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Review Article

Hylonome

In vivo investigation of tendon responses to mechanical loading

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Abstract

Tendons transmit skeletal muscle forces 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 material 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

Introduction

Tendons link skeletal muscle to bone and are essential in all movements initiated by muscle contraction. But not only are tendons essential for movement - movement is also essential for tendons. In 1977 Beckham and co-workers found that the formation of chick tendons during embryogenesis was incomplete when muscle contraction was inhibited[1]. This underlines the close relationship between tendon function and mechanical stimulus, which also exists in mature tissue. The adaptation of tendon tissue to mechanical loading will be the topic of this overview, with the main focus on *in vivo* studies in humans.

Tendon composition

Tendon tissue is characterized by a large extra cellular matrix (ECM) and a relatively low number of cells, which are primarily fibroblasts[2]. 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[3]. 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[2]. 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[4] (Figure 4), and presumably such an integrin-based attachment also exists in tendon tissue[5]. 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[4].

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-

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Abbreviations

CSA: Cross sectional area
ECM: Extra cellular matrix
IGF-I: Insulin like growth factor-I
LOX: Lysyl oxidase
MRI: Magnetic resonance imaging
PET: Positron emission tomography
PINP: Pro-collagen I N-terminal pro-peptide
PICP: Pro-collagen I C-terminal pro-peptide
TGF-β-1: Transforming growth factor-b-1
US: Ultrasound

ing. Male runners were found to have ~30 % larger achilles tendon cross sectional areas (CSA) than non-runners[6], 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 leg[8]. 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 controls[9]. 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 high[10-12].

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 loading[13-19]. However, Kongsgaard et al. found a 4-7% increase in CSA of the patella tendon after 12 weeks of knee extension strength training[20], and with similar interventions Seynnes et al. and Arampatzis et al. also observed increases in CSAs of the patella- and achilles tendons respectively[21,22]. 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 tendon[8]. Furthermore, several studies indicate that MRI gives relatively precise measures of tissue cross sectional areas[23-26], 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 tendon[28].

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 (L_0):
   Strain = $\Delta L / L_0$
2) Tendon stress, which describes the tendon force (F_t) relative to the tendon CSA
   Stress = F_t / CSA
3) Tendon stiffness, which describes the change in tendon length (ΔL) (deformation) in relation to the force applied to the tendon (ΔF_t). 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: $\Delta F_t / \Delta L$ (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 tendons[29]. In recent years, however, the use of ultrasound has advanced the *in vivo* measures of tendon mechanics and made studies on human tendons possible[28,30].

Typically the elongation of the tendon is measured with ultrasound during a ramp isometric (static) contraction ending in maximal voluntary contraction (MVC) (Figure 2). This is done simultaneously with a measure of force output. Correcting for joint moment arm length and muscle antagonist activation, the

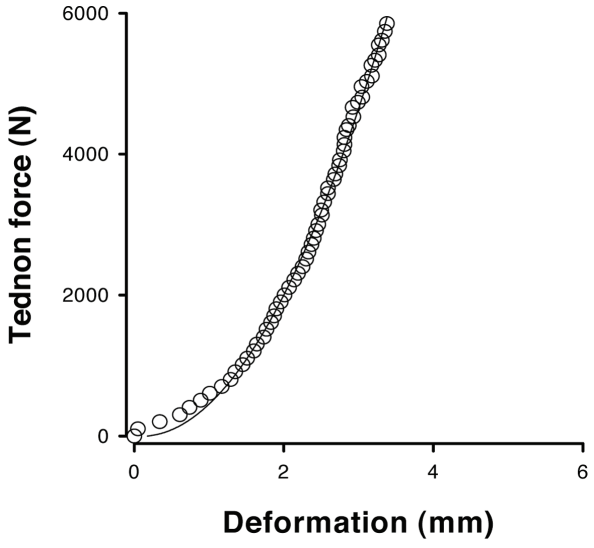

Figure 1. Patella tendon force-derformation curve. Relationship between the force applied to the tendon (F_t) and the tendon deformation (elongation (ΔL)).

