 1) is thought to be a key regulatory enzyme in the control of matrix turnover, and a decline in this enzyme may represent a failure in the normal remodelling process. Thus, tendinopathy may result from a failure to repair or adequately maintain the tendon matrix in response to mechanical strain or repeated micro-trauma.

A study of synovial fluids from the glenohumeral joint of patients with rotator cuff pathology showed high levels of expression of both MMP-1 and MMP-3 in patients with ruptured tendons, with no change in the levels of TIMP-1, and the levels of enzyme correlated with the size of tear [120]. GAG levels were also higher in fluids from massive tears compared with partial tears, consistent with increased turnover of matrix proteoglycans [120]. Immunolocalization studies have shown MMP-1 expressed at the edge of the tear in ruptured supraspinatus [121] and increased expression in patellar tendinosis [122].

The importance of matrix turnover in tendon pathology was also demonstrated in a molecular study of Achilles tendinopathy using cDNA arrays [9]. Out of 265 genes that were analysed, 17 genes were up-regulated and 23 were down-regulated in degenerate tissue samples. The absence of inflammation within the tendon was confirmed, and there were large increases in the expression of matrix genes such as collagen type I and type III. The proteoglycans versican, biglycan and perlecan were increased, but there was no change in decorin. Glycoproteins such as laminin, SPARC (secreted protein acidic and rich in cysteine) and tenascin-C

were also increased; the latter observation was consistent with earlier biochemical studies [118]. In addition to matrix genes, there were substantial changes in MMP expression. MMP-2, MMP-3, MMP-14, MMP-16 and MMP-19 were all detected in normal tendon. The greatest difference between normal and pathological specimens was in the level of MMP-3 mRNA (also reflected in the level of MMP-3 protein), which was absent or less abundant in painful tendinopathy and ruptured tendon [9]. Expression of MMP-1 mRNA was not detected, either in normal tendons or in chronic tendon lesions. Because MMP-1 and MMP-3 are normally regulated co-ordinately, the difference in expression is an interesting observation that is yet to be explained. It is possible that MMP-3 activity is required for normal tendon maintenance, at least in highly stressed tendons, such as the supraspinatus and the Achilles. The loss of MMP-3 activity in tendinopathy could account for the increase in proteoglycan commonly found in tendon lesions, as these are potential substrates for the enzyme. However, more work is required to investigate the role of other proteoglycan-degrading enzymes, such as the aggrecanases (ADAMTS-1, 4 and 5), in tendon pathology.

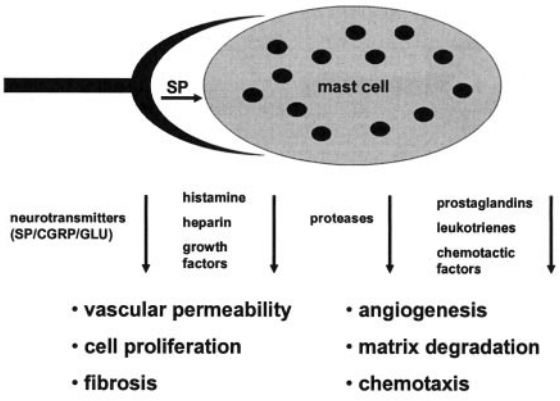
The important role of MMPs in tendon pathology is further supported by the side-effect profile of two very different drug compounds. First, a broad-spectrum inhibitor of matrix metalloproteinases was found to induce a painful tendinopathy in patients, usually in the shoulders or hands [123]. Detailed studies are required to determine which metalloproteinase activities are implicated—whether an MMP, ADAM or ADAMTS enzyme. Secondly, fluoroquinolone antibiotics can also induce tendinopathy in some patients [124], and these drugs have recently been shown to modulate MMP activity, at least *in vitro* [125, 126]. Studies on canine tenocytes showed a stimulation of caseinase activity by canine tenocytes treated with ciprofloxacin. In studies of human tenocytes we have shown a small stimulation of MMP-3 expression by ciprofloxacin [126]. In addition, pretreatment with ciprofloxacin potentiated the stimulatory effect of IL-1 on both MMP-1 and MMP-3 gene expression, and there was a corresponding increase in synthesis of MMP-3 [126]. Because fluoroquinolones may also stimulate inflammatory pathways in or around the tendon [127], the combined effect on tendon matrix turnover may account for the onset of tendinopathy in some patients.

Regulation of matrix turnover in tendinopathy

Despite many elegant hypotheses and *in vitro* studies, the factors that induce pathological matrix remodelling in tendinopathy have not been fully characterized. There is evidence that cell activities are substantially altered in chronic tendon lesions. Cells in ruptured tendons showed evidence of hypoxic changes [5], and levels of lactate were increased in the peritendinous fluid of patients with Achilles tendinopathy, consistent with increased anaerobic activity [128]. There was evidence of increased apoptosis in degenerate rotator cuff tendons (almost 2.5 times higher than in controls), implicating programmed cell death in the pathology [129].

