Scand J Med Sci Sports 2009: 19: 500–510
doi: 10.1111/j.1600-0838.2009.00986.x



SCANDINAVIAN JOURNAL OF
MEDICINE & SCIENCE
IN SPORTS

# From mechanical loading to collagen synthesis, structural changes and function in human tendon

**M. Kjær, H. Langberg, K. Heinemeier, M. L. Bayer, M. Hansen, L. Holm, S. Doessing, M. Kongsgaard, M. R. Krogsgaard, S. P. Magnusson**

*Department of Orthopaedics, Institute of Sports Medicine, Bispebjerg Hospital and Centre for Healthy Ageing, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark*
*Corresponding author: Michael Kjær, MD, PhD, Department of Orthopaedics, Institute of Sports Medicine, Bispebjerg Hospital and Centre for Healthy Ageing, Faculty of Health Sciences, University of Copenhagen, DK-2400 Copenhagen NV, Denmark. Tel: +45 3531 6089, Fax: +45 3531 2733, E-mail: m.kjaer@mfi.ku.dk*

Accepted for publication 22 May 2009

**The adaptive response of connective tissue to loading requires increased synthesis and turnover of matrix proteins, with special emphasis on collagen. Collagen formation and degradation in the tendon increases with both acute and chronic loading, and data suggest that a gender difference exists, in that females respond less than males with regard to an increase in collagen formation after exercise. It is suggested that estrogen may contribute toward a diminished collagen synthesis response in females. Conversely, the stimulation of collagen synthesis by other growth factors can be shown in both animal and human models where insulin-like growth factor 1 (IGF-I) and transforming growth factor-β-1 (TGF-β-1) expression increases to accompany or precede an increase in procollagen expression and collagen synthesis. In humans, it can be demonstrated that an increase in the interstitial concentration of TGF-β, PGE2, IGF-I plus its binding proteins and interleukin-6 takes place after exercise. The increase in IGF-I expression in tendon includes the isoform that has so far been thought only to exist in skeletal muscle (mechano growth factor). The increase in IGF-I and procollagen expression showed a similar response whether the tendon was stimulated by concentric, isometric or eccentric muscle contraction, suggesting that strain rather that stress/torque determines the collagen-synthesis stimulating response seen with exercise. The adaptation time to chronic loading is longer in tendon tissue compared with contractile elements of skeletal muscle or the heart, and only with very prolonged loading are significant changes in gross dimensions of the tendon observed, suggesting that habitual loading is associated with a robust change in the size and mechanical properties of human tendons. An intimate interplay between mechanical signalling and biochemical changes in the matrix is needed in tendon, such that chemical changes can be converted into adaptations in the morphology, structure and material properties.**

Mechanical loading of tendon tissue has been the focus of biomechanical interest for several years – particularly its force-transmitting and energy-storing capacity. More lately, however, the biochemical and molecular adaptive responses of the tendon to loading have drawn more attention, and have challenged the traditional view of the relatively inert nature of the tendon. Thus, studies on the influence of a mechanical load on tendon tissue adaptation will be important for an understanding of the structural and functional changes that can occur with changes in tendon loading, and ultimately for an understanding of tendon overuse and injury healing. Human movement comes about from the force created by contracting muscles, which is transmitted to bone via aponeurosis and tendon. The human tendon is loaded with tremendous forces, and is therefore often subjected to overload injuries, such as patellar or Achilles tendinopathy. The prevalence of patellar tendinopathy among athletes has been estimated to be ~ 15% and that in elite jumping athletes (volleyball players) as high as 50% (Ferretti et al., 1983; Ferretti, 1986; Lian et al., 1996). Recent data in humans suggest that tendon tissue is metabolically responsive to tensile loading (Kalliokoski et al. 2005; Bojsen-Moller et al., 2006).

## Collagen as a main player in mechanically loaded tendon

Tendon tissue is subjected to tensile loading and consists predominantly of fibrillar collagen molecules – primarily type I and III collagen. Although other matrix proteins are also important components of tendon, it seems logical to focus first on collagen tissue adaptation with loading and to evaluate whether increased loading will lead to increased collagen protein synthesis (Kjær, 2004). Collagen is produced in tendon fibroblasts, which are arranged in parallel

