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TOPICAL REVIEW

The impact of loading, unloading, ageing and injury on the human tendon

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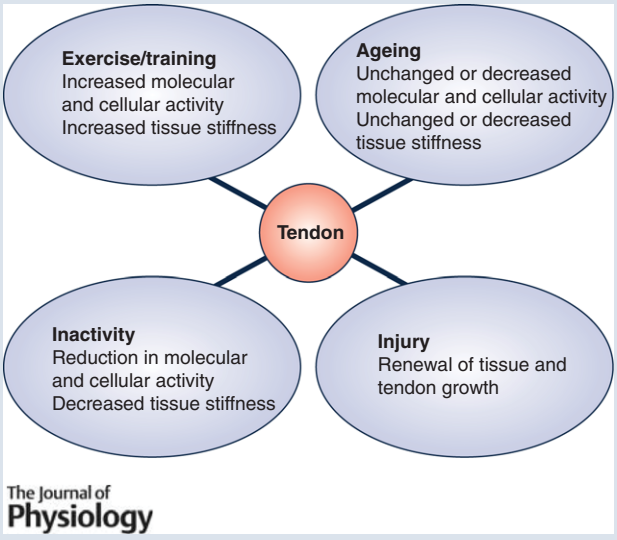

Abstract A tendon transfers force from the contracting muscle to the skeletal system to produce movement and is therefore a crucial component of the entire muscle-tendon complex and its function. However, tendon research has for some time focused on mechanical properties without any major appreciation of potential cellular and molecular changes. At the same time, methodological developments have permitted determination of the mechanical properties of human tendons *in vivo*, which was previously not possible. Here we review the current understanding of how tendons respond to loading, unloading, ageing and injury from cellular, molecular

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and mechanical points of view. A mechanistic understanding of tendon tissue adaptation will be vital for development of adequate guidelines in physical training and rehabilitation, as well as for optimal injury treatment.

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Abstract figure legend The overall changes that occur in tendon with training, ageing, inactivity and injury.

Introduction

Cellular and molecular aspects. The tendon is a connective tissue characterized by a relatively low abundance of cells (primarily fibroblasts) that are elongated in the direction of the collagen fibrils, and a richness of matrix proteins dominated by fibrillar collagen (especially collagen type I) embedded with proteoglycans (PGs), glycoproteins (GPs) and glycosaminoglycans (GAGs) (Kjaer, 2004). The larger PG molecules are dominated by versican and aggrecan, and the smaller PGs (small leucine-rich proteoglycans, SLRPs) contain decorin, biglycan, lumican and fibromodulin. In addition, cartilage oligomorphometric protein (COMP), lubricant, tenascin-C, fibronectin, elastin and cross-link molecules of both an enzymatic and a non-enzymatic nature each have a role in the tissue's mechanical properties and thus in tendon strength, stiffness and elasticity (Halper & Kjaer, 2014).

For many years the focus in tendon physiology has been on its biomechanical properties without any major appreciation of potential cellular biochemical changes in response to loading or unloading. Conversely, the focus in skeletal muscle research has been on the cellular biochemistry and protein synthesis of structural contractile components, but little attention has been given to the dynamics of intramuscular connective tissue and its biomechanical properties. Interestingly, early work by Vihko and colleagues (Savolainen *et al.* 1988; Karpakka *et al.* 1990) focusing on connective tissue in skeletal muscle demonstrated in a rat model that mechanical loading and unloading influenced the activity of enzymes involved in collagen synthesis in skeletal muscle and tendon (Savolainen *et al.* 1988; Karpakka *et al.* 1990). As summarized by Michael Rennie in the 1996 *Handbook of Physiology* (Rennie, 1996), it has been reported that whereas de-tensioning of skeletal muscle in a shortening (rather than in a lengthening) position led to a marked decrease in the enzymatic activity of the collagen synthesizing enzymes propyl 4-hydroxylase (PH) and galactosylhydroxylysyl glucosyltransferase (GGT) (Karpakka *et al.* 1990), mild mechanical loading after muscle-tendon immobilization did not, in their model, alter PH or GGT activity (Savolainen *et al.* 1988). These early studies indicated that loading of tendon is important for maintenance of matrix protein synthesis, and indirectly suggested that a certain magnitude of loading is needed to obtain an increase in matrix protein synthesis in tendon.