Matrix interactions, insoluble deposits, mechanical strain and locally released cytokines and signalling molecules (to name but a few factors) may have a direct effect on tenocyte activity and the expression of tendon matrix genes and enzymes. For example, amyloid, calcific deposits and breakdown products of proteins such as tenascin-C have been shown to accumulate in degenerate tendon [118, 130, 131]. Insoluble deposits are capable of increasing the expression of enzyme activities and stimulating matrix turnover, at least *in vitro* [132, 133]. Although inflammatory cytokines such as IL-1 have not been detected in the tendon itself, there was greater expression of IL-1β (and IL-1 receptor antagonist) in the synovium of patients with perforating rotator cuff tears compared with those with non-perforating tears, although the degree of shoulder pain was inversely correlated with the levels of gene expression [134, 135]. The inducible cyclooxygenase enzyme COX-2, a key regulator in the pathway of prostaglandin synthesis, was significantly increased in patellar tendinosis, and there was increased expression of transforming growth factor β (TGF-β1) [136]. Increased cellularity at the site of the tendon lesion was associated with the levels of expression of platelet-derived growth factor (PDGF) receptor, and cells derived from lesions showed increased rates of cell proliferation and a greater response to PDGF compared with normal tendon cells [137]. Other mediators of inflammation and vascular perfusion that are stimulated by exercise, such as bradykinin, adenosine, IL-6, thromboxane and prostaglandin E_2, may also play a role [138] (see above).

Because substance P and other neuropeptides have been detected in tendon and in fluids collected from around painful tendons [139–141], there is evidence for a 'neurogenic' hypothesis of tendon overuse injury [142] (Fig. 3). Nerve endings and mast cells may function as units to modulate tendon homeostasis and mediate adaptive responses to mechanical strain. Excessive stimulation as a result of overuse may result in pathological changes to the tendon matrix. In support of this hypothesis, substance P has been shown to modulate the expression of several (rabbit) tendon matrix



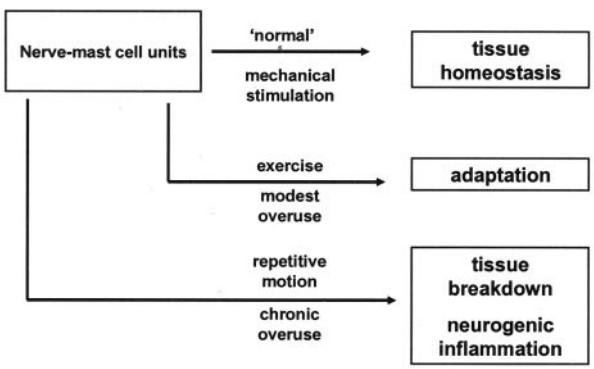


FIG. 3. Neurogenic hypothesis of tendon 'overuse' injury. (A) Nerve endings and mast cells in the matrix exist as functional units in the tendon matrix. The release of neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP) stimulates the degranulation of mast cells, releasing a panoply of agents which modulate a variety of cell activities in the matrix. (B) Nerve and mast cell units function to modulate homeostatic and adaptive responses in the normal tendon, although excessive stimulation leads to tissue breakdown and degeneration. Adapted from Hart *et al.* [142].

genes and enzymes, including MMP-1, although the precise effects were dependent on gender and hormonal status [143].

A study of TGF-β expression in the Achilles showed that one isoform (TGF-β2) was predominant in the fibrillar matrix of both normal and pathological tendons [144]. Although TGF-β2 was increased in Achilles tendinopathy, the absence of one of the TGF-β signalling receptors (TGF-βR1) was consistent with the hypothesis that TGF-β signalling was not actually taking place in the tendon. Consequently, failure to control matrix degradation may result from failure to up-regulate TIMPs in response to TGF-β. This observation suggests that the addition of growth factors such as TGF-β to facilitate tendon repair may be ineffective in chronic tendinopathy. It is also consistent with the hypothesis that the chronic nature of tendinopathy represents a failure to regulate cell activities appropriately during repair or matrix remodelling. Currently there are no therapies for tendinopathy that specifically address this problem.

Animal models, gene knockouts and tendon pathology

Studies of tendinopathy are hampered by the absence of suitable animal models that mimic the chronic condition seen in humans. Investigations of equine tendon lesions have demonstrated accumulated damage after prolonged, high-intensity exercise, with changes in the matrix similar to those described in human tendinopathy [82, 145, 146]. Attempts to recreate tendon lesions in smaller animals using exercise regimes have been generally less successful [147]. Electrical stimulation of rabbit triceps surae muscles to provoke kicking activity can induce changes in the paratenon, and possibly also degeneration of the tendon matrix, although these lesions have proved difficult to reproduce [148, 149]. Various surgical procedures have been used in rabbit and rodent models, as has the injection of enzymes (collagenase) or inflammatory mediators such as prostaglandins [150–152]. Treatment of rodents with fluoroquinolone antibiotics can rapidly induce tendon lesions, with inflammation of the paratenon preceding degenerative changes in the tendon matrix [153, 154]. Because these drugs can affect MMP expression by tendon cells, this model may provide useful information about the disease [126, 155].

The role of specific proteins has been investigated in gene-knockout animal models. Mice deficient in any one of the small proteoglycans (decorin, biglycan, fibromodulin or lumican) manifest with smaller-diameter collagen fibrils, joint laxity and tendon weakness [156, 157]. Recently, inhibition of decorin expression has been used to increase the diameter of collagen fibres in healing ligaments, and this approach may have some promise in the treatment of tendon injury, improving the mechanical properties of the scar tissue [158]. Mice with a mutated collagen cleavage site that renders type I collagen resistant to collagenase have thicker tendons than normal, and the tendon insertion fails to migrate with bone growth, demonstrating the importance of collagen turnover, at least during development [159, 160]. Mice lacking MMP-13, the rodent equivalent of MMP-1, showed severely delayed wound healing in the skin, although the effects on tendon healing have not been studied [161]. Future studies combining models of tendon pathology with specific gene-knockouts are likely to yield important information on the role of specific matrix proteins and enzymes in tendon development and disease.