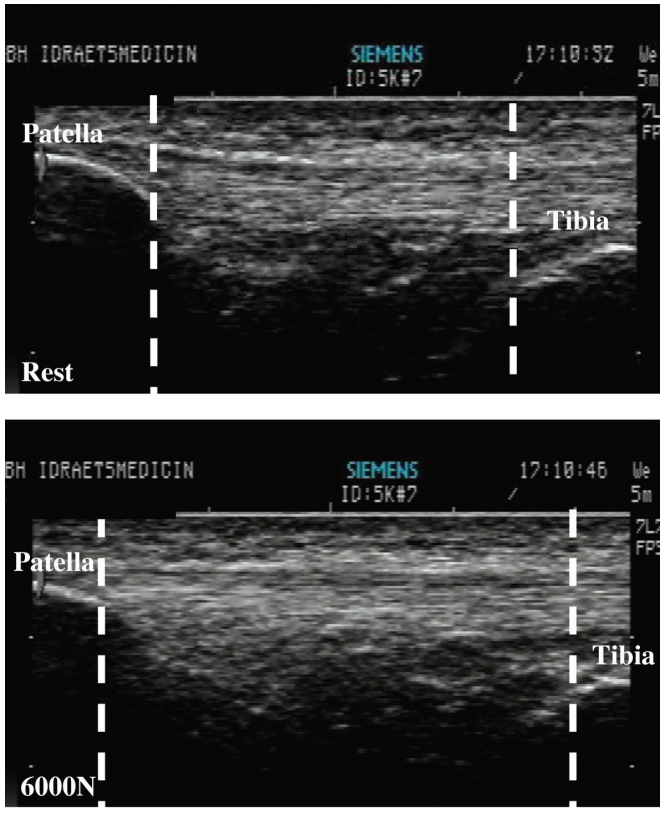

Figure 2. Ultrasound image of the patella tendon (including patella and tibia) at rest (top) and subjected to 6000 Newton (bottom).

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 modulus[30].

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 loading[13,16-18,20-22,31,32].

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 men[20,32]. 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 others[20,32].

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)[13,16,32]. Others find increases in both modulus and CSA[20-22] (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

and -volumes were equal[13,21]. Similarly, Kongsgaard et al. found that heavy resistance training led to increased patella tendon stiffness in young men, while light resistance training of equal volume did not have this effect[20]. This indicates that a certain strain or stress level is necessary to induce tendon adaptation, and taking into account the *in vitro* evidence of tendon damage in response to loading above certain stress levels[33], it is likely that no great difference separates beneficial loading (which leads to improved tendon properties) from tendon overloading/overuse.

Tendon morphology and loading

The loading induced enhanced mechanical tendon properties may relate to an increased collagen content[34]. In support of this there are several indications of a loading induced collagen synthesis in tendon in *in vivo* human experiments[10,35-40].

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)[41] (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[42].

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[43], while in young horses (21 months old) they saw no effect of the same regimen[44]. 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[45]. 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)[42] and by the natural progressive change in fibril size during both maturation and aging[41].

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[46]. 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 helixes to each other, and are essential in preventing slippage between molecules when the tissue is mechanically loaded[47]. Thus, an increase in the formation of cross-links in response to loading could be a part of the tendon adaptation process.

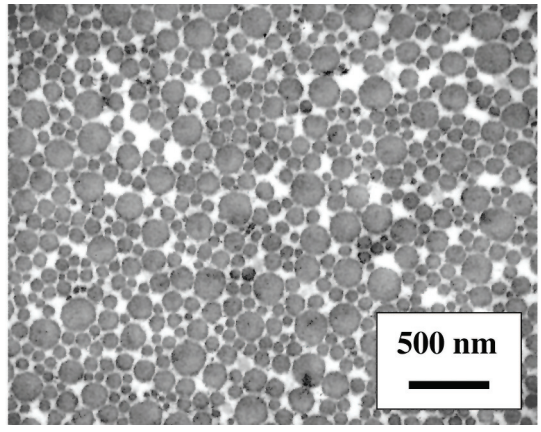

Figure 3. Transverse Electron microscope image of human patella tendon collagen fibrils.

Especially the enzymatically derived cross-links, which are synthesized trough the activity of lysyl oxidase (LOX)[47], 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[48]. 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[49].

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[10,37-40].

To get a more direct measure of collagen synthesis inside the actual tendon, an alternative approach is to measure incor-