The study of protein dynamics in tendon has developed markedly in recent years. Initially indirect determinations of protein synthesis were made using circulating or tissue concentrations of hydroxyprolin and collagen regulating enzymes (Weiss & Klein, 1969; Hart *et al.* 1976). Later, the ability to determine the cleavage products of collagen synthesis or degradation allowed estimates of tissue turnover (Shinmei *et al.* 1993), and the development of the microdialysis method allowed sampling of these substances in the proximity of the tendon, including in humans (Langberg *et al.* 1999; Miller *et al.* 2005; Hansen *et al.* 2009*a*; Miller *et al.* 2011). Some of these studies indicated a relatively fast turnover of tendon collagen tissue (Langberg *et al.* 1999). Subsequently the use of stable isotope infusion of labelled amino/imino acids or heavy water (deuterium) and tendon biopsy sampling allowed a more accurate evaluation of collagen dynamics both in relation to more acute exercise and immobilization interventions. Some studies in humans have found relatively high collagen synthesis rates in tendons of ∼1% per 24 h (Miller *et al.* 2005), while others have found more moderate values of around 0.2% per 24 h (de Boer *et al.* 2007*b*; Nielsen *et al.* 2014). Finally, tissue sampling of tendon autopsies, in both humans and animals, and analysis of ^{14}C content (bomb pulse method) and L-to-D conversion of amino acids (racemization) have permitted 'lifetime' estimation of the matrix tissue turnover in tendon (Libby *et al.* 1964; Helfman & Bada, 1976; Lynnerup *et al.* 2008; Alkass *et al.* 2013). Using racemization, it has been demonstrated that the half-life of horse tendon is around 200 years in high strain, injury-prone superficial digital flexor tendon, and far less (∼34 years) in low strain and rarely injured common digital extensor tendon (Thorpe *et al.* 2010). The human Achilles tendon can be considered a high strain and injury-prone tendon, and data obtained using the bomb pulse method indicates that the collagen structure of the core of the human Achilles tendon changes until about the age of 17 years and thereafter the tissue structure is stable (Heinemeier *et al.* 2013*b*). The latter findings are supported by data from rats showing that growth and

turnover of tendon tissue ceased shortly after 60 days of life and remained relative stable throughout the entire lifespan of around 500 days (Bechshoft *et al.* 2017).

This leaves us with a challenge regarding our understanding of tendon tissue turnover in human tendons. It is suggested that the tendon collagen tissue consists of two pools of tissue, one that is relatively stable with relatively dormant cells, which is formed during childhood and adolescence, and one much smaller pool with a fast turnover of cells which provides a form of 'daily maintenance' of tissue homeostasis. Theoretically, such a model could work with all available methodologies, and fits with the view that a major part of the tendon is developed during childhood and adolescence, while at the same time allowing for a daily adjustment mechanism and thus the ability to adapt to changes in mechanical loading patterns. Presently, the anatomical locations of the proposed pools of tissue remain an enigma.

Effect of loading on tendon

Cellular and molecular aspects. Mechanical stimuli can lead to tendon cell responses that result in changes in the extracellular matrix. Cell culture studies on tendon show that fibroblasts respond to mechanical stretch by increasing production and secretion of certain growth factors that in turn act on the fibroblasts in an autocrine or paracrine fashion to induce expression and synthesis of collagen (Chiquet *et al.* 2009). Early growth response transcription factor 1(EGR-1) is an important factor for embryonic and postnatal tendon development (Lejard *et al.* 2011). It is also known that EGR-1 is regulated by mechanical loading (Olesen *et al.* 2006), and that a lack of EGR-1 is associated with weaker tendon formation (Guerquin *et al.* 2013). With respect to other tendon growth factors, it has been shown, using a model that creates tendon-like structures from isolated animal and human cells within 2–3 weeks (Bayer *et al.* 2010; Paxton *et al.* 2010), that phosphorylation of p38, S6K1 and ERK1/2 is associated with mechanical loading of tendon constructs (Paxton *et al.* 2010, 2012). Whether these factors are crucial for tendon growth can be debated, as blockade of, for example, p38 was found not to be important for tendon growth during development (Schwartz *et al.* 2015), but probably plays a role in mechanotransduction (Paxton *et al.* 2012). Interestingly, a study of the relation between loading and signalling for collagen synthesis based on the phosphorylation of ERK1/2 suggested that 10 min of 2.5%stretch every 6 h was most favourable for improving tendon function(Paxton *et al.* 2012). In addition, it should be noted that in a rat model it has been demonstrated that the stimulatory effect of loading on collagen and IGF-I expression was similar whether the muscle contraction mode was concentric, isometric or eccentric, irrespective of the fact that the force-time integral was far greater for the eccentric contraction (Heinemeier *et al.* 2007*a*).

The enzyme responsible for cross-link formation, lysyl oxidase (LOX), has been demonstrated to be necessary for optimal tendon fibrillogenesis, since blocking of LOX does not influence the total amount of collagen synthesized, but markedly weakens the tendon structure and makes it more compliant (Herchenhan *et al.* 2015*b*). Several experiments on small animals with electrically induced muscle training or synergist ablation leads to substantial increases in mRNA expression of the collagen-inducing growth factors IGF-I and TGFb1, in parallel with increased mRNA expression of collagen type I and III in the tendon tissue (Olesen *et al.* 2006; Heinemeier *et al.* 2007*a*,*b*). In addition, repeated bouts of treadmill running have been shown to elevate levels of IGF-I protein in rat tendons (Hansson *et al.* 1988). Thus, it seems likely that the tendon cells respond to loading by increasing growth factor production, and that the action of these growth factors leads to induction of collagen expression, although this remains to be proven.