Summary

The tendon matrix is ideally constructed to perform its major function, the transfer of force from muscle to bone. It is not a static tissue, and the matrix will adapt according to the level, direction and frequency of the applied load, a process of remodelling mediated by the tendon fibroblasts. The tendon matrix is constantly remodelled throughout life, although higher rates of turnover are found at sites exposed to high levels of strain, compression or shear forces, such as the Achilles and the supraspinatus. Matrix changes in tendon pathology could be attributed to intrinsic factors, such as changes in cell activity with age, or extrinsic factors, such as 'overuse', repetitive strain and microtrauma. In degenerate tendon there is an increased rate of matrix remodelling, leading to a qualitatively different and mechanically less stable tendon, which is susceptible to damage. Thus tendon degeneration may result from a failure to regulate specific MMP activities in response to repeated injury or mechanical strain. Whether tendon repair can be improved merits further investigation of the factors controlling matrix remodelling in tendon. These factors include mechanical strain, growth factors, cytokines and neuropeptides, all of which have been implicated in the control of tendon homeostasis, adaptation and repair.

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References

1. Józsa L, Kannus P. Tendon alterations in inherited diseases. In: Józsa L, Kannus P, eds. Human tendons: anatomy, physiology and pathology. Champaigns: Human Kinetics, 1997:390–402.
2. Józsa L, Kannus P. Tendon alterations in endocrinologic and metabolic diseases. In: Józsa L, Kannus P, eds. Human tendons: anatomy, physiology and pathology. Champaign: Human Kinetics, 1997:403–12.
3. Józsa L, Kannus P. Tendon alterations in rheumatic diseases. In: Józsa L, Kannus P, eds. Human tendons: anatomy, physiology and pathology. Champaign: Human Kinetics, 1997:412–29.
4. Puddu G, Ippolito E, Postacchini F. A classification of Achilles tendon disease. Am J Sports Med 1976;4:145–50.
5. Kannus P, Józsa L. Histopathological changes preceding spontaneous rupture of a tendon. A controlled study of 891 patients. J Bone Joint Surg Am 1991;73A:1507–25.
6. Åström M, Rausing A. Chronic Achilles tendinopathy. A survey of surgical and histopathologic findings. Clin Orthop 1995;316:151–64.
7. Maffulli N, Khan KM, Puddu G. Overuse tendon conditions: time to change a confusing terminology. Arthroscopy 1998;14:840–3.
8. Khan KM, Cook JL, Bonar F, Harcourt P, Åström M. Histopathology of common tendinopathies—Update and implications for clinical management. Sports Med 1999;27:393–408.
9. Ireland D, Harrall RL, Holloway G, Hackney R, Hazleman BL, Riley GP. Multiple changes in gene expression in chronic human Achilles tendinopathy. Matrix Biol 2001;20:159–69.
10. Józsa L, Kannus P. Spontaneous rupture of tendons. In: Józsa L, Kannus P, eds. Human tendons: anatomy, physiology and pathology. Champaign: Human Kinetics, 1997:254–325.
11. Gibson W. Are 'spontaneous' Achilles tendon ruptures truly spontaneous? Br J Sports Med 1998;32:266.
12. Chard MD, Cawston TE, Riley GP, Gresham A, Hazleman BL. Rotator cuff degeneration and lateral epicondylitis: a comparative histological study. Ann Rheum Dis 1994;53:30–4.
13. Movin T, Gad A, Reinholt P, Rolf C. Tendon pathology in long-standing achillodynia—Biopsy findings in 40 patients. Acta Orthopaed Scand 1997;68:170–5.
14. Riley GP, Goddard MJ, Hazleman BL. Histopathological assessment and pathological significance of matrix degeneration in supraspinatus tendons. Rheumatology 2001;40:229–30.
15. Kannus P. Etiology and pathophysiology of chronic tendon disorders in sports. Scand J Med Sci Sports 1997;7:78–85.
16. Kannus P. Tendon pathology: Basic science and clinical applications. Sports Exercise Inj 1997;3:62–75.