along the main direction of tension; the cells have an elongated shape with flattened and elongated nuclei and long, actin-based cytoplasmic protrusions. The tendon fibroblasts interact with the extracellular matrix (ECM) through cell–matrix coupling and form a cellular network throughout the tendon. Theoretically, collagen synthesis can be studied in several ways. Animal studies have used approaches with indirect determination of collagen synthesis by measuring the enzymes involved in the formation and processing of collagen (e.g. prolyl-4-hydroxylase), and from such studies, it has been clearly demonstrated that increased and decreased loading could increase and reduce the collagen synthesis, respectively (Kovanen, 1989). Using the microdialysis technique, catheters are placed in the region of interest (e.g. around or through a tendon) and the interstitial concentrations of the procollagen propeptides that are cleaved off in the maturation from procollagen to collagen (PICP or PINP) of both previously loaded and unloaded tendons are determined (Langberg et al., 1999, 2001). This technique provides the possibility of determining local changes of PICP or PINP in proximity to a tendon that would otherwise only contribute marginally to any change in these parameters globally (i.e. changes in the concentrations of PINP or PICP in the circulating blood stream) (Langberg et al., 2001). More recently, the use of non-radioactive stable isotopes also provided advantages for a more direct determination of collagen synthesis in human tissue. Given that representative samples of the tissue (like tendon) can be obtained, the direct protein synthesis can be determined as incorporation of the tracer into the connective tissue (Babraj et al., 2005; Miller et al., 2006a, ). Tendon tissue sampling can be performed in humans by percutaneous tendon biopsies, and has been used in protocols where protein turnover, mRNA transcription and collagen fibril diameter are determined in both young and elderly patients and healthy subjects (Miller et al., 2006a, b). The use of stable isotope techniques to study the incorporation of labelled amino acids into tissue in order to study the kinetics of collagen has been attempted in tendon, ligament and bone (Babraj et al., 2005). Briefly, the principle of the direct incorporation technique using the precursor-product approach applicable on tendon tissue is to label the amino acid, proline, e.g. L-$^{13}$C-proline or L-$^{15}$N-proline. Proline is abundant in collagen, and is incorporated directly into new collagen proteins. Newly synthesized procollagens are posttranslationally hydroxylated at the proline residues (forming hydroxyproline) before being assembled into the triplehelical structure. Thus, the collagen-specific hydroxyproline will be labelled. Measurement of the enrichment of hydroxyproline from a tendon sample, provides a very specific synthesis measure of collagen protein in the tendon tissue (Babraj et al., 2005). Using this method, acute exercise has been shown to increase the fractional synthesis rate of collagen in the patellar tendon from approximately 0.05%/h to around 0.10%/h within 24 h after exercise, showing a significant increase already after 6 h post-exercise (Miller et al., 2005). This corresponds to a collagen synthesis that, on a 24-h level, increases from around 1% at rest to 2–3% after exercise. The collagen synthesis rate remains elevated for at least 2–3 days after acute exercise. An interesting finding is that the magnitude of increase in collagen synthesis with acute mechanical loading seems to be quite uniform and relatively independent of the magnitude of exercise. As illustrated in Fig. 1, a doubling of the collagen synthesis occurs in human tendon (patellar and Achilles) 24 h after exercise irrespective of whether heavy strength training, enduring leg kicking activity or prolonged running was under-

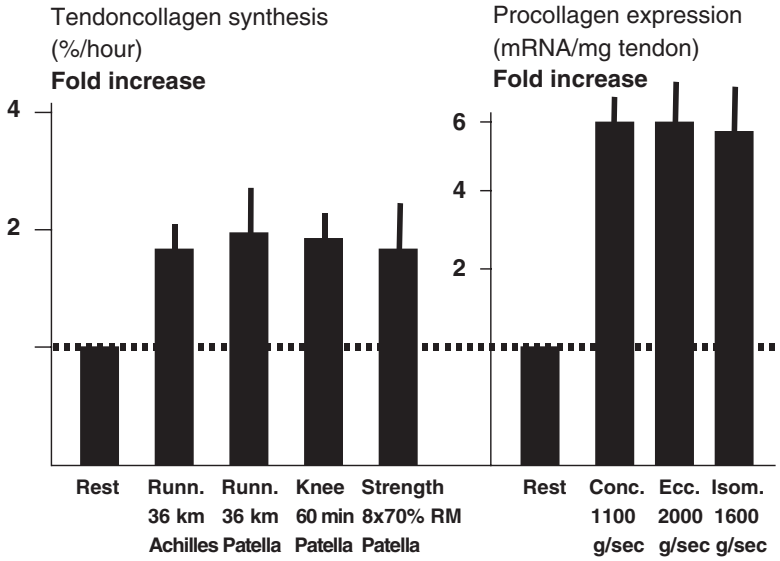


*Fig. 1.* Collagen synthesis in human tendon determined either by microdialysis determined interstitial procollagen propetide concentrations or by stable isotope injected tendon incorporation of labeled proline measured in the resting state or 24 h after an acute bout of exercise. The changes are expressed as fold increase and exercise is either 36 km of running in athletes (Runn), 1 h of knee extension exercise against resistance to fatigue (Knee) or three series of eight repetitions of leg press/knee extension at a load of 70% of one repetition maximum (Strength). In addition, data for mRNA of procollagen are provided from animal experiments where limb muscle was either subjected to concentric, isometric or eccentric electrically induced exercise. Data are taken from Langberg et al. (1999), Heinemeier et al. (2007a, b), Miller et al. (2005) and H. Langberg and L. Holm, unpublished observation.