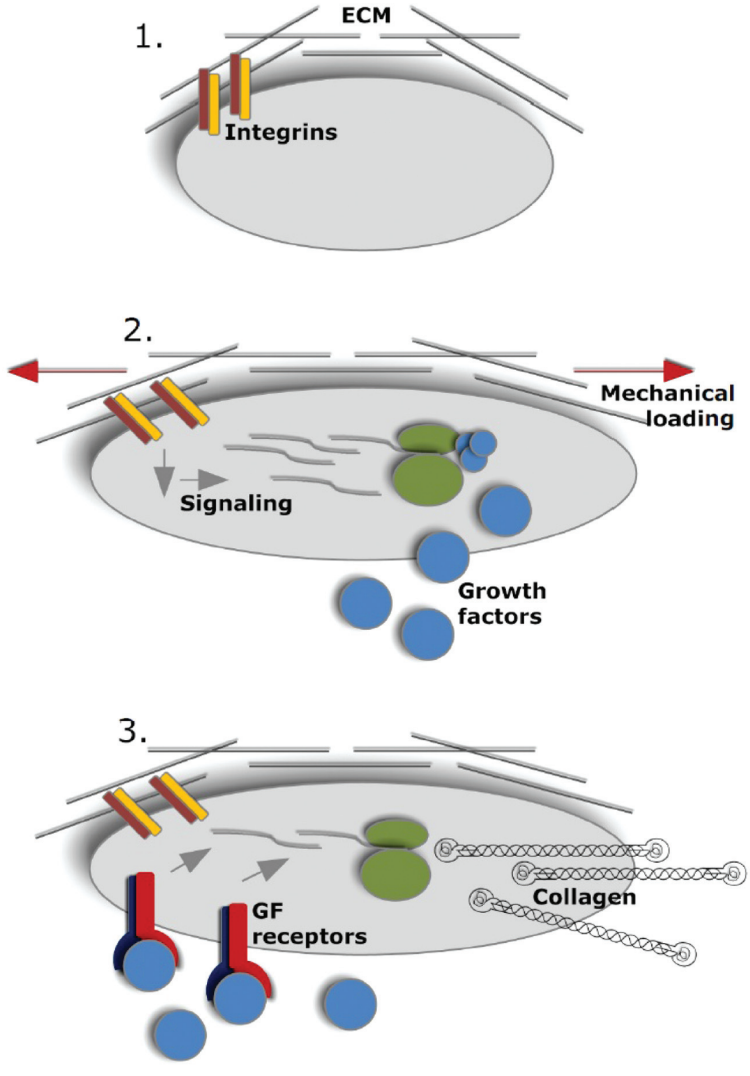

Figure 4. Possible mechanism for loading induced collagen synthesis: 1) Fibroblast connected to extra cellular matrix via integrins. 2) Transcription and synthesis of growth factors induced by mechanical loading via changed intracellular signaling. 3) Autocrine/paracrine action of growth factors leading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

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[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[35,36], while no response was seen in women after similar exercise[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[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[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[36] it should also be noted that the study included both male (n=3) and female (n=3) participants[51]. In other words, further studies are needed on humans to fully elucidate the effect of acute loading on tendon collagen expression.

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)[52], connective tissue growth factor (CTGF)[53,54], insulin like growth factor-I (IGF-I)[55-57] and IL-6[58-60].

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 fibroblast[52]. 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-1[63]. In addition, tendon mRNA expression of TGF-β-1 was markedly induced by short-term training in rats[48], 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 fibroblasts[64,65], 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 loading[53], but a later study using the same model found no effect on the tendon CTGF mRNA levels[66]. Similarly no significant changes were found in tendon expression of CTGF in response to 4 days of resistance type training[48] or in response to 12 weeks of different types of exercise training in rats[67]. 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 explants[56,68], and recently systemic growth hormone supplementation was shown to induce a simultaneous increase in the expression of IGF-I and collagen I in human tendon tissue *in vivo*[69]. 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 stimulus[55,70], and more recently loading of rat tendon was found to induce IGF-I mRNA expression simultaneously with type I collagen[50,71]. 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 fibroblasts[58,59], and since running exercise has been shown to induce relatively large increases in peri-tendon levels of IL-6[60], 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 microdalysis-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

1. Beckham C, Dimond R, Greenlee TK Jr. The role of movement in the development of a digital flexor tendon. Am J Anat 1977;150(3):443-59.
2. Wang JH. Mechanobiology of tendon. J Biomech 2006;39(9):1563-82.
3. Kannus P. Structure of the tendon connective tissue. Scand J Med Sci Sports 2000;10(6):312-20.
4. Chiquet M, Gelman L, Lutz R, Maier S. From mechanotransduction to extracellular matrix gene expression in fibroblasts. Biochim Biophys Acta 2009;1793(5):911-20.
5. Bayer ML, Yeung CY, Kadler KE, et al. The initiation of embryonic-like collagen fibrillogenesis by adult human tendon fibroblasts when cultured under tension. Biomaterials 2010;31(18):4889-97.
6. Rosager S, Aagaard P, Dyhre-Poulsen P, Neergaard K, Kjaer M, Magnusson SP. Load-displacement properties