17. Nirschl RP. Mesenchymal syndrome. Va Med Mon (1918) 1969;96:659–62.
18. Brooks CH, Revell WJ, Heatley FW. A quantitative histological study of the vascularity of the rotator cuff tendon. J Bone Joint Surg 1992;74B:151–3.
19. Ahmed IM, Lagopoulos M, McConnell P, Soames RW, Sefton GK. Blood supply of the Achilles tendon. J Orthop Res 1998;16:591–6.
20. Leadbetter WB. Cell-matrix response in tendon injury. Clin Sports Med 1992;11:533–78.
21. Fenwick SA, Hazleman BL, Riley GP. The vasculature and its role in the damaged and healing tendon. Arthritis Res 2002;4:252–60.
22. Nirschl RP, Pettrone FA. Tennis elbow. J Bone Joint Surg 1973;61A:832–9.
23. Åström M, Westlin N. Blood-flow in chronic Achilles tendinopathy. Clin Orthop Rel Res 1994;308:166–72.
24. Åström M. Laser Doppler flowmetry in the assessment of tendon blood flow. Scand J Med Sci Sports 2000;10:365–7.
25. Herring SA, Nilson KL. Introduction to overuse injuries. Clin Sports Med 1987;6:225–39.
26. Józsa L, Kannus P. Overuse injuries of tendons. In: Józsa L, Kannus P, eds. Human tendons: anatomy, physiology and pathology. Champaign: Human Kinetics, 1997:164–253.
27. Selvanetti A, Cipolla M, Puddu G. Overuse tendon injuries: Basic science and classification. Operative Tech Sports Med 1997;5:110–7.
28. Kjaer M. The treatment of overuse injuries in sports. Scand J Med Sci Sports 2001;11:195–6.
29. Butler DL, Grood ES, Noyes FR, Zernicke RF. Biomechanics of ligaments and tendons. Exerc Sport Sci Rev 1978;6:125–81.
30. Elliott DH. Structure and function of mammalian tendon. Biol Rev 1965;40:392–421.
31. Almekinders LC, Temple JD. Etiology, diagnosis, and treatment of tendonitis: an analysis of the literature. Med Sci Sports Exerc 1998;30:1183–90.
32. Khan KM, Cook JL, Maffulli N, Kannus P. Where is the pain coming from in tendinopathy? It may be biochemical, not only structural, in origin. Br J Sports Med 2000;34:81–3.
33. Khan KM, Cook JL, Kannus P, Maffulli N, Bonar SF. Time to abandon the 'tendinitis' myth. BMJ 2002;324:626–7.
34. Kastelic J, Galeski A, Baer E. The multicomposite structure of tendon. Connect Tissue Res 1978;6:11–23.
35. Miller EJ, Gay S. Collagen: an overview. Methods Enzymol 1982;82:3–32.
36. Burgeson RE, Nimni ME. Collagen types: Molecular structure and tissue distribution. Clin Orthop 1992;282:250–72.
37. Greenlee TK, Ross R. The development of the rat flexor digital tendon: a fine structure study. J Ultrastruct Res 1967;18:353–76.
38. Parry DAD, Barnes GRG, Craig AS. A comparison of the size distribution of collagen fibrils in connective tissues as a function of age and a possible relation between fibril size distribution and mechanical properties. Proc R Soc Lond B Biol Sci 1978;203: 305–21.
39. Moore MJ, De Beaux A. A quantitative ultrastructural study of rat tendon from birth to maturity. J Anat 1987;153:163–9.
40. Józsa L, Kannus P. Structure and metabolism of normal tendons. In: Józsa L, Kannus P, eds. Human tendons: anatomy physiology and pathology. Champaign: Human Kinetics, 1997:46–95.
41. Parry DAD, Flint MH, Gillard GC, Craig AS. A role for glycosaminoglycans in the development of collagen fibrils. FEBS Lett 1982;149:1–7.
42. Ochiai N, Matsui T, Miyaji N, Merklin RJ, Hunter JM. Vascular anatomy of flexor tendons. I. Vincular system and blood supply of the profundus tendon in the digital sheath. J Hand Surg Am 1979; 4:321–30.
43. Józsa L, Kannus P, Balint JB, Reffy A. Three-dimensional ultrastructure of human tendons. Acta Anat (Basel) 1991;142: 306–12.
44. Williams IF, Craig AS, Parry DAD, Goodship AE, Shah J, Silver IA. Development of collagen fibril organization and collagen crimp patterns during tendon healing. Int J Biol Macromol 1985;7:275–82.