Growth factors involved in modulating the signalling cascade include transforming growth factor b1 (TGFb1), plasma-derived growth factor (PDGF) and connective tissue growth factor (CTGF) (Schild & Trueb, 2002; Yang *et al.* 2004; Sugg *et al.* 2017). In addition, insulin like growth factor-I (IGF-I) can also act as a link between mechanical load and collagen synthesis in tendon tissue (Hansson *et al.* 1988; Abrahamsson & Lohmander, 1996; Herchenhan *et al.* 2015*a*). In humans the administration of growth hormone for 14 days, or local injection into tendon of IGF-1 resulted in increased collagen synthesis (Doessing *et al.* 2010; Nielsen *et al.* 2014). Further, oestrogen also influences tendon protein metabolism, and can inhibit collagen synthesis (Hansen *et al.* 2009*b*) and lower the expression of lysyl oxidase (LOX), which is important for cross-link formation (Lee *et al.* 2015). Very recently, the addition of vitamin C-enriched gelatin (along with many amino acids) produced an increase in collagen synthesis in engineered ligament and amino terminal propeptide of collagen I in the blood of humans (Shaw *et al.* 2017). Regarding degradation, it has been demonstrated that matrix metalloproteinase (MMP) activity is increased with exercise (Koskinen *et al.* 2004), and that the activation of ERK1/2 seems important for this MMP response (Sugg *et al.* 2017). Finally, an investigation of the breakdown products of collagen revealed a higher release of these in response to exercise and prolonged physical training (Langberg *et al.* 1999, 2001).

The role of inflammation in acute physiological changes in tendon has been investigated in several ways. It has been demonstrated that inhibition of COX-2 specific pathways abolishes the exercise-induced rise in tendon blood flow in humans (Langberg *et al.* 2003). Further,

stretching of tendon cells has resulted in prostaglandin E2 release in a dose-dependent manner (Wang *et al.* 2003; Legerlotz *et al.* 2013), and in humans blockade of inflammation during acute exercise completely inhibited the rise in collagen synthesis normally seen in response to exercise (Christensen *et al.* 2011). In bovine models, tendon stretching has resulted in a rise in both IL-6 and collagen type I mRNA (Legerlotz *et al.* 2013), while in humans a marked peritendinous rise in the interstitial tissue concentration of IL-6 (and collagen synthesis) was found in association with exercise (Andersen *et al.* 2011), and an infusion of IL-6 into the peritendinous tissue in the resting state caused a rise in collagen synthesis (Andersen *et al.* 2011). This suggests that a rise in inflammatory mediators is important for collagen turnover and for tendon adaptation to exercise. This mandatory effect of an increase in inflammatory parameters on activation of collagen synthesis may be independent of the potentially inhibiting effect on collagen synthesis of a chronic elevated inflammatory state (e.g. in ageing, disease or immobilization). In de-tensioning of tendon, expression of collagen I is immediately reduced, together with a rise in expression of inflammatory signalling markers (Bayer et al 2014).

Comparable loading-induced collagen synthesis in adult human tendon has been reported. Microdialysis studies showedincreased levels of markers for collagen synthesis in the peritendinous tissue surrounding the Achilles tendon in response to both acute exercise and long-term training (Langberg *et al.* 1999, 2001). However, these microdialysis data probably reflect collagen synthesis at the very periphery of the tendon and might not reveal changes within tendon tissue. Using stable isotope infusion with tendon biopsy sampling, an increased rate of collagen synthesis was observed in patellar tendons of young men in response to acute kicking exercise (Miller *et al.* 2005). However, several other studies using the same technique, and the same exercise model, could not robustly confirm this loading-induced collagen synthesis in adult human tendon (Hansen *et al.* 2009*a*,*b*; Petersen *et al.* 2010; Dideriksen *et al.* 2013).

Several studies have investigated gene expression in human patellar tendon tissue in response to acute exercise. In two of these investigations a decrease or no change in growth factor and collagen mRNA expression in tendon biopsies from the mid-portion of the tendon was found (Sullivan *et al.* 2009; Heinemeier *et al.* 2013*a*), while one study found modest increases in collagen and CTGF mRNA expression in tissue from the proximal part of the patellar tendon in response to exercise (Dideriksen *et al.* 2013). In other words, the response of adult human tendon tissue to acute loading does not seem to mimic that of rodent tendon tissue. This suggests that adult human tendon tissue is far less responsive than that of small animals, and such differences may relate to the fact that rats and mice are still in a growth phase when they are typically used in experiments (typically at 10–12 weeks of age for rats) (Olesen *et al.* 2006; Heinemeier *et al.* 2007*a*,*b*). This hypothesis is supported by recent data on 6-month-old mice, which showed that overload-induced plantaris tendon hypertrophy was based on growth and cell proliferation only in the most superficial layers of tendon tissue, while the 'original' core tendon remained relatively constant (Gumucio *et al.* 2014). A greater potential for growth at the tendon periphery is further suggested by an early study that showed higher levels of IGF-I protein expression in cells located in the rat Achilles tendon periphery compared to the more central parts of the tendon(Hansson *et al.* 1988). More recent studies also suggest greater potential for growth and cell proliferation in the superficial parts of the tendon in rodents (Mendias *et al.* 2012; Tan *et al.* 2013). In other words, it may be speculated that a new layer of the collagen matrix is added at the periphery, analogous to growth of a tree, when the tendon grows in response to loading. An alternative explanation for contradictory results regarding adaptability of tendon tissue to loading and overall metabolic activity, could be that large differences exist between different types of tendons. Data from horses show that high strain-injury prone tendons have slower turnover than low strain rarely injured tendons (Thorpe *et al.* 2010). Albeit counterintuitive, it may be that high-strain tendons simply cannot 'afford' to have a constant ongoing remodelling as this may reduce the tendon strength. Therefore, the high-strain Achilles tendon may well have slower turnover than tendons that are loaded less, such as the patellar tendon.