of the human triceps surae aponeurosis and tendon in runners and non-runners. Scand J Med Sci Sports 2002; 12(2):90-8.
7. Kongsgaard M, Aagaard P, Kjaer M, Magnusson SP. Structural Achilles tendon properties in athletes subjected to different exercise modes and in Achilles tendon rupture patients. J Appl Physiol 2005;99(5):1965-71.
8. Couppe C, Kongsgaard M, Aagaard P et al. Habitual loading results in tendon hypertrophy and increased stiffness of the human patellar tendon. J Appl Physiol 2008; 105(3):805-10.
9. Westh E, Kongsgaard M, Bojsen-Moller J, et al. Effect of habitual exercise on the structural and mechanical properties of human tendon, *in vivo*, in men and women. Scand J Med Sci Sports 2008;18(1):23-30.
10. Hansen M, Koskinen SO, Petersen SG, et al. Ethinyl oestradiol administration in women suppresses synthesis of collagen in tendon in response to exercise. J Physiol 2008;586(Pt 12):3005-16.
11. Hansen M, Kongsgaard M, Holm L, et al. Effect of estrogen on tendon collagen synthesis, tendon structural characteristics, and biomechanical properties in postmenopausal women. J Appl Physiol 2009; 106(4):1385-93.
12. Hansen M, Miller BF, Holm L, et al. Effect of administration of oral contraceptives *in vivo* on collagen synthesis in tendon and muscle connective tissue in young women. J Appl Physiol 2009;106(4):1435-43.
13. Arampatzis A, Peper A, Bierbaum S, Albracht K. Plasticity of human Achilles tendon mechanical and morphological properties in response to cyclic strain. J Biomech 2010.
14. Foure A, Nordez A, Cornu C. Plyometric training effects on Achilles tendon stiffness and dissipative properties. J Appl Physiol 2010;109(3):849-54.
15. Hansen P, Aagaard P, Kjaer M, Larsson B, Magnusson SP. Effect of habitual running on human Achilles tendon load-deformation properties and cross-sectional area. J Appl Physiol 2003;95(6):2375-80.
16. Kubo K, Kanehisa H, Ito M, Fukunaga T. Effects of isometric training on the elasticity of human tendon structures *in vivo*. J Appl Physiol 2001;91(1):26-32.
17. Kubo K, Ikebukuro T, Yaeshima K, Yata H, Tsunoda N, Kanehisa H. Effects of static and dynamic training on the stiffness and blood volume of tendon *in vivo*. J Appl Physiol 2009;106(2):412-7.
18. Kubo K, Ikebukuro T, Yata H, Tsunoda N, Kanehisa H. Time course of changes in muscle and tendon properties during strength training and detraining. J Strength Cond Res 2010;24(2):322-31.
19. Urlando A, Hawkins D. Achilles tendon adaptation during strength training in young adults. Med Sci Sports Exerc 2007;39(7):1147-52.
20. Kongsgaard M, Reitelseder S, Pedersen TG, et al. Region specific patellar tendon hypertrophy in humans following resistance training. Acta Physiol (Oxf) 2007;191(2):111-21.
21. Arampatzis A, Karamanidis K, Albracht K. Adaptational responses of the human Achilles tendon by modulation of the applied cyclic strain magnitude. J Exp Biol 2007; 210(Pt15):2743-53.
22. Seynnes OR, Erskine RM, Maganaris CN, et al. Training-induced changes in structural and mechanical properties of the patellar tendon are related to muscle hypertrophy but not to strength gains. J Appl Physiol 2009;107(2):523-30.
23. Mitsiopoulos N, Baumgartner RN, Heymsfield SB, Lyons W, Gallagher D, Ross R. Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. J Appl Physiol 1998;85(1):115-22.
24. Wakefield RJ, O'Connor PJ, Conaghan PG, et al. Finger tendon disease in untreated early rheumatoid arthritis: a comparison of ultrasound and magnetic resonance imaging. Arthritis Rheum 2007;57(7):1158-64.
25. Chang CB, Seong SC, Kim TK. Preoperative magnetic resonance assessment of patellar tendon dimensions for graft selection in anterior cruciate ligament reconstruction. Am J Sports Med 2009;37(2):376-82.
26. Reeves ND, Maganaris CN, Maffulli N, Rittweger J. Human patellar tendon stiffness is restored following graft harvest for anterior cruciate ligament surgery. J Biomech 2009;42(7):797-803.
27. Roberts CS, King DH, Goldsmith LJ. A statistical analysis of the accuracy of sonography of the patellar tendon. Arthroscopy 1999;15(4):388-91.
28. Magnusson SP, Narici MV, Maganaris CN, Kjaer M. Human tendon behaviour and adaptation, *in vivo*. J Physiol 2008;586(1):71-81.
29. Woo SL, Ritter MA, Amiel D, et al. The biomechanical and biochemical properties of swine tendons—long term effects of exercise on the digital extensors. Connect Tissue Res 1980;7(3):177-83.
30. Hansen P, Bojsen-Moller J, Aagaard P, Kjaer M, Magnusson SP. Mechanical properties of the human patellar tendon, *in vivo*. Clin Biomech (Bristol, Avon) 2006;21(1):54-8.
31. Burgess KE, Connick MJ, Graham-Smith P, Pearson SJ. Plyometric vs. isometric training influences on tendon properties and muscle output. J Strength Cond Res 2007; 21(3):986-989.
32. Reeves ND, Maganaris CN, Narici MV. Effect of strength training on human patella tendon mechanical properties of older individuals. J Physiol 2003;548(Pt 3):971-81.
33. Wang XT, Ker RF, Alexander RM. Fatigue rupture of wallaby tail tendons. J Exp Biol 1995;198(Pt3):847-52.
34. Parry DA, Barnes GR, Craig AS. A comparison of the size distribution of collagen fibrils in connective tissues as a function of age and a possible relation between fibril size distribution and mechanical properties. Proc R Soc Lond B Biol Sci 1978;203(1152):305-21.
35. Miller BF, Olesen JL, Hansen M, et al. Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. J Physiol 2005;567(Pt3):1021-33.
36. Miller BF, Hansen M, Olesen JL, et al. Tendon collagen synthesis at rest and after exercise in women. J Appl Physiol 2007;102(2):541-6.