taken. However, the present data do not reveal the lower limit that exists for stimulation of tendon collagen synthesis; they suggest that mechanical loading sets collagen synthesis at a new level, suggesting an ''on-off'' switch for connective tissue with regard to the need for mechanical loading in order to synthesize new collagen optimally. Both a single loading bout as well as long-term habitual loading produce a markedly elevated collagen synthesis response (Langberg et al., 1999, 2001; Miller et al., 2005). However, to what extent this elevated synthesis yields incorporation of collagen into the load-bearing structure of tendon, and therefore either an increase in tendon size (hypertrophy) or an altered composition and a change in mechanical function, has remained unknown. Similar to collagen synthesis, there is an indication that protein degradation is activated after exercise, in that local levels of matrix metalloproteinases in tendon and muscle tissue are increased after acute exercise (Koskinen et al. 2004). No good direct determination of collagen degradation has been provided so far, and therefore the present data only indirectly point toward an increased collagen turnover with exercise. It is difficult to state how much of this increased collagen turnover is in fact turned into assembled load-bearing collagen, but there is – from data on connective tissue in the lung – reason to believe that at least a certain percentage of the newly formed collagen will end up as insoluble collagen type I in the final tendon structure (Laurent, 1987).

## The tendon cell in humans: the indispensable cellular network of actin cytoskeleton and junctional complexes

Clearly, the tendon fibroblast is considered a key player in tendon maintenance, adaptation to changes in homeostasis and remodeling in case of minor or more severe disturbances to tendon tissue. In postnatal tendons, the fibroblast is the major mechanoresponsive cell in the tissue (Yang et al., 2005). Although elaborate studies on this cell type have been published during the last few years, the tendon fibroblasts have remained a somewhat under-researched topic, especially in postnatal human tendon. To date, the human tendon fibroblasts have been examined with regard to the cells' role in the differentiation potential (De Mos et al. 2007), the existence and niche of tendon progenitor cells (Bi et al., 2007), expression patterns during *in vitro* culturing (Almarza et al., 2008), cell morphology (Ujihara et al., 2008), cell shape with regard to collagen expression (Li et al., 2008) and the capability of tendon fibroblasts to form a cellular network in tendon (Wall et al., 2007).

Collagen fibrils are aligned in a well-organized parallel order in tendon, and so are the cell-associated actin filaments, following the crimp pattern of the tendon. This was visualized by Ralphs et al. (2002) in chicken tendon, and also observed in our laboratory in human samples (M. Bayer, unpublished observation). Notably, the organized arrangement of actin filaments in human tendon is severely disturbed in injured tendon samples. To study the actin cytoskeleton and associated molecules *in vitro*, tendon fibroblasts in culture have been examined and shown to form a dense actin cytoskeleton with filaments arranged in parallel. This structure undergoes rapid re-structuring following interference of cellular integrity, e.g. through experimentally inducing cell detachment and cell rounding through trypsin. The proteolytic function of trypsin forces adherent cells to detach and to float in suspension. Tendon fibroblasts in this state reveal a rounded cellular shape and a loss of the elongated actin-based filaments (Fig. 2(a–c)). Within 1 h after trypsination and re-seeding, tendon fibroblasts adhere to culture plastic and begin to rebuild their characteristic morphology, which is an elongated shape with long actin-based cytoplasmic protrusions that interact through actin-associated proteins with adjacent cells. In this process, cells spread over the surface, form gradually growing lamellipodia and establish multiple cell contacts (Figs 2(g–i) and 3; Li et al., 2008). In tendon tissue, both adherens and gap junctions are found (McNeilly et al., 1996; Banes et al., 1999; Ralphs et al., 2002; Waggett et al., 2006; Richardson et al., 2007; Wall et al., 2007, unpublished observation).

Adherens junctions are cadherin–catenin-mediated cell adhesion sites. Cadherin molecules are transmembrane proteins and bind with their extracellular part through homophilic interactions to cadherins on neighboring cells. $Ca^{2+}$ ions are linked at the junctions between cells, and thereby enable a rigid rod by increasing stability to the adhesion site. On the intracellular side, cadherins are linked through catenin-β and catenin-α to the actin cytoskeleton. During development, cadherins play an essential role in a variety of biological processes, such as cell sorting, cell polarization, proliferation, apoptosis and tissue morphogenesis. In postnatal tissue, adherens junctions control cell polarity, cell migration and proliferation. Notably, cadherin-mediated adherens junctions are suggested to show a force-dependent behavior, and are likely involved in mechanostransduction (Goodsell, 2002; Pettitt, 2005; Ehrlich et al., 2006; Schwartz & DeSimone, 2008).