Structural and mechanical aspects. The chief function of tendon is force transmission, and yet the specific pathway of force transmission remains largely unresolved. The collagen fibrils of tendon, which have a diameter range of 30–200 nm (Williams *et al.* 1985; Magnusson *et al.* 2002; Lavagnino *et al.* 2005), are considered the principle load transmitting structure of tendons (Figure 1). The fibrils are surrounded by an extracellular matrix consisting of water, proteoglycans, glycosaminoglycans (GAGs), elastin and glycoproteins (Wang, 2006), and this structural composition is not unlike that of a composite material in which fibres transmit force laterally to adjacent fibres through the matrix. Fibre slippage at the microscopic level (Screen *et al.* 2004*a*,*b*; Screen, 2008) and X-ray diffraction studies showing that the constituent fibrils are stretched less than the whole structure (Fratzl *et al.* 1998; Puxkandl *et al.* 2002; Krauss *et al.* 2009) support such a composite concept. It has been suggested that force is transferred laterally between adjacent fibrils via proteoglycans and their associated GAG chains, including chondroitin- and dermatan-sulfate (Scott & Thomlinson,

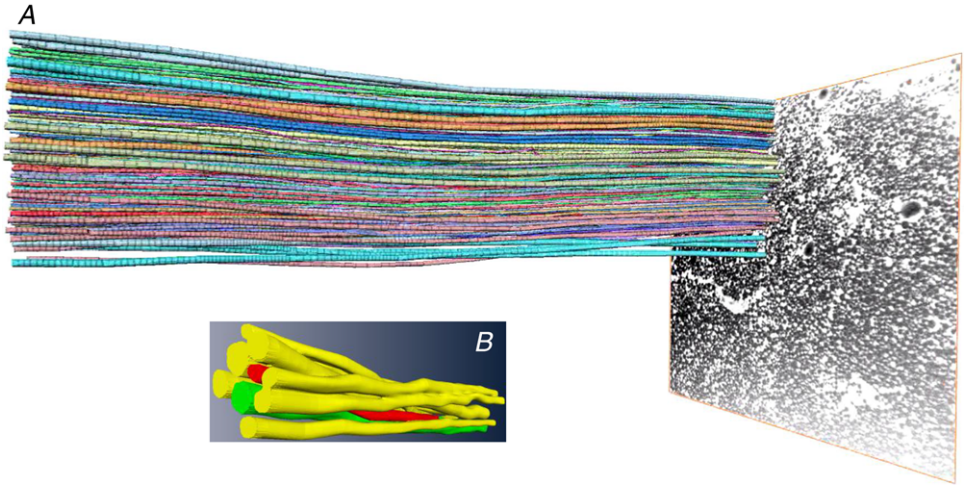

Figure 1. The principle load transmitting structure of tendons
A, an illustration of tracing of collagen fibrils from a human patellar tendon obtained with FIB-SEM on a nanoscale level, suggesting that the fibrils are continuous. *B*, close-up of individual fibrils that typically range between 30 and 200 nm in diameter. For further detail, see Svensson *et al*. 2017.

1998; Ryan *et al.* 2015). However, removing this complex in human tendon (Svensson *et al.* 2011) and ligament (Lujan *et al.* 2007, 2009) does not appreciably affect the mechanical properties of the tissue.

The notion that there is a lateral pathway of force transmission largely rests on the assumption that fibrils in mature tissue are discontinuous, but determining the actual structural length of fibrils with diameters of 30–200 nm has proven rather challenging for several technical reasons. Studies based on direct structural observations indicate that fibrils may be structurally continuous whereas those based on more indirect methods tend to suggest that fibrils are discontinuous (Folkhard *et al.* 1987; Craig *et al.* 1989; Silver *et al.* 2000; Provenzano & Vanderby, 2006; Szczesny *et al.* 2015). However, in a recent study using serial block face-scanning electron microscopy on tissue from adult human patellar and hamstring tendons, it was possible to track 2700 fibrils over a defined distance and only detect one single fibril tip (Svensson *et al.* 2017). Based on these data it was estimated that the fibril length was 67.5 mm, which strongly supports the notion that fibrils are continuous (Svensson *et al.* 2017).