37. Langberg H, Skovgaard D, Petersen LJ, Bulow J, Kjaer M. Type I collagen synthesis and degradation in peritendinous tissue after exercise determined by microdialysis in humans. J Physiol 1999;521:299-306.
38. Langberg H, Rosendal L, Kjaer M. Training-induced changes in peritendinous type I collagen turnover determined by microdialysis in humans. J Physiol 2001; 534(Pt1):297-302.
39. Olesen JL, Heinemeier KM, Gemmer C, Kjaer M, Flyvbjerg A, Langberg H. Exercise dependent IGF-I, IGF-BPs and type-I collagen changes in human peritendinous connective tissue determined by microdialysis. J Appl Physiol 2006.
40. Christensen B, Dandanell S, Kjaer M, Langberg H. Effect of anti-inflammatory medication on the running induced rise in tendon collagen synthesis in humans. J Appl Physiol 2010.
41. Parry DA. The molecular and fibrillar structure of collagen and its relationship to the mechanical properties of connective tissue. Biophys Chem 1988;29(1-2):195-209.
42. Birch HL. Tendon matrix composition and turnover in relation to functional requirements. Int J Exp Pathol 2007; 88(4):241-8.
43. Patterson-Kane JC, Wilson AM, Firth EC, Parry DA, Goodship AE. Comparison of collagen fibril populations in the superficial digital flexor tendons of exercised and nonexercised thoroughbreds. Equine Vet J 1997;29(2):121-5.
44. Patterson-Kane JC, Firth EC, Parry DA, Wilson AM, Goodship AE. Effects of training on collagen fibril populations in the suspensory ligament and deep digital flexor tendon of young thoroughbreds. Am J Vet Res 1998; 59(1):64-8.
45. Michna H. Morphometric analysis of loading-induced changes in collagen-fibril populations in young tendons. Cell Tissue Res 1984;236(2):465-70.
46. Kongsgaard M, Qvortrup K, Larsen J, et al. Fibril morphology and tendon mechanical properties in patellar tendinopathy: effects of heavy slow resistance training. Am J Sports Med 2010;38(4):749-56.
47. Avery NC, Bailey AJ. Enzymic and non-enzymic cross-linking mechanisms in relation to turnover of collagen: relevance to aging and exercise. Scand J Med Sci Sports 2005;15(4):231-40.
48. Heinemeier KM, Olesen JL, Haddad F, et al. Expression of collagen and related growth factors in rat tendon and skeletal muscle in response to specific contraction types. J Physiol 2007;582(Pt3):1303-16.
49. Couppe C, Hansen P, Kongsgaard M, et al. Mechanical properties and collagen cross-linking of the patellar tendon in old