As mentioned above, cadherin molecules and the actin cytoskeleton are linked together. The cytoplasmic-located protein catenin-α binds to the actin cytoskeleton; the co-localization of cadherin 11 and actin can be visualized on tendon fibroblasts (Fig. 3(f) and (i)). During the process of contact formation, it seems as if the growing lamellipodia precedes the formation of adherens junctions, i.e. the elongation of actin-based

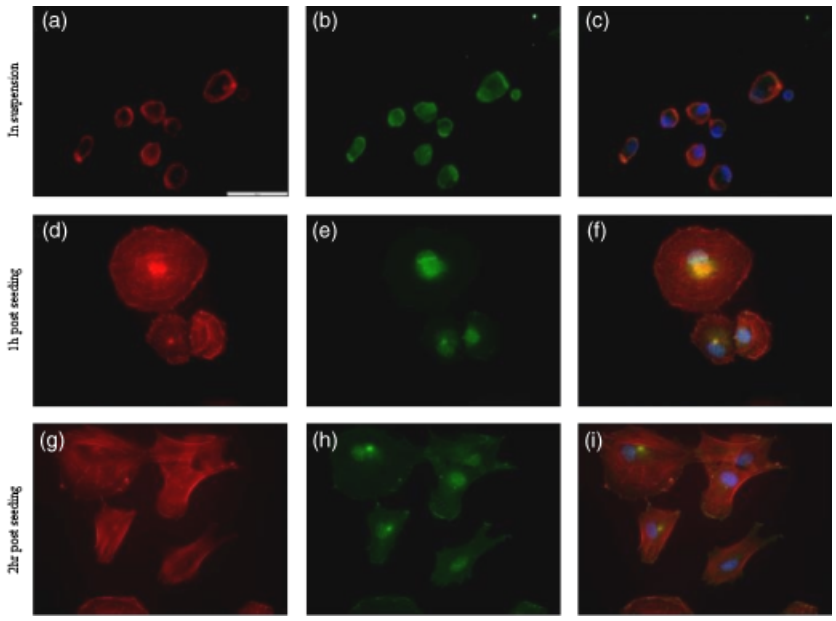


*Fig. 2.* Cellular morphology and Cadherin 11 localization in tendon fibroblasts following detachment. (a–c) Cells in suspension: rounded cell morphology with Cadherin 11 staining of the whole-cell body. (d–f) 1 h postseeding: circular cell morphology and prominent perinuclear staining for Cadherin 11. (g–e) 2 h postseeding: Tendon fibroblasts start to flatten, and begin to form lammeliopodia. (a), (d), (g): red color corresponds to phalloidin staining; (b), (e), (h): green color corresponds to Cadherin 11 staining; (c), (f), (i): computer-generated merged images of the individually captured images; blue color corresponds to nuclear stain with DAPI × 40 magnification.

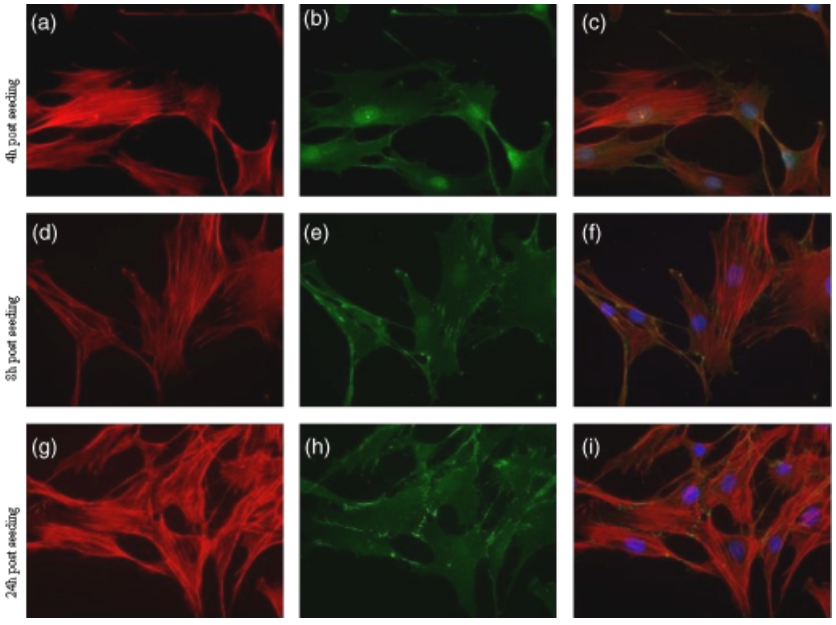


*Fig. 3.* Cellular morphology and formation of adherens junctions in tendon fibroblasts following detachment. (a–c) 4 h postseeding: tendon fibroblasts with expanding lamelliopodia, Cadherin 11 staining both perinuclear and localized to the plasma membrane. (d–f) 8 h postseeding: fibroblasts with distinct actin filaments and formation of adherens junctions (comb-like appearance). The perinuclear localization is absent. (g–e) 24 h postseeding: Fibroblasts with distinct actin filaments and adherens junctions with the typical comb-like structure. The perinuclear localization is absent. (a), (d), (g): red color corresponds to phalloidin staining; (b), (e), (h): green color corresponds to Cadherin 11 staining; (c), (f), (i): computer-generated merged images of the individually captured images; blue color corresponds to nuclear stain with DAPI. Scale bar = 50 μm, × 40 magnification.

protrusions is observed when cadherin 11 primarily appears perinuclear (Fig. 3(a–c)). It could, however, also be a simultaneous process of highly dynamically expanding lamellipodia, cadherin adhesion formation in which the cadherin and catenin molecules enable further actin dynamics as a "pushing force" to establish multiple strong cell adhesions (Bryant and Stow, 2004, Mège et al., 2006).

