Introduction

Tendons link skeletal muscle to bone and are essential in all voluntary movement. In turn, movement appears to affect tendon properties, and in recent years considerable effort has been put into discovering how tendon tissue responds to mechanical stimuli in vivo. Months and years of mechanical loading can influence the gross morphology of tendon, seen as an increase tendon cross-sectional area (CSA). Similarly, tendon stiffness appears to be affected by weeks to months of loading. Increased stiffness can relate to changes in CSA and/or tendon material properties (modulus), though the relative contribution of these parameters is largely unclear. The possible mechanisms behind alterations in tendon mechanical properties include changes in collagen fibril morphology and levels of cross-linking between collagen molecules. Furthermore, increased levels of collagen synthesis and expression are seen as a response to acute exercise and training, and may be a central parameter in tendon adaptation to loading. There are indications that this collagen-induction relates to the auto-/paracrine action of collagen-stimulating growth factors, such as TGFβ-1 and IGF-I, which are expressed in response to mechanical stimuli.

Keywords: Tendon, Exercise, Collagen, TGF-beta, IGF-I, Material Properties

Tendon composition

Tendon tissue is characterized by a large extracellular matrix (ECM) and a relatively low number of cells, which are primarily fibroblasts. The major component of the tendon ECM is type I collagen assembled in type I collagen fibrils. The collagen fibrils are arranged mainly in the longitudinal direction of the force imposed on the tendon during muscle contraction. In mature tendon tissue, the fibroblasts are typically arranged in rows along the force-transmitting axis of the tendon and can be connected to each other by gap-junctions. In general, connective tissue fibroblasts are attached to the ECM via integrins that span the cell membrane and connect the intracellular cytoskeleton with the surrounding matrix, and presumably such an integrin-based attachment also exists in tendon tissue. The physical link between fibroblasts and ECM permits the cells to sense and respond to mechanical stimuli and appears vital for fibroblast function. Likewise the connection between tendon fibroblasts and the tendon matrix is thought to be essential for the ability of tendon tissue to sense and thus adapt to changes in the mechanical loading pattern.

In the past decades considerable effort has been put into discovering how tendon tissue responds to mechanical stimuli in vivo in relation to morphological, viscoelastic and metabolic parameters.

Long-term loading and tendon size in humans

Indications of tendon adaptation to physical loading have been found in cross sectional studies investigating the thickness of tendons in persons performing long-term habitual load-
ing. Male runners were found to have ~30 % larger achilles tendon cross sectional areas (CSA) than non-runners6, and similarly male athletes who perform frequent weight-bearing exercise (running, jumping) have relatively thick tendons compared to athletes in non-weight bearing sports (kayakers) (~20 % larger CSA)7. It could of course be argued that these differences are based on natural selection. However, a recent study found greater CSAs of patella tendons in the leading leg of male athletes competing for at least 5 years in sports with a side-to-side difference (badminton and fencing). In the proximal part of the tendon there was an almost 30 % difference in CSA between the leading leg and the non-leading leg8. This must be considered as relatively strong evidence that tendons undergo hypertrophy, at least after very long-term mechanical loading. Interestingly, these findings seems to be gender specific, in that marked differences in tendon CSA has not consistently been found between female athletes and sedentary controls9. This suggests that gender specific humoral factors may be involved in the training induced adaptive morphological response of human tendon, and several studies do in fact indicate that the exercise related adaptation of the tendon tissue is blunted when levels of estrogen are high10-12.

**Short-term loading and tendon size**

Several attempts have been made to detect tendon hypertrophy in response to more short-term loading. A major part of these studies do not find any change in tendon CSA after 2-3 months, or more, of increased physical loading13-19. However, Kongsgaard et al. found a 4-7% increase in CSA of the patella tendon after 12 weeks of knee extension strength training20, and Kongsgaard et al. found a 4-7% increase in CSA of the patella controls9. This suggests that gender specific humoral factors consistently been found between female athletes and sedentary controls9. However, a recent study found greater CSAs of patella tendons in the leading leg of male athletes competing for at least 5 years in sports with a side-to-side difference (badminton and fencing). In the proximal part of the tendon there was an almost 30 % difference in CSA between the leading leg and the non-leading leg8. This must be considered as relatively strong evidence that tendons undergo hypertrophy, at least after very long-term mechanical loading. Interestingly, these findings seems to be gender specific, in that marked differences in tendon CSA has not consistently been found between female athletes and sedentary controls9. This suggests that gender specific humoral factors may be involved in the training induced adaptive morphological response of human tendon, and several studies do in fact indicate that the exercise related adaptation of the tendon tissue is blunted when levels of estrogen are high10-12.