45. Wilmink J, Wilson AM, Goodship AE. Functional significance of the morphology and micromechanics of collagen fibres in relation to partial rupture of the superficial digital flexor tendon in racehorses. Res Vet Sci 1992;53:354–9.
46. Evans JH, Barbenel JC. Structural and mechanical properties of tendon related to function. Equine Vet J 1975;7:1–8.
47. von der Mark K. Localization of collagen types in tissues. Int Rev Connect Tissue Res 1981;9:265–324.
48. Riley GP, Harrall RL, Constant CR, Chard MD, Cawston TE, Hazleman BL. Tendon degeneration and chronic shoulder pain: Changes in the collagen composition of the human rotator cuff tendons in rotator cuff tendinitis. Ann Rheum Dis 1994;53: 359–66.
49. Aumailley M, Gayraud B. Structure and biological activity of the extracellular matrix. J Mol Med 1998;76:253–65.
50. Duance VC, Restall DJ, Beard H, Bourne FJ, Bailey AJ. The location of three collagen types in skeletal muscle. FEBS Lett 1977;79:248–52.
51. Fan L, Sarkar K, Franks DJ, Uhthoff HK. Estimation of total collagen and types I and III collagen in canine rotator cuff tendons. Calcif Tissue Int 1997;61:223–9.
52. Lapiere CM, Nusgens B, Pierard GE. Interaction between collagen type I and type III in conditioning bundles organization. Connect Tissue Res 1977;5:21–9.
53. Linsenmayer TF, Fitch JM, Birk DE. Heterotypic collagen fibrils and stabilizing collagens: Controlling elements in corneal morphogenesis. Ann NY Acad Sci 1990;580:143–60.
54. Birk DE, Fitch JM, Babiarz JP, Doane KJ, Linsenmayer TF. Collagen fibrillogenesis *in vitro*: Interaction of types I and V collagen regulates fibril diameter. J Cell Sci 1990;95:649–57.
55. Waggett AD, Ralphs JR, Kwan APL, Woodnutt D, Benjamin M. Characterization of collagens and proteoglycans at the insertion of the human Achilles tendon. Matrix Biol 1998;16:457–70.
56. Shaw LM, Olsen BR. FACIT collagens: Diverse molecular bridges in extracellular matrices. Trends Biochem Sci 1991;16:191–4.
57. Fukuta S, Oyama M, Kavalkovich K, Fu FH, Niyibizi C. Identification of types II, IX and X collagens at the insertion site of the bovine Achilles tendon. Matrix Biol 1998;17:65–73.
58. Eyre DR, Paz MA, Gallop PM. Cross-linking in collagen and elastin. Annu Rev Biochem 1984;53:717–48.
59. Bailey AJ, Paul RG, Knott L. Mechanisms of maturation and ageing of collagen. Mech Ageing Dev 1998;106:1–56.
60. Knott L, Bailey AJ. Collagen cross-links in mineralizing tissues: A review of their chemistry, function, and clinical relevance. Bone 1998;22:181–7.
61. Bank RA, TeKoppele JM, Oostingh G, Hazleman BL, Riley GP. Lysylhydroxylation and non-reducible cross-linking of human supraspinatus tendon collagen: changes with age and in chronic rotator cuff tendinitis. Ann Rheum Dis 1999;58:35–41.
62. Vogel KG, Koob TJ. Structural specialisation in tendons under compression. Int Rev Cytol 1989;115:267–93.
63. Riley GP, Harrall RL, Constant CR, Chard MD, Cawston TE, Hazleman BL. Glycosaminoglycans of human rotator cuff tendons: Changes with age and in chronic rotator cuff tendinitis. Ann Rheum Dis 1994;53:367–76.
64. Berenson MC, Blevins FT, Plaas AHK, Vogel KG. Proteoglycans of human rotator cuff tendons. J Orthop Res 1996;14:518–25.
65. Monnier VM, Sell DR, Nagaraj RH *et al*. Maillard reaction-mediated molecular damage to extracellular matrix and other tissue proteins in diabetes, aging, and uremia. Diabetes 1992;41(Suppl. 2):36–41.
66. DeGroot J, Verzijl N, Jacobs KM *et al*. Accumulation of advanced glycation endproducts reduces chondrocyte-mediated extracellular matrix turnover in human articular cartilage. Osteoarthritis Cartilage 2001;9:720–6.
67. Bank RA, Verzijl N, Lafeber FP, TeKoppele JM. Putative role of lysyl hydroxylation and pyridinoline cross-linking during adolescence in the occurrence of osteoarthritis at old age. Osteoarthritis Cartilage 2002;10:127–34.