While a detailed expression profile throughout tendon development and tissue maintenance is lacking, two types of cadherins have been described, i.e. N-cadherin and cadherin 11, both in embryonic and postnatal tendon (Ralphs et al., 2002; Richardson et al., 2007; M. Bayer, unpublished observation). Ralphs and co-workers visualized the association of cadherins and the actin cytoskeleton through co-localization of N-cadherin and actin-based filaments in tissue obtained from chicken tendon. Recently, we observed that cadherin 11 is present in human tendon tissue, and that tendon fibroblasts rapidly form cadherin-mediated adherens junctions *in vitro* to form a tight network of fibroblasts (Fig. 3). Although it has not been sufficiently investigated, the dynamic expression of different cadherin proteins as a result of various environmental challenges could indicate a role of adherens junctions in physiological and pathological adaptations in mature tendon (Follonier et al., 2008).

In contrast to adherens junctions (and tight junctions), gap junctions do not seal membrane-form adjacent cells together, but build channels between neighboring cells to allow shuffling of small molecules, thereby enabling an active exchange through the intercellular network. In postnatal tendon tissue, tendon fibroblasts express connexin 32 and connexin 43 and the junctional proteins are co-localized with actin. This was reported in avian as well as in human tendon (Wall & Banes, 2005; Wall et al., 2007, unpublished observations). In postnatal tendon, age does not have a significant effect on the abundance of connexin expression; however, there is a distinct difference between fetal tendon and postnatal tissue. The amount of connexin in the tendon during development is significantly higher, at least in equine tendon (Stanley et al., 2007).

Tendons are constantly exposed to mechanical strain, and it is noteworthy that the actin–connexin co-localization is affected by mechanical loading, as it was shown to increase concomitantly with an increase of mechanical load. These findings suggest a higher turnover of junctional proteins, or an increase in the formation of gap junctions between tendon fibroblasts exposed to strain, or an increased demand of stability of junctions between cells, exposed to strain, or a combination of these. Blocking gap junctions, on the other hand, resulted in a decrease of collagen type I production, supporting that gap junctions play an indispensable role in maintaining cellular function in tendon tissue (Banes et al., 1999; Waggett et al., 2006; Wall et al., 2007).

The importance of the actin cytoskeleton in promoting the formation of cell junctions is one of many indispensable roles of the filamentous network. Among various functions, one of them is the link between the actin cytoskeleton and mechanotransduction. Although the activation of actin-linked signal transduction has not been studied sufficiently in the tendon fibroblasts, investigations on other cell types show that fibroblasts sense, transduce and respond to changes in mechanical strain in the environment through interaction with the matrix, mainly through focal adhesion. Thereby, the cell creates a link through receptors on the cells with ligands in the ECM and the actin cytoskeleton, and enables adequate reactions through activation of downstream effectors. The findings by Ralphs et al. (2002) support a role of focal adhesions by reporting a co-localization of actin filaments with the focal adhesion protein vinculin in the tendon (Woods & Couchman, 2001; Ralphs et al., 2002; Huveneers et al., 2008).

An increase in the knowledge of tendon fibroblasts in human tissue appears to be a necessity. It is important to obtain a comprehensive view of cellular processes in healthy tendon, to find cues that might play a critical role in diseased, degenerated and injured tendon. The work on isolated human tendon fibroblasts has several limitations, both with regard to ethical and methodological issues. However, opportunities in cell culture systems to mimic *in vivo* conditions are considered as adequate tools for further research. Based on existing knowledge of tendon fibroblasts, new insights and the urgent need to enhance the results of the cells in healthy and diseased mature tendon, we suggest that understanding the cellular network and the link between tendon fibroblasts with the matrix are areas in tendon research that require profound investigations in the nearer future.