### Measuring tendon mechanics

A number of studies have investigated changes in the *in vivo* biomechanics of human tendon tissue in response to loading. Typical parameters describing the tendon mechanical properties are:

1. **Tendon strain**, which describes the elongation/deformation of the tendon (ΔL) relative to the normal length (L₀): Strain = ΔL / L₀
2. **Tendon stress**, which describes the tendon force (Fₜ) relative to the tendon CSA Stress = Fₜ / CSA
3. **Tendon stiffness**, which describes the change in tendon length (ΔL) (deformation) in relation to the force applied to the tendon (ΔFₜ). This parameter is dependent on the CSA and length of the tendon. (Greater CSA and shorter length will lead to greater stiffness) (Figure 1) Stiffness: ΔFₜ / ΔL (Figure 1)
4. **Tendon modulus**, which describes the relation between tendon stress and tendon strain. In other words, modulus represents the properties of the actual tendon material independently of the CSA. This makes it possible to compare structures with different dimensions. A high tendon modulus indicates a relatively stiff tissue. Modulus = Stress/strain

Previously these tendon mechanical properties were typically measured in mechanical testing devices on *ex vivo* animal tendons22. In recent years, however, the use of ultrasound has advanced the *in vivo* measures of tendon mechanics and made studies on human tendons possible28,30.

Typically the elongation of the tendon is measured with ultrasound (US) and with similar interventions Seynnes et al. and Arampatzis et al. also observed increases in CSAs of the patella- and achilles tendons respectively21,22. Common for these 3 studies was that CSA was measured with use of magnetic resonance imaging (MRI) (as opposed to ultrasound (US)) and that the increases seen in CSA were only regional. In other words the tendon only hypertrophied at certain lengths, indicating that it could be important to measure in the right location in order to detect these changes. This is supported by the above described findings of Couppé et al., who also found that tendon hypertrophy in athletes was only present in proximal and distal parts of the tendon6. Furthermore, several studies indicate that MRI gives relatively precise measures of tissue cross sectional areas23,26, and this method might be more precise than ultrasound (US)27, and thus enable detection of smaller tendon CSA changes.

In summary, it appears that tendons do hypertrophy in response to both long- and short-term loading, but that short-term changes in CSA are relatively small and seemingly occur only at specific regions of the tendon.

The CSA of a tendon is important for its biomechanical properties. A tendon with a large CSA will elongate less than a smaller tendon at the same force, provided that the tendon tissue material properties are the same. In this way, a loading induced increase in CSA will change the tendon mechanics, which intuitively makes sense for example in relation to large increases in muscle strength. In addition to tendon hypertrophy, an alteration of the tissue material properties is another way for the tendon to adapt to loading, which will also influence the mechanical properties of the tendon28.

**Abbreviations**

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>CSA</td>
<td>Cross sectional area</td>
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<td>ECM</td>
<td>Extra cellular matrix</td>
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<td>IGF-I</td>
<td>Insulin like growth factor-I</td>
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<td>LOX</td>
<td>Lysyl oxidase</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>PET</td>
<td>Positron emission tomography</td>
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<tr>
<td>PINP</td>
<td>Pro-collagen I N-terminal pro-peptide</td>
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<td>PICP</td>
<td>Pro-collagen I C-terminal pro-peptide</td>
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<tr>
<td>TGF-β-1</td>
<td>Transforming growth factor-b-1</td>
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actual force placed on the tendon is estimated and related to the simultaneous measures of tendon elongation (Figure 1). Based on these measures, strain and stiffness can be calculated, and if tendon CSA is measured (with either US or MRI) it is additionally possible to calculate stress and modulus30.