68. Sell DR, Monnier VM. Structural elucidation of a senescence cross-link from human extra-cellular matrix. Implication of pentoses in the aging process. J Biol Chem 1989;264:21594–602.
69. Hardingham TE, Fosang AJ. Proteoglycans: many forms and many functions. FASEB J 1992;6:861–70.
70. Koob TJ, Vogel KG. Site related variations in glycosaminoglycan content and swelling properties of bovine flexor tendon. J Orthop Res 1987;5:414–24.
71. Vogel KG, Heinegård D. Characterisation of proteoglycans from adult bovine tendon. J Biol Chem 1985;260:9298–306.
72. Vogel KG, Sandy JD, Pogány G, Robbins JR. Aggrecan in bovine tendon. Matrix 1994;14:171–9.
73. Okuda Y, Gorski JP, An K-N, Amadio PC. Biochemical histological and biomechanical analyses of canine tendon. J Orthop Res 1987;5:60–8.
74. Merrilees MJ, Flint MH. Ultrastructural study of tension and pressure zones in a rabbit flexor tendon. Am J Anat 1980;157:87–106.
75. Gillard GC, Reilly HC, Bell-Booth PG, Flint MH. The influence of mechanical forces on the glycosaminoglycan content of the rabbit flexor digitorum profundus tendon. Connect Tissue Res 1979;7: 37–46.
76. Benjamin M, Qin S, Ralphs JR. Fibrocartilage associated with human tendons and their pulleys. J Anat 1995;187:625–33.
77. Vogel KG, Ördög A, Pogány G, Oláh J. Proteoglycans in the compressed region of human tibialis posterior tendon and in ligaments. J Orthop Res 1993;11:68–77.
78. Ippolito E, Natali PG, Postacchini F, Accini L, de Martino C. Morphological, immunological and biochemical study of rabbit Achilles tendon at various ages. J Bone Joint Surg 1980;62A:583–98.
79. Vogel HG. Age dependence of mechanical properties of rat tail tendons (hysteresis experiments). Akt Gerontol 1983;13:22–7.
80. Carlstedt CA. Mechanical and chemical factors in tendon healing. Acta Orthop Scand 1987;58(Suppl. 224):7–75.
81. Paul RG, Bailey AJ. Glycation of collagen: The basis of its central role in the late complications of ageing and diabetes. Int J Biochem Cell Biol 1996;28:1297–310.
82. Smith RK, Birch H, Patterson-Kane J *et al*. Should equine athletes commence training during skeletal development? Changes in tendon matrix associated with development, ageing, function and exercise. Equine Vet J Suppl 1999;30:201–9.
83. Jarvinen M, Józsa L, Kannus P, Jarvinen TLN, Kvist M, Leadbetter W. Histopathological findings in chronic tendon disorders. Scand J Med Sci Sports 1997;7:86–95.
84. Chard MD, Wright JK, Hazleman BL. Isolation and growth characteristics of adult human tendon fibroblasts. Ann Rheum Dis 1987;46:385–90.
85. Riley GP, Cox M, Harrall RL, Clements S, Hazleman BL. Inhibition of tendon cell proliferation and matrix glycosaminoglycan synthesis by non-steroidal anti-inflammatory drugs *in vitro*. J Hand Surg Br 2001;26:224–8.
86. Riley GP, Curry V, DeGroot J *et al*. Matrix metalloproteinase activities and their relationship with collagen remodelling in tendon pathology. Matrix Biol 2002;21:185–95.
87. Langberg H, Skovgaard D, Petersen LJ, Bulow J, Kjaer M. Type I collagen synthesis and degradation in peritendinous tissue after exercise determined by microdialysis in humans. J Physiol 1999; 521:299–306.
88. Langberg H, Skovgaard D, Asp S, Kjaer M. Time pattern of exercise-induced changes in type I collagen turnover after prolonged endurance exercise in humans. Calcif Tissue Int 2000;67:41–4.
89. Langberg H, Rosendal L, Kjaer M. Training-induced changes in peritendinous type I collagen turnover determined by microdialysis in humans. J Physiol 2001;534:297–302.
90. Langberg H, Skovgaard D, Karamouzis M, Bulow J, Kjaer M. Metabolism and inflammatory mediators in the peritendinous space measured by microdialysis during intermittent isometric exercise in humans. J Physiol 1999;515:919–27.
91. Karamouzis M, Langberg H, Skovgaard D, Bulow J, Kjaer M, Saltin B. In situ microdialysis of intramuscular prostaglandin and thromboxane in contracting skeletal muscle in humans. Acta Physiol Scand 2001;171:71–6.
92. Langberg H, Bjorn C, Boushel R, Hellsten Y, Kjaer M. Exercise-induced increase in interstitial bradykinin and adenosine concentrations in skeletal muscle and peritendinous tissue in humans. J Physiol 2002;542:977–83.
93. Langberg H, Olesen JL, Gemmer C, Kjaer M. Substantial elevation of interleukin-6 concentration in peritendinous tissue, in contrast to muscle, following prolonged exercise in humans. J Physiol 2002; 542:985–90.
94. Verzijl N, DeGroot J, Thorpe SR *et al*. Effect of collagen turnover on the accumulation of advanced glycation end products. J Biol Chem 2000;275:39027–31.
95. Verzijl N, DeGroot J, Oldehinkel E *et al*. Age-related accumulation of Maillard reaction products in human articular cartilage collagen. Biochem J 2000;350:381–7.
96. Everts V, Van der Zee E, Creemers L, Beertsen W. Phagocytosis and intracellular digestion of collagen, its role in turnover and remodelling. Histochem J 1996;28:229–45.
97. Creemers LB, Jansen IDC, Docherty AJP, Reynolds JJ, Beertsen W, Everts V. Gelatinase A (MMP-2) and cysteine proteinases are essential for the degradation of collagen in soft connective tissue. Matrix Biol 1998;17:35–46.
98. Matrisian LM. The matrix-degrading metalloproteinases. BioEssays 1992;14:455–63.
99. Cawston TE. Proteinases and inhibitors. Br Med Bull 1995;51: 385–401.
100. Nagase H, Woessner JF. Matrix metalloproteinases. J Biol Chem 1999;274:21491–4.
101. Knäuper V, López-Otin C, Smith B, Knight G, Murphy G. Biochemical characterization of human collagenase-3. J Biol Chem 1996;271:1544–50.
102. Dalton SE, Cawston TE, Riley GP, Bayley IJL, Hazleman BL. Human tendon biopsy samples in organ culture produce procollagenase and tissue inhibitor of metalloproteinases. Ann Rheum Dis 1995;54:571–7.
103. Cawston TE, Curry VA, Summers CA *et al*. The role of oncostatin M in animal and human connective tissue collagen turnover and its localization within the rheumatoid joint. Arthritis Rheum 1998; 41:1760–71.
104. Kaushal GP, Shah SV. The new kids on the block: ADAMTSs, potentially multifunctional metalloproteinases of the ADAM family. J Clin Invest 2000;105:1335–7.
105. Tortorella MD, Burn TC, Pratta MA *et al*. Purification and cloning of aggrecanase-1: a member of the ADAMTS family of proteins. Science 1999;284:1664–6.
106. Abbaszade I, Liu RQ, Yang F *et al*. Cloning and characterization of ADAMTS11, an aggrecanase from the ADAMTS family. J Biol Chem 1999;274:23443–50.
107. Kuno K, Okada Y, Kawashima H *et al*. ADAMTS-1 cleaves a cartilage proteoglycan, aggrecan. FEBS Lett 2000;478:241–5.
108. Nakamura H, Fujii Y, Inoki I *et al*. Brevican is degraded by matrix metalloproteinases and aggrecanase-1 (ADAMTS4) at different sites. J Biol Chem 2000;275:38885–90.
109. Campbell MA, Winter AD, Ilic MZ, Handley CJ. Catabolism and loss of proteoglycans from cultures of bovine collateral ligament. Arch Biochem Biophys 1996;328:64–72.
110. Rees SG, Flannery CR, Little CB, Hughes CE, Caterson B, Dent CM. Catabolism of aggrecan, decorin and biglycan in tendon. Biochem J 2000;350:181–8.
111. Birch HL, Bailey AJ, Goodship AE. Macroscopic 'degeneration' of equine superficial digital flexor tendon is accompanied by a change in extracellular matrix composition. Equine Vet J 1998;30:534–9.
112. Williams IF, Heaton A, McCullagh KG. Cell morphology and collagen types in equine tendon scar. Res Vet Sci 1980;28: 302–10.
113. Silver IA, Brown PN, Goodship AE *et al*. A clinical and experimental study of tendon injury, healing and treatment in the horse. Equine Vet J Suppl. 1983;1:1–24.