The cross-sectional area of the tendon will impact on the average stress (N/m^2) that the tendon sees at a given force. It is therefore important to know if the tendon can hypertrophy in response to exercise. Animal studies have not provided conclusive data to answer this question (Sommer, 1987; Buchanan & Marsh, 2001; Heinemeier *et al.* 2012). In humans, cross-sectional data suggests that endurance training is associated with a larger Achilles tendon cross-sectional area that is more pronounced close to the insertion (Magnusson & Kjaer, 2003; Kongsgaard *et al.* 2005; Arampatzis *et al.* 2007; Couppe *et al.* 2014), and that resistance training yields fairly modest increases in tendon cross-sectional area (Kongsgaard *et al.* 2007; Seynnes *et al.* 2009). However, a study performed on athletes who for years loaded one side more than the other has overcome some study limitations such as (i) training history, selection bias and inter-subject variations, (ii) the relatively short duration of earlier training studies, and (iii) the lack of assessment of region specificity in existing training studies (Couppe *et al.* 2008). The data showed that subjects with a side-to-side difference (22%) in knee extensor strength as a result of habitual sport specific high loading also have a greater tendon cross-sectional area (20%) on the stronger side (Couppe *et al.* 2008). These data are perhaps currently some of the most convincing evidence that tendons undergo tissue hypertrophy in response to loading. However, whether the hypertrophy represents added tensile bearing components, i.e. principally collagen fibrils, remains a largely unanswered question.

Relatively little is known about the effect of exercise on the collagen fibril itself. Animal data are essentially inconclusive (Michna, 1984; Patterson-Kane *et al.* 1997, 1998). In recent years it has been possible to obtain human percutaneous tendon biopsies, which has opened up for new possibilities, although obtaining repeated biopsies remains a challenge (Heinemeier *et al.* 2016). It has been shown that the collagen of the Achilles tendon is not renewed after 17 years of age (Heinemeier *et al.* 2013*b*), and it has been proposed that exercise during skeletal maturation can influence the tendon fibril development (Smith *et al.* 2002). However, a recent study showed that the fibril morphology of long distance runners who were physically active did not differ from those who were physically inactive during their maturation (Lenskjold *et al.* 2015). Similarly, life-long

habitual running did not appear to appreciably influence fibril morphology compared to age-matched non-runners (Couppe *et al.* 2014). Albeit inconclusive, these relatively scant cross-sectional human data suggest that the collagen fibrils are largely unaffected by exercise.

Although it is well known that the tendon cells (fibroblasts) respond to strain, the dose response to strain magnitude is not well established. Most (Webb *et al.* 2006; Joshi & Webb, 2008; Gauvin *et al.* 2011), but not all (Feng *et al.* 2006) studies have found that the adaptive response of fibroblasts to dynamic load is superior to that of static load. In healthy human Achilles tendons, it has been shown that exercising at 90% of maximum voluntary contraction (MVC; ∼5% tendon strain) yields increased stiffness and cross-sectional area compared to working at 55% MVC (∼3% tendon strain), and that the tendon was more responsive to long duration loading(6 s cycle) than high faster loads (2 s cycle) (Arampatzis *et al.* 2007, 2010). Studies specifically addressing the effects of recovery and how it affects tendon adaptation are lacking.

Unlike muscle, the tendon is a mechanically passive structure that lengthens or shortens in response to the load placed on it, and it is therefore unlikely that direction of the movement of the muscle-tendon unit will have any distinct effect on the tissue of the tendon. In fact, the magnitude of human Achilles tendon stretch is identical during the concentric and eccentric component of a heel rise/drop against body weight (Rees *et al.* 2008; Chaudhry *et al.* 2015). The cellular response to concentric or eccentric contractions to the same force level is similar with respect to the expression of collagen (Garma *et al.* 2007). In healthy subjects 12 weeks of resistance training comprising either concentric or eccentric knee extensions produced similar magnitudes of tendon hypertrophy (Farup *et al.* 2014). These findings reinforce the notion that the cellular and tissue response in healthy tendon is independent of contraction mode. Finally, some of the confounding results with regard to changes in tendon mechanical properties in response to mechanical loading may have to do with the different tendons examined (e.g. patellar *vs.* Achilles *vs.* supraspinatus etc.), in addition to the methods, different animal/human models, and inherent biology.