## Role of growth factors in tendon adaptation to changes in loading

An important question in relation to increased collagen turnover is how tendon senses the external loading during muscular contraction and more specifically what factors are involved in this regulation. The first example of factors involved in collagen synthesis regulation comes from the observation that apparently a gender difference exists, in that females respond less than males with regard to the increase in collagen formation after exercise (Miller et al., 2006a, b). The expected increase in collagen synthesis to exercise was less pronounced in women than in males, and furthermore the basal collagen synthesis rate was also lower in females compared with male counterparts (Miller et al., 2006a, b). Further experiments in females who have varying levels of sex hormones (e.g. oral contraceptives) suggest that estradiol may contribute to a diminished collagen synthesis response in females (Hansen et al., 2008). Interestingly, this finding of a lower collagen synthesis rate both at rest and in response to exercise in women vs males is correlated to a demonstration of lower stress to failure tolerance in patella tendons of females compared with males (Haraldsson et al., 2005). Overall, this may contribute to our understanding with regard to the fact that women experience a larger number of soft tissue injuries in e.g. cruciate ligaments than men, and may also be important in understanding how rapidly humans of both genders adapt to not only physical training but also how well they may resist prolonged periods of reduced activity. The exact mechanism behind this gender-specific response is not definitively known, but the finding of a correlation between increased estrogen levels and a reduction in collagen synthesis in humans is supported by *in vitro* studies, where it has been shown that estradiol receptors are present in ligaments (Sciore et al., 1998) and the finding that estradiol *per se* can exert a collagen synthesis-inhibiting effect in tendons and ligaments (Liu et al., 1996; Yu et al., 2001). In addition to a suggested direct inhibiting effect of estradiol upon collagen synthesis,

it may also be that estradiol levels exert an indirect effect by influencing other hormonal components of the human endocrine system. As an example, more recently, high levels of circulating estradiol in women have been shown to be associated with low levels of circulating insulin-like growth factor (IGF-I), a substance that may be directly coupled to the degree of collagen synthesis increase with exercise. Thus, the gender differences may not be an effect of estradiol directly, but rather an influence of estradiol on IGF-I.

An important regulating factor in collagen synthesis is the growth hormone (GH)–IGF-I axis, where *in vitro* data have shown a role for collagen formation (Døssing & Kjær, 2005). As an example, it has been shown that IGF-I (and IGF-II) administration in rabbits accelerates the protein synthesis in tendons (Abrahamsson, 1997), and likewise that the recovery after tendon injury was accelerated when IGF-I was administered (Kurtz et al., 1999). Although GH in skeletal muscle has been shown to exert an effect on muscle growth in GH-deficient individuals, the effect of GH supplementation on muscle protein synthesis is absent in both young and elderly humans (Lange et al., 2002). Despite this, it seems that GH/IGF-I influences connective tissue, and administration of GH over 3 weeks has been shown to elevate circulation blood levels of procollagen propeptides (Longobardi et al. 2000). Thus, it may be tempting to speculate that GH/IGF-I has a stimulating effect on tendon tissue. In dwarf rats, GH administration has been shown to increase the expression of both collagen type I and III in intramuscular fibroblasts (Wilson et al., 1995). Recently, it has been shown that IGF-I is present in human Achilles tendon linked directly to fibroblasts, and furthermore a detectable interstitial concentration has been demonstrated in human tendon (Olesen et al., 2006). Although this supports the possibility that IGF-I may play a role in human tendon, the following question, however, remains: to what extent is IGF-I upregulated with exercise?

Induction of collagen expression in response to increased loading is seen in many cell and tissue types, and has been suggested to depend on a mechanically induced expression/secretion of collagen-inducing growth factors. These growth factors are then thought to work in an auto/paracrine manner to induce ECM protein production (Sarasa-Renedo & Chiquet, 2005). Several growth factors, which stimulate collagen synthesis, are expressed in response to mechanical loading. These include transforming growth factor-β-1 (TGF-β-1) (Schild & Trueb, 2002), connective tissue growth factor (CTGF) (Frazier et al., 1996; Schild & Trueb, 2002) and IGF-I (Hansson et al., 1988; Abrahamsson & Lohmander, 1996; Butt & Bishop, 1997). Importantly, loading-induced type I and/or type III-collagen expression appear to depend directly on TGF-β-1 activity in human ligaments (Kim et al., 2002; Nakatani et al., 2002) and patella tendon fibroblasts (Yang et al., 2004). Thus, these growth factors, especially TGF-β-1, may play an essential role in mediating the adaptation response of tendon and muscle connective tissue to changes in mechanical loading.

We have recently investigated mRNA expression of the stress-responsive growth factors, TGF-β-1, IGF-I and CTGF, along with type I and III collagen, in rat tendon and muscle after a short-term strength training involving either pure shortening, lengthening or static contractions of plantar extensors (Heinemeier et al., 2007a, b). In the Achilles tendon, the expression of TGF-β-1 and IGF-I (but not CTGF) was increased in response to strength training, but no difference was seen between contraction types, even though the force production was markedly higher during lengthening contractions compared with shortening contractions. In gastrocnemius muscle, a similar regulation of gene expression was observed, but, in contrast to the tendon response, the effect of lengthening contractions was significantly greater than the effect of shortening contractions with regard to the expression of growth factors. Interestingly, the expression of type I and III collagen mRNA followed a pattern very similar to that of TGF-β-1 and IGF-I expression in both tissue types. These results underline that mechanical loading of tendon and muscle induces collagen expression, and they support a role for TGF-β-1 and IGF-I as mediators of this effect. Importantly, it is also indicated that tendon tissue, although it reacts to loading, is less sensitive to differences in the mechanical stimulus compared with skeletal muscle.