114. Williams IF, McCullagh KG, Silver IA. The distribution of types I and III collagen and fibronectin in the healing equine tendon. Connect Tissue Res 1984;12:211–22.
115. Watkins JP, Auer JA, Gay S, Morgan SJ. Healing of surgically created defects in the equine superficial digital flexor tendon: collagen-type transformation and tissue morphologic reorganization. Am J Vet Res 1985;46:2091–6.
116. Bailey AJ, Bazin S, Sims TJ, LeLous M, Nicoletis C, Delaunay A. Characterisation of the collagen of human hypertrophic and normal scars. Biochim Biophys Acta 1975;405:412–21.
117. Maffulli N, Waterston SW, Ewen SW. Ruptured Achilles tendons show increased lectin stainability. Med Sci Sports Exerc 2002; 34:1057–64.
118. Riley GP, Harrall RL, Cawston TE, Hazleman BL, Mackie EJ. Tenascin-C and human tendon degeneration. Am J Pathol 1996; 149:933–43.
119. Tillander B, Franzen L, Norlin R. Fibronectin, MMP-1 and histologic changes in rotator cuff disease. J Orthop Res 2002; 20:1358–64.
120. Yoshihara Y, Hamada K, Nakajima T, Fujikawa K, Fukuda H. Biochemical markers in the synovial fluid of glenohumeral joints from patients with rotator cuff tear. J Orthop Res 2001;19:573–9.
121. Gotoh M, Hamada K, Yamakawa H, Tomonaga A, Inoue A, Fukuda H. Significance of granulation tissue in torn supraspinatus insertions: an immunohistochemical study with antibodies against interleukin-1 beta, cathepsin D, and matrix metalloprotease-1. J Orthop Res 1997;15:33–9.
122. Fu SC, Chan BP, Wang W, Pau HM, Chan KM, Rolf CG. Increased expression of matrix metalloproteinase 1 (MMP1) in 11 patients with patellar tendinosis. Acta Orthop Scand 2002;73: 658–62.
123. Millar AW, Brown PD, Moore J *et al*. Results of single and repeat dose studies of the oral matrix metalloproteinase inhibitor marimastat in healthy male volunteers. Br J Clin Pharmacol 1998; 45:21–6.
124. van der Linden PD, van Puijenbroek EP, Feenstra J *et al*. Tendon disorders attributed to fluoroquinolones: a study on 42 spontaneous reports in the period 1988 to 1998. Arthritis Rheum 2001;45:235–9.
125. Williams RJ, Attia E, Wickiewicz TL, Hannafin JA. The effect of ciprofloxacin on tendon, paratenon, and capsular fibroblast metabolism. Am J Sports Med 2000;28:364–9.
126. Corps AN, Harrall RL, Curry VA, Fenwick SA, Hazleman BL, Riley GP. Ciprofloxacin enhances the stimulation of matrix metalloproteinase 3 expression by interleukin-1beta in human tendon-derived cells. Arthritis Rheum 2002;46:3034–40.
127. Kashida Y, Kato M. Characterization of fluoroquinolone-induced Achilles tendon toxicity in rats: Comparison of toxicities of 10 fluoroquinolones and effects of anti-inflammatory compounds. Antimicrob Agents Chemother 1997;41:2389–93.
128. Alfredson H, Bjur D, Thorsen K, Lorentzon R, Sandstrom P. High intratendinous lactate levels in painful chronic Achilles tendinosis. An investigation using microdialysis technique. J Orthop Res 2002;20:934–8.
129. Yuan J, Murrell GA, Wei AQ, Wang MX. Apoptosis in rotator cuff tendonopathy. J Orthop Res 2002;20:1372–9.
130. Cole AS, Cordiner-Lawrie S, Carr AJ, Athanasou NA. Localised deposition of amyloid in tears of the rotator cuff. J Bone Joint Surg Br 2001;83:561–4.
131. Riley GP, Harrall RL, Constant CR, Cawston TE, Hazleman BL. Prevalence and possible pathological significance of calcium phosphate salt accumulation in tendon matrix degeneration. Ann Rheum Dis 1996;55:109–15.
132. McCarthy GM, Mitchell PG, Struve JA, Cheung HS. Basic calcium phosphate crystals cause coordinate induction and secretion of collagenase and stromelysin. J Cell Physiol 1992;153:140–6.
133. Deb S, Gottschall PE. Increased production of matrix metalloproteinases in enriched astrocyte and mixed hippocampal cultures treated with β-amyloid peptides. J Neurochem 1996;66:1641–7.
134. Gotoh M, Hamada K, Yamakawa H *et al*. Perforation of rotator cuff increases interleukin 1beta production in the synovium of glenohumeral joint in rotator cuff diseases. J Rheumatol 2000; 27:2886–92.
135. Gotoh M, Hamada K, Yamakawa H *et al*. Interleukin-1-induced subacromial synovitis and shoulder pain in rotator cuff diseases. Rheumatology 2001;40:995–1001.
136. Fu SC, Wang W, Pau HM, Wong YP, Chan KM, Rolf CG. Increased expression of transforming growth factor-beta1 in patellar tendinosis. Clin Orthop 2002;174–83.
137. Rolf CG, Fu BSC, Pau A, Wang W, Chan B. Increased cell proliferation and associated expression of PDGFRβ causing hypercellularity in patellar tendinosis. Rheumatology 2001;40: 256–61.
138. Kjaer M, Langberg H, Skovgaard D *et al*. *In vivo* studies of peritendinous tissue in exercise. Scand J Med Sci Sports 2000; 10:326–31.
139. Ljung BO, Forsgren S, Fridén J. Substance P and calcitonin gene-related peptide expression at the extensor carpi radialis brevis muscle origin: implications for the etiology of tennis elbow. J Orthop Res 1999;17:554–9.
140. Gotoh M, Hamada K, Yamakawa H, Inoue A, Fukuda H. Increased substance P in subacromial bursa and shoulder pain in rotator cuff diseases. J Orthop Res 1998;16:618–21.
141. Ackermann PW, Finn A, Ahmed M. Sensory neuropeptidergic pattern in tendon, ligament and joint capsule. A study in the rat. Neuroreport 1999;10:2055–60.
142. Hart DA, Frank CB, Bray RC. Inflammatory processes in repetitive motion and overuse syndromes: potential role of neurogenic mechanisms in tendons and ligaments. In: Gordon SL, Blair SJ, Fine LJ, eds. Repetitive motion disorders of the upper extremity. Rosemont: American Academy of Orthopaedic Surgeons, 1995:247–62.
143. Hart DA, Kydd A, Reno C. Gender and pregnancy affect neuropeptide responses of the rabbit Achilles tendon. Clin Orthop 1999;365:237–46.
144. Fenwick SA, Curry V, Harrall RL, Hazleman BL, Hackney R, Riley GP. Expression of transforming growth factor-beta isoforms and their receptors in chronic tendinosis. J Anat 2001;199:231–40.
145. Patterson-Kane JC, Parry DA, Birch HL, Goodship AE, Firth EC. An age-related study of morphology and cross-link composition of collagen fibrils in the digital flexor tendons of young thoroughbred horses. Connect Tissue Res 1997;36:253–60.
146. Patterson-Kane JC, Wilson AM, Firth EC, Parry DAD, Goodship AE. Comparison of collagen fibril populations in the superficial digital flexor tendons of exercised and nonexercised thoroughbreds. Equine Vet J 1997;29:121–5.
147. Messner K, Wei Y, Andersson B, Gillquist J, Rasanen T. Rat model of Achilles tendon disorder. A pilot study. Cells Tissues Organs 1999;165:30–9.
148. Backman C, Boqvist L, Fridén J, Lorentzon R, Toolanen G. Chronic achilles paratenonitis with tendinosis: an experimental model in the rabbit. J Orthop Res 1990;8:541–7.
149. Archambault JM, Hart DA, Herzog W. Response of rabbit Achilles tendon to chronic repetitive loading. Connect Tissue Res 2001;42:13–23.
150. Carpenter JE, Thomopoulos S, Flanagan CL, DeBano CM, Soslowsky LJ. Rotator cuff defect healing: a biomechanical and histologic analysis in an animal model. J Shoulder Elbow Surg 1998;7:599–605.
151. Sullo A, Maffulli N, Capasso G, Testa V. The effects of prolonged peritendinous administration of PGE1 to the rat Achilles tendon: a possible animal model of chronic Achilles tendinopathy. J Orthop Sci 2001;6:349–57.
152. Dahlgren LA, van der Meulen MC, Bertram JE, Starrak GS, Nixon AJ. Insulin-like growth factor-I improves cellular and molecular aspects of healing in a collagenase-induced model of flexor tendinitis. J Orthop Res 2002;20:910–9.
153. Kato M, Takada S, Kashida Y, Nomura M. Histological examination on Achilles tendon lesions induced by quinolone antibacterial agents in juvenile rats. Toxicol Pathol 1995;23:385–92.
154. Kashida Y, Kato M. Characterization of fluoroquinolone-induced