Unloading

Cellular and molecular aspects. De-tensioning of tissue removes the constant signal for mechanotransduction in tendon. In tendon constructs made from human tenocytes it has been demonstrated that even a few days after removing tensile loading there is a downregulation of mRNA for both tenomodulin and collagen, and this is accompanied by a disorganization of the aligned fibrils in the tendon structure (Bayer *et al.* 2014). This down-regulation could not be counteracted by addition of TGF-beta to the medium. The findings indicate that tension influences the phenotypical characteristics of tendon. Clearly, protein signalling and tissue turnover in this *in vitro* model of tendon may occur much more rapidly and pronouncedly than *in vivo*, but even 2–3 weeks of immobilization of a lower limb in both young and old individuals will lead to an 80% reduction in synthesis rate of collagen (de Boer *et al.* 2007*b*; Dideriksen *et al.* 2017). In line with these changes, 2 weeks of immobilization resulted in a downregulation of LOX and scleraxis and an upregulation of MMP2 in human patella tendon (Boesen *et al.* 2013; Dideriksen, 2014). It is interesting that administration of both growth hormone and NSAID counteracted some of the signalling, and, taken together, it appears that inactivity in humans favours reduced collagen synthesis, reduced collagen synthesis stimulating signalling and accelerated proteolytic activity. Both growth hormone and, to some extent, NSAID are able to act as matrix stabilizers during immobilization during rehabilitation (Boesen *et al.* 2013, 2014; Dideriksen *et al.* 2017).

Structural and mechanical aspects. The effects of immobilization on the tendon have been studied in animal models, and they overwhelming show a decline in mechanical properties (Woo *et al.* 1982; Yamamoto *et al.* 1993; Hannafin *et al.* 1995; Almeida-Silveira *et al.* 2000; Matsumoto *et al.* 2003; Rumian *et al.* 2009). It seems that the human data on the effects of immobilization on tendon properties largely mirror those of the animal models (Reeves *et al.* 2005; de Boer *et al.* 2007*a*; Seynnes *et al.* 2008; Shin *et al.* 2008; Kinugasa *et al.* 2010; Couppe *et al.* 2012). Interestingly, rather short-term (1–2 weeks) limb unloading seems to significantly reduce the stiffness of the tendon. Moreover, the changes in mechanical properties take place in the absence of any change in cross-section of the tendon (Reeves *et al.* 2005; de Boer *et al.* 2007*a*; Christensen *et al.* 2008; Shin *et al.* 2008; Couppe *et al.* 2012). In fact, it appears that the cross-section of tendon will only be reduced during extremely protracted periods of inactivity (Maganaris *et al.* 2006). The mechanism for this relatively rapid change in mechanical properties in the absence of an overall change in the (MRI determined) size of the structure is elusive, but may relate to LOX-derived cross-links.

Ageing

Cellular and molecular aspects. Tendon cell numbers and density have so far not been studied systematically in humans. Instead animal models have mostly been used, making it difficult to separate changes in the number of tendon cells during development and growth from those related to ageing (Svensson *et al.* 2016). Within the

confines of these limitations, it has been shown that cell density is reduced with age in rabbits and rats (Nagy *et al.* 1969; Nakagawa *et al.* 1994), whereas in horses there is no decline in cell density (or DNA content) from 2 to 30 years of age (Stanley *et al.* 2007; Thorpe *et al.* 2015). Interestingly, there is very little or no difference in metabolic activity of cultured cells from rats and in collagen synthesis capacity in horse tendons (Tsai *et al.* 2011; Thorpe *et al.* 2015) in regard to ageing. This suggests that there is only a limited decline in synthesis capacity of tendon cells with ageing, and observations in animal models may reflect an effect of maturation than ageing per se. When cell function is evaluated by migration and proliferation capacity, an age-related reduced cell proliferation has been shown in injured human supraspinatus tendon (Klatte-Schulz *et al.* 2012), and in rat and mouse tendon a reduction in both migration and proliferation was shown (Arnesen & Lawson, 2006; Torricelli *et al.* 2013). Further, data using progenitor cells from aged tendons in humans and animals suggest both lower proliferation and migration with ageing (Zhou *et al.* 2010; Kohler *et al.* 2013; Zhang & Wang, 2015). This indicates that ageing does impair cell capacity in a way that probably cannot be counteracted by changes in the circulating environment. In the study on human tendon progenitor cells, it was shown that the potential for differentiation into adipogenic, chondrogenic and osteogenic cells was unaltered with ageing (Kohler *et al.* 2013). Given the very limited human data, it is difficult to firmly conclude anything, and the findings in animal models are still impaired by the limited ability to separate ageing and tissue maturation. In regard to expression and synthesis of proteins from tendon cells, it has been shown that older rats demonstrated a reduced mRNA expression of collagen (I, III and V) whereas the protein content of collagen types estimated by immunohistochemistry was unchanged with ageing (Goh *et al.* 2008; Dyment & Galloway, 2015; Zhang *et al.* 2016). Further, ageing in rats led to decreased expression of elastin but no change in expression of the factors involved in tissue growth (CTGF and TGF-beta; Kostrominova & Brooks, 2013). Finally, high levels of extracellular proteoglycan, calcium deposits and lipid have been found in elderly mice (Zhang & Wang, 2015). In a recent study with young and old rats it was demonstrated that ageing was associated with a downregulation of genes that regulate matrix remodelling (Marqueti *et al.* 2018). Further, aged rats show a reduced density of blood vessels and a few examples of calcification in older tendons were shown (Marqueti *et al.* 2018). Interestingly, the intervention of regular strength training in older rats upregulated mRNA expression of CTGF, decorin and VEGF, and no calcifications were found in tendons of old trained rats (Marqueti *et al.* 2018). Although the study found differences between young and old rats, it also demonstrated that physical training can counteract several age-related changes in tendon connective tissue, which is similar to findings in relation to ageing and oxidative and metabolic capacity in skeletal muscle (Larsen *et al.* 2012).