**Loading and tendon mechanics**

A number of studies have investigated changes in tendon mechanical properties in response to resistance type training (6-14 weeks), and a large part of these observe an increase in tendon stiffness as a result of the increased loading5,6,13,16,20-22,31,32. There are, however, quite large discrepancies between the findings in these studies. For example Reeves et al. found an increase of 65% in tendon stiffness in elderly people after strength training, while Kongsgaard et al. only found a 15% increase after an apparently more intense strength training period in young men20,32. This may of course relate to differences in the subjects, but it is very likely that the complexity of measuring tendon mechanical properties could also contribute to the divergence. At least there appears to be a considerable variance in the methodological approaches used by the investigators in this field. These differences relate to: 1) the method for US imaging of tendons during measurement of elongation (use of external marker or inclusion of two bony ends in US image) (Figure 2), 2) the duration of the ramp isometric contraction used for measuring corresponding levels of tendon elongation and tendon force (varying from 4- to 10 seconds), 3) the methods for estimation of the actual tendon force (e.g. correction for antagonist activation and joint moment arm) and several others20,32.

In spite of these methodological differences, it appears that the tendon stiffness can be altered in response to chronic loading. This increase in stiffness may be a result of tendon hypertrophy, as stiffness is directly dependent on CSA, or of changes in the material properties (modulus), or a combination of these. Several authors find an increase in modulus (varying from 10- to 65 %) without any change in CSA, indicating that the stiffness change is based solely on an alteration of material properties or that small CSA changes have gone undetected (discussed above)15,16,32. Others find increases in both modulus and CSA20-22 (only tendency to increase in modulus in Kongsgaard et al. 2007). The relative contribution of CSA changes versus changes in material properties to the increased stiffness is very difficult to determine, especially when taking into account the methodological differences between studies. Interestingly, Couppé et al. found that athletes competing in sports with at side-to-side difference had markedly stiffer tendons on the leading leg (~35%), and this seemed to be exclusively due to an increase in CSA, as no difference was observed in tendon modulus.

The triggering of tendon mechanical adaptation may relate closely to the type and degree of loading applied. Experiments performed by Arampatzis et al. indicate that a certain degree of strain is necessary to induce tendon adaptation. They found an increased human achilles tendon stiffness in response to 14 weeks of plantar flexor training at strain levels of ~4.5%, but not at strain levels of ~3%, even though loading frequencies...
Tendon morphology and loading

The loading induced enhanced mechanical tendon properties may relate to an increased collagen content. In support of this there are several indications of a loading induced collagen synthesis in tendon in vivo human experiments. As a part of this response, a change in the size of collagen fibrils has been suggested as a mechanism for adaptation of tendon to mechanical stimulus. In theory fibrils with large diameters should increase stiffness because of the greater possibility of cross-links (see below) (Figure 3). This suggestion is supported by findings of larger mass-average fibril diameters in horse tendons with high modulus compared to tendons with lower modulus within the same animal.

Evaluation of loading related changes in fibril morphology of animal tendons, measured with electron microscopy (Figure 3), shows diverging results. Patterson-Kane et al. found a reducing effect of training on fibril diameter in mature horses, while in young horses (21 months old) they saw no effect of the same regimen. In mice a u-shaped response was found over time, with a marked increase in fibril diameter in the initial loading period, followed by a decrease in diameter and finally an increase after 10 weeks of training. The results from mice may indicate a splitting, and possibly fusion, of fibrils of a certain size and this possibility underlines the complexity of investigating this phenomenon. The complexity is further increased by the variation in fibril morphology between tendons (within individuals) and by the natural progressive change in fibril size during both maturation and aging.

To our knowledge no human studies on healthy subjects have yet been published regarding a loading-induced change in tendon fibril morphology. However, it has been shown in humans with patella tendinopathy that heavy strength training may normalize an otherwise pathological distribution of fibril diameters. This suggests that loading can influence fibril morphology in humans, though further studies are needed to determine if this is also the case in healthy tendons.