Achilles tendon toxicity in rats: comparison of toxicities of 10 fluoroquinolones and effects of anti-inflammatory compounds. Antimicrob Agents Chemother 1997;41:2389–93.
155. Williams RJ III, Attia E, Wickiewicz TL, Hannafin JA. The effect of ciprofloxacin on tendon, paratenon, and capsular fibroblast metabolism. Am J Sports Med 2000;28:364–9.
156. Ameye L, Aria D, Jepsen K, Oldberg A, Xu T, Young MF. Abnormal collagen fibrils in tendons of biglycan/fibromodulin-deficient mice lead to gait impairment, ectopic ossification, and osteoarthritis. FASEB J 2002;16:673–80.
157. Jepsen KJ, Wu F, Peragallo JH *et al*. A syndrome of joint laxity and impaired tendon integrity in lumican- and fibromodulin-deficient mice. J Biol Chem 2002;277:35532–40.
158. Nakamura N, Hart DA, Boorman RS *et al*. Decorin antisense gene therapy improves functional healing of early rabbit ligament scar with enhanced collagen fibrillogenesis in vivo. J Orthop Res 2000; 18:517–23.
159. Liu X, Wu H, Byrne M, Jeffrey J, Krane S, Jaenisch R. A targeted mutation at the known collagenase cleavage site in mouse type I collagen impairs tissue remodeling. J Cell Biol 1995;130:227–37.
160. Krane SMZW. Collagenase in embryonic development and postnatal remodeling of connective tissues. In: Hoeffler W, ed. Collagenases. Austin: R.G. Landes, 1999:171–87.
161. Beare AH, O'Kane S, Krane SM, Ferguson MW. Severely impaired wound healing in the collagenase-resistant mouse. J Invest Dermatol 2003;120:153–63.