With the effect of loading on tendon and muscle connective tissue in mind, it is relevant to consider whether unloading will lead to an opposite response – such as a decrease in the expression of collagen and collagen-inducing growth factors (De Boer et al., 2007). Additionally, it would be interesting to investigate whether tendon, also in an unloading situation, will react less markedly than muscle. To study the coordinated response of tendon and muscle to unloading, we measured tissue mass, as well as mRNA expression levels for collagen and growth factors, of rat Achilles tendon and soleus muscle after 7 and 14 days of hindlimb suspension. In accordance with earlier studies, the soleus muscle mass decreased to 50% of the control levels after 14 days of unloading (Hirose et al., 2007). Achilles tendon mass, on the other hand, was not affected by suspension, and although this may seem surprising, previous studies have shown unchanged cross-sectional areas of rat Achilles tendons after several weeks of unloading in both human and animal studies (Bikle et al., 1994; Almeida-Silveira et al. 2000; Matsumoto et al., 2003). With regard to gene expression, the response to

unloading was clearly not opposite to that of loading. The expression of collagen I and III, TGF-β-1 and CTGF was not affected in either tendon or muscle by hindlimb suspension (although collagen III tended to decrease in muscle after 7 days). Furthermore, we found an increased expression level of IGF-I in tendon tissue in response to unloading. This was perhaps unexpected, but a similar effect of unloading has been shown in bone tissue, and the IGF-I mRNA increase may represent a secondary response to an unloading-induced IGF-I resistance (Bikle et al., 1994; Sakata et al., 2004).

With return to normal cage activity (reload) after the hindlimb suspension period, the skeletal muscle expression of collagen I and III was markedly induced ($>10$-fold), while the expression of growth factors was moderately induced. This indicates an overall anabolic response in the muscle tissue, which is a logical response when considering the marked loss of soleus muscle mass seen after suspension (50% at day 14 HS) compared with the moderate loss of body mass. In tendon tissue, the response to reload was far less pronounced, and the expression of growth factors corresponded to control levels during the entire reload period. The moderate response to reload supports that the changes induced by suspension were limited in tendon, compared with muscle, as indicated by the stable tissue mass and the unchanged expression of matrix-related genes during unloading. Meanwhile, the expression of both collagen I and III was actually induced in tendon after 4 days of reload, indicating that some changes must have taken place in the tendon tissue during the suspension period. This is supported by previous results showing a pronounced disuse-induced change in tendon mechanical properties (Almeida-Silveira et al. 2000; Matsumoto et al., 2003), and it could be speculated that changes in the tendon matrix induced by unloading will affect the tendon–cell response to the resumption of a normal loading pattern and thus lead to increased collagen expression.

In conclusion, the general response of both tendon and muscle tissue to unloading does not appear to follow a pattern opposite to that of a loading response, and in general the tendon response appears to be less pronounced than the muscle response. This could indicate that tendon tissue is protected from rapid changes in tissue mass, while muscle, which is known to act as a protein store for the organism, is subject to substantial and fast changes in tissue mass.

### Effect of loading on the properties of the human patellar tendon *in vivo*

In humans, cross-sectional data suggest that endurance training is associated with a larger Achilles tendon cross-sectional area, which appears to be site specific (Rosager et al., 2002; Magnusson & Kjær, 2003; Kongsgaard et al., 2005). However, in an intervention study it was shown that 9 months of endurance training in untrained persons left the Achilles tendon CSA unchanged (Hansen et al., 2003). On the other hand, animal data have shown that muscle strength/size is related to the tendon size (Elliott, 1965), suggesting that perhaps the magnitude of loading influences tendon size. In animal studies, the cross-sectional area of tendon often adapts differentially between tendon types and thus indicates that responses may be tendon specific. Several human studies have shown that resistance training over 12–14 weeks that produces increases in muscle strength of up to 21% does not result in an accompanying increase in tendon CSA (Reeves et al. 2003a, b; Kubo et al., 2006), but rather a markedly altered modulus, which implies that there is a change in the composition of the structure rather than the size. In contrast, we recently showed that, in humans, resistance training for 12 weeks yielded region-specific increases in PT CSA, without a change in modulus (Kongsgaard et al., 2007), which was the first human intervention study to report such tendon hypertrophy. It is possible that previous studies have overlooked the tendon hypertrophy because it occurs in the distal and proximal end of the tendon and not in the middle, where it is commonly measured. We subsequently followed up on these findings with an alternative design: cross-sectional studies are obviously limited due to issues of training history, selection bias and inter-subject variations. Longitudinal training studies may be of insufficient duration to produce a robust tendon hypertrophy response, and the existing training studies have examined tendon size in a region that appears to be unresponsive to training-associated adaptation. Some of these limitations may be partially overcome by examination of region-specific PT properties in persons who engage in sport where one lower extremity is habitually subjected to more loading than the contralateral (control) side, such as in fencing or badminton. When we examined patellar tendon size and mechanical properties in subjects who display a side-to-side strength difference of $\geq 15\%$ due to persistent sport-induced loading over several years, we found a regional variation in CSA along the patellar tendon, which markedly influenced average stress along the tendon (Couppe et al., 2008). The habitually more loaded tendon of the stronger extremity had a greater cross-sectional area compared with the contralateral side. The lead extremity also displayed greater stiffness than the contralateral side, while the modulus did not differ significantly. In sum, these data show that a habitual loading is associated with a robust change of the patellar tendon size and mechanical properties.