In addition to alterations in fibril morphology, the degree of cross-linking between collagen molecules within the fibrils is an obvious parameter to investigate in relation to tendon mechanics. Cross-links connect the individual collagen triple helices to each other, and are essential in preventing slippage between molecules when the tissue is mechanically loaded. Thus, an increase in the formation of cross-links in response to loading could be a part of the tendon adaptation process.

Especially the enzymatically derived cross-links, which are synthesized trough the activity of lysyl oxidase (LOX), seem likely to be influenced by loading, as the production/activity of LOX may be regulated by mechanical stimuli. In support of this, short-term training in rats markedly increased levels of lysyl oxidase expression in the loaded tendon tissue. No data are yet published in relation to changes in LOX enzyme- or actual cross-link levels in response to loading of healthy human tendons. Indirectly, however, a recent study in elderly men indicates the importance of cross-links for tendon mechanics. It showed that elderly men had 30% less collagen in their tendons compared to young, but no differences were found in tendon mechanical properties, as this was seemingly compensated for by an increased amount of both enzymic- and AGE (advanced glycation end product) cross-links.

In summary, the precise mechanism for changes in tendon mechanical properties as a response to long-term loading are still unknown. There are however strong indications that an increased collagen synthesis is part of the tendon adaptation response to mechanical loading.

Tendon collagen synthesis in response to loading

Different approaches have been taken to estimate the in vivo tendon collagen synthesis in response to mechanical stimulus. Microdialysis has been employed in human subjects for measuring changes in peri-tendinous levels of collagen I pro-peptides (PINP and PICP, Pro-collagen I N- and C- terminal pro-peptides). These pro-peptides, which are cleaved off from newly synthesized collagen molecules, are used as markers of collagen synthesis. Studies investigating both acute exercise and prolonged training have consistently shown elevated levels of these markers in peri-tendinous tissue of the loaded tendons – indicating elevated collagen synthesis in response to mechanical stimuli.

To get a more direct measure of collagen synthesis inside the actual tendon, an alternative approach is to measure incor-
poration of stable isotope labeled proline into tendon tissue (e.g. C-13-proline). By supplementing participants with labeled proline followed by sampling of tendon biopsies, the extent of proline incorporation in the tissue, measured by gas chromatography-mass spectrometry, gives an indication of the collagen synthesis rate in the tendon\textsuperscript{35}. Using this method an increase in the collagen fractional synthesis rate was seen in patella tendons of young males in response to acute kicking exercise\textsuperscript{35,36}, while no response was seen in women after similar exercise\textsuperscript{36}. This supports the possibility of loading induced collagen synthesis in tendon, but also underlines that this adaptation response may be dependent on certain hormonal levels.

An obvious third approach to estimate induction of collagen synthesis by tendon loading is to investigate alterations in mRNA expression of collagen. In rats this approach revealed a marked increase in achilles tendon type I and III collagen mRNA in response to both short-term resistance training and tendon overload by muscle ablation\textsuperscript{48,50}. In humans knowledge on this subject is extremely limited and only one study is published with regard gene-expression changes in healthy human tendon after exercise. In this study a decrease was found in the expression of collagen I and III mRNA 4 hours after resistance type exercise, while levels were back to normal after 24 h\textsuperscript{51}. These results indicate that loading induced collagen synthesis is not based on increased transcription rates, which contradicts the animal data. This may relate of course to species differences, but also to the amount of loading, and timing of measurements. Considering the apparent sex-difference in the tendon response to loading\textsuperscript{36} it should also be noted that the study included both male (n=3) and female (n=3) participants\textsuperscript{51}. In other words, further studies are needed on humans to fully elucidate the effect of acute loading on tendon collagen expression.

\textbf{Figure 4.} Possible mechanism for loading induced collagen synthesis: 1) Fibroblast connected to extra cellular matrix via integrins. 2) Transcription and synthesis of growth factors induced by mechanical loading via changed intracellular signaling. 3) Autocrine/paracrine action of growth factors leading to increased collagen transcription and synthesis.
Mechanisms for induction of collagen synthesis

The mechanism linking mechanical loading of tendon tissue to the apparent increase in collagen synthesis is still unknown. A number of in vitro studies indicate that the mechanical stimulation of collagen expression may be dependent on the auto/para-crine effect of certain growth factors expressed in response to loading (Figure 4). These growth factors include transforming growth factor-β-1 (TGF-β-1)⁶², connective tissue growth factor (CTGF)⁵³,⁵⁴, insulin like growth factor-I (IGF-I)⁵⁵,⁵⁶ and IL-6⁶⁰,⁶¹.