Structural and mechanical aspects. The effect of ageing is challenging to study because it is hard to separate the effects of age *per se* from the related inactivity associated with ageing (Lazarus & Harridge, 2017). Animal data show that tendon cross-sectional area (CSA) may increase (Nakagawa *et al.* 1996; Birch *et al.* 1999) or remain unchanged (Wood *et al.* 2011) with age, and, similarly, cross-sectional studies in humans indicate that tendon CSA may increase (Magnusson *et al.* 2003; Stenroth *et al.* 2012; Couppe *et al.* 2014) or remain unchanged (Carroll *et al.* 2008; Couppe *et al.* 2009*a*, 2014) with ageing. However, not accounting for reduced physical activity with ageing (Lazarus & Harridge, 2017) and its potential effects on tendon CSA may be a key limitation. It was recently shown that young and old men of similar height, weight and activity level also displayed similar tendon CSA (Couppe *et al.* 2014), which suggests that, unlike muscle, there is no loss of tendon tissue with increasing age. In support of this, the total content of collagen fibrils (volume fraction) remains largely unaltered with ageing in animal and human models (Parry & Craig, 1978; Wood *et al.* 2011; Couppe *et al.* 2014).

Factors such as the rate of force development, electromechanical delay and the elastic energy return of tendon influence the overall function of the muscle-tendon complex, and can be affected by the mechanical properties of tendon (Bojsen-Moller *et al.* 2005; Magnusson *et al.* 2008; Nordez *et al.* 2009; Quinlan *et al.* 2017). Again, data from animal models do not provide a coherent picture, as some show increased modulus and strength (Viidik *et al.* 1996; Nielsen *et al.* 1998; Wood *et al.* 2011), some a weaker and more compliant tendon (Vogel, 1978; Simonsen *et al.* 1995; Dressler *et al.* 2002; LaCroix *et al.* 2013), and yet others demonstrate no change with age (Haut, 1983; Haut *et al.* 1992; Nakagawa *et al.* 1996). *In vitro* data on human tendons suggest that there are no changes or a reduction in the mechanical properties of the tendon with ageing (Hubbard & Soutas-Little, 1984; Johnson *et al.* 1994; Flahiff *et al.* 1995), while *in vivo* human studies have reported unchanged (Carroll *et al.* 2008; Couppe *et al.* 2009*a*) or reduced (Kubo *et al.* 2003; Onambele *et al.* 2006; Stenroth *et al.* 2012; Couppe *et al.* 2014; Quinlan *et al.* 2017) modulus values with ageing. Some of the discrepancies in the human *in vivo* data may relate to data analysis: strength tends to decline with age, and therefore a reduction in tendon strain may be a consequence of a lower force placed on the tendon (and as a result less strain). Therefore, it is necessary to evaluate strain to a common force when comparing mechanical properties between age groups. As previously mentioned it is probably of

paramount importance to account for age-related activity levels. It was recently shown that old subjects (66 years) had a lower modulus compared to young subjects (26 years), but when old and young were matched for activity level, there was no difference in mechanical properties (Couppe *et al.* 2014). Collectively, the data suggest that with age there is no change or a decrease in the modulus of the tendon. What is somewhat puzzling about these findings is that there is an increase in advance glycation endproducts (AGE) with age (Couppe *et al.* 2009*b*, 2014; Hansen *et al.* 2010), which can be mitigated by physical activity (Couppe *et al.* 2014), and an elevation of AGEs would typically increase tendon stiffness (Bai *et al.* 1992; Li *et al.* 2013; Svensson *et al.* 2018). It is unclear, but it is possible that the increase in AGEs is partly countered by a reduction in collagen content with age (Couppe *et al.* 2009*b*).

Injured tendon

Cellular and molecular aspect. The exact pathogenesis behind development of tendon overuse – tendinopathy – is still not completely solved. It is known that tendinopathic (and painful) tendon areas are associated with more rounded fibroblasts and cell accumulation (Glazebrook *et al.* 2008; de Mos *et al.* 2009). Further, there is an upregulation of mRNA for collagen I and II plus TGF-beta (Pingel *et al.* 2013*a*, 2014), and increased amounts of proteoglycans, and of the proteins versican, aggrecan and fibromodulin, whereas decorin, which normally is associated with aligned tendon fibrils, was unchanged in content in tendinpathic tendons (Parkinson *et al.* 2010). To what extend tendinopathy is associated with inflammation is still debated, but in general long-term chronic tendinopathy is not dominated by inflammation, whereas different indicators of inflammation have been demonstrated early in tendinopathy (Dakin *et al.* 2015).