## Damage and healing of tendon – recovery and rehabilitation of human tendon

Overuse injuries of tendons are a highly frequent and disabling condition affecting both professional and recreational athletes as well as workplace employees undertaking forceful and repetitive tasks (Langberg & Kongsgaard, 2008; Tan & Chan, 2008). The prevalence of tendinopathies has been reported to range from 2% among the employment-active part of the population in western countries to as high as 55% among certain jumping athletes (Lian et al., 2006). Tendinopathy can occur in almost any tendon. Common examples include Achilles tendinitis, patellar tendinitis, tennis elbow, golfer's elbow and supraspinatus tendinitis. The high prevalence, together with the fact that the pathologies often become chronic, makes tendinopathies a large socioeconomic problem. In spite of the extremely high prevalence of tendinopathies, knowledge regarding effective rehabilitation regimes and medical treatments is limited (Langberg & Kongsgaard, 2008). One of the reasons is the lack of knowledge regarding the pathogenesis of the tendinopathies.

Activity-related pain, focal tendon tenderness and decreased strength and movement in the affected area characterize tendinopathy. Previously, it was believed that inammation was the key contributor to tendon overuse problems, but, although still debated, there is growing evidence suggesting this is not the case (Movin et al., 1997; Alfredson et al., 1999). At present, tendinopatheis are classified as a primarily degenerative condition with histologic examination of tendinosis tissues showing a disrupted collagen matrix, increased cellularity and proteoglycan concentration, but lack of inammatory cell inltration (Fu et al., 2002; Riley, 2008). Unpublished data, though, indicate that the strength-bearing structures, the fibrils, are unchanged to normal conditions in diameter and distribution in chronic overuse conditions (J. Pingel, unpublished observation), suggesting that the problem is mainly situated between the fibrils. Multiple studies have demonstrated neovascularity in the overused tendons (Ohberg et al., 2004) along with ingrowths of nerve endings. In spite of these observations, the molecular and biomechanical mechanisms underlying the cause and progression of tendinopathy as well as the healing of the connective tissue are not understood (Riley, 2008).

During the last decade, treatment involving heavy-load eccentric training has been shown to provide good clinical results in the treatment of both Achilles tendinosis (Alfredson et al., 1998; Langberg et al., 2007) as well as in the case of patella tendinopathy (Jonsson & Alfredson, 2005; Kongsgaard et al., 2006; Frohm et al., 2007). At present, not much is known about the mechanism behind this positive clinical effect of eccentric rehabilitation. A few studies have suggested potential structural, functional and molecular explanations (Ohberg & Alfredson, 2004; Ohberg et al., 2004; Langberg et al., 2007; Rees et al., 2008). After tendon injury, most repair activity is associated with cells from the epitenon and endotenon migrating to the lesion and synthesizing a new matrix (Jones et al., 2003; Kajikawa et al., 2007). This migration and matrix synthesis may also be stimulated by eccentric loading as controlled eccentric rehabilitation exercises have been found to lead to increased collagen synthesis in injured tendons only, in contrast to the healthy leg (Langberg et al., 2007). This increase in collagen synthesis is correlated with a decrease in pain during activity, indicating that the condition emerged from a mismatch between the tissue strength and the load during activity (Langberg et al., 2007). Alternatively, the rehabilitation training resulted in a reduced release of pain-inducing factors. In a recent study on the effect of experimental induced pain (injections of hypertonic saline in the Achilles tendon), we showed that the muscular control of the calf muscle is disturbed as a result of the pain (M. Henriksen et al., unpublished observation). When loaded eccentrically, the pain leads to increased physiological tremors not present during concentric loading. This could indicate that the fibroblasts migrating into the injured tissue (Jones et al., 2003) are exposed to a different load pattern when subjected to eccentric contraction, leading to an increase in collagen synthesis and thus stimulating the healing of the injured tissue. However, despite this sparse information, the number of unanswered questions are still substantial, and much more research is needed before the etiology of tendinopathy is understood and the correct rehabilitation is determined.

## Perspectives

The increased knowledge of the collagen response of human tendon toward loading is important for recommendations in training and rehabilitation regimens in order to maximize effect without overloading tendons. It is also important for the understanding of long-term tissue changes in relation to chronic training. Clearly, it represents only one side of the coin, and studies on other matrix proteins and structures (e.g. cross bindings) will be important to explain the conversion of biochemical changes in tendon into structural and functional adaptations.

**Key words:** fibroblast, growth factors, exercise, physical training.

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