TGF-β-1 has been implicated in regulation of tendon collagen expression, as loading-induced type I and/or type III-collagen expression appears to depend directly on TGF-β-1 activity in human ligament.⁶¹,⁶² and patella tendon fibroblast³². However, this is based on cell culture studies, and in vivo data are very scarce on this subject, although one human study shows a probable exercise-induced increased in peritendon levels of TGF-β-1⁶³. In addition, tendon mRNA expression of TGF-β-1 was markedly induced by short-term training in rats⁴⁸, and this was seen simultaneously with an increased collagen I expression. Combined with the in vitro based knowledge, this supports a role for TGF-β-1 in mechanically induced tendon collagen synthesis, though further studies are needed to define a causal relationship in vivo.

CTGF has been suggested as a downstream mediator of the TGF-β-1 collagen-inducing actions in fibroblasts⁶⁴,⁶⁵, but only few data exist on the role of CTGF in tendon. One study found an increase in the amount of CTGF positive (immuno-stained) cells in rabbit tendon after 80 accumulated hours of low force repetitive loading⁶³, but a later study using the same model found no effect on the tendon CTGF mRNA levels⁶⁶. Similarly no significant changes were found in tendon expression of CTGF in response to 4 days of resistance type training⁴⁸ or in response to 12 weeks of different types of exercise training in rats⁶⁷. Thus an important role for CTGF in tendon adaptation to loading seems unlikely based on the current knowledge, however, no studies have yet been published with regard to CTGF in human tendons.

IGF-I is another likely candidate to induce collagen synthesis in loaded tendon tissue. In vitro studies show that IGF-I induces type I collagen synthesis in rabbit tendon explants⁶⁰,⁶⁸, and recently systemic growth hormone supplementation was shown to induce a simultaneous increase in the expression of IGF-I and collagen I in human ligament.⁶¹,⁶² and patella tendon fibroblast³². With regard to the connection between mechanical loading and IGF-I expression, two early in vivo studies indicate that the presence of IGF-I protein in rat tendon is induced by mechanical stimulus⁵⁵,⁵⁶, and more recently loading of rat tendon was found to induce IGF-I mRNA expression simultaneously with type I collagen⁴⁸. Thus, IGF-I in addition to TGF-β-1, may be important for the loading induced changes seen in tendon collagen expression and synthesis.

Finally, IL-6 has been found to induce collagen synthesis in fibroblasts⁵⁸,⁵⁹, and since running exercise has been shown to induce relatively large increases in peri-tendon levels of IL-6⁶⁰, this factor may also act as a mediator of loading induced collagen synthesis. This is supported by recent unpublished data, indicating that tendon collagen synthesis is increased with microdaly-sis-based local infusion of IL-6 (Bisgaard et al. in review).

Conclusion

In conclusion, mechanical loading appears to induce changes in gross morphology, mechanical properties as well as biochemical parameters of tendon tissue.

Both long-term (years)- and relatively short-term (months) loading induces tendon hypertrophy, although CSA-changes in response to short-term loading-regimes are not observed by all investigators. This may be partly due to the fact that the degree of hypertrophy is relatively small and seems to occur only in certain tendon regions.

With regard to tendon mechanical properties, increased tendon stiffness is generally observed in response to large volumes of loading. The extent of this adaptation is highly variable, however, and may depend on the methodological approach. Likewise no consensus exists with regard to the relative contribution of CSA changes versus changes in tendon modulus to this increase in stiffness.

Alterations in tendon material properties are suggested to include changes in collagen fibril morphology as well as cross-link content, but further studies are needed in this context, as only indirect indications of this type of tendon adaptation in humans exist at the moment.

An increased collagen synthesis is consistently observed as a part of the tendon adaptation response to mechanical loading, and there are indications the this effect relates to the action of collagen stimulating growth factors such as TGFβ-1 and IGF-I.

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References