After tendon rupture, a broad anabolic and inflammatory signalling occurs. A marked inflammatory response has been demonstrated both in the early, the proliferative and the remodelling phase of tendon healing in animal models (Eliasson *et al.* 2009; Schulze-Tanzil *et al.* 2011). Interestingly, the addition of mechanical loading and activity to the tendon during healing stimulated the NO response (Eliasson *et al.* 2012) and lowered the inflammatory response, as indicated by TNF-alpha and IL-1. At the same it time caused an improvement in the synthesis of tendon matrix tissue (Eliasson *et al.* 2009).

Structural and mechanical aspects. In addition to rounding of fibroblasts, increased cell number, an increase in the content of proteoglycans glycosaminoglycans (GAGs), water and hypervascularization (with nerve ingrowth), the collagen fibrils appear disorganized (Glazebrook *et al.* 2008; Kongsgaard *et al.* 2009; Pingel *et al.* 2013*b*). The increase in water content, along with the hypervascularization, leaves the tendon with an overall increase in CSA. Tendon stiffness has been reported to decrease with tendinopathy (Arya & Kulig, 2010; Child *et al.* 2010; Helland *et al.* 2013), or remain unchanged (Kongsgaard *et al.* 2010). It remains unknown if such a change relates to the pathology itself or if it is an injury-related response to inactivity (unloading).

Loading-based interventions have become a principal theme in the treatment of tendinopathies (Malliaras *et al.* 2013), with positive clinical (Alfredson *et al.* 1996; Alfredson & Lorentzon, 2000; Mafi *et al.* 2001; Silbernagel *et al.* 2007), structural, and biochemical outcomes (Couppe *et al.* 2009*b*; Kongsgaard *et al.* 2010). An isolated eccentric loading protocol has mostly been regarded as the treatment of choice, although there is a lack of support for its superior effective (Malliaras *et al.* 2013). Other loading-based exercise regimes, including isolated concentric training (Mafi *et al.* 2001), heavy slow resistance training (Kongsgaard *et al.* 2009; Beyer *et al.* 2015) and concentric-eccentric progressing to eccentric training (Silbernagel *et al.* 2001, 2007) also appear to be clinically effective. How a loading regime influences tendon composition in patients with tendinopathy has been very little investigated. It has been shown that fibril morphology is abnormal in tendinopathy, but that heavy slow resistance training can change fibril morphology towards normal fibril density and mean fibril area (Kongsgaard *et al.* 2010). It has also been shown that the cross-link composition can be changed with heavy slow resistance training in this patient group (Kongsgaard *et al.* 2009).

Despite the tendon's remarkable ability to transfer large magnitudes of muscular force to the skeletal system for locomotion purposes, there are instances when the tendon ruptures completely. The aetiology remains largely unknown, and much research has focused on tendon repair, and rehabilitation and clinical outcome in order to rapidly return patients to normal function. However, it was recently shown that cellular activity, as measured by the glucose uptake associated with ambulation, is higher in repaired than in intact Achilles tendons at 3 months (6x), 6 months (3x) and even 12 months (1.6x) after a surgical repair (Eliasson *et al.* 2016). Furthermore, it was recently shown that the repaired Achilles tendon increases its stiffness up to at least 6 months (P. Eliasson and others, unpublished data). Collectively, these data indicate that the tendon response to loading is rather slow and is not normalized until sometime after 6–12 months after injury (Eliasson *et al.* 2016).

In conclusion, methodological improvements in the last 20 years or so have permitted determination of cellular, molecular and mechanical changes in response to loading or unloading of tendon tissue (summarized in Table 1), which was previously difficult to study. This

Table 1. Tendon responses to loading, unloading, ageing and injury: arrows indicate the overall direction

	Cellular/molecular			Structural/mechanical	
	Anabolic/catabolic	Cell number	Tissue synthesis	CSA	Stiffness
Loading	↑	→	↑	↑	↑
Unloading	↓	→	↓	→	↓
Ageing	→↘	↓*	→	→	→↘
Injury	↑	↓	↑	↑	→↘**

*Only animal data. **Only tendinopathy.

new knowledge of tendon physiology will undoubtedly contribute to the unravelling of the optimal adaptation of tendon to loading throughout life, as well as the aetiology of tendon injuries. With that knowledge, it will be possible to develop more effective treatment and prevention strategies than we have today. To ensure optimal guidelines for both normal physiological adaptation to physical training and for rehabilitation after tendon injury, we need a tight interplay between the description of clinical presentation and associated tissue changes and a basic biomedical understanding of the mechanisms behind adaptive changes in tendon to mechanical challenges throughout life.

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Additional information

Competing interests

None declared.

Author contributions

Both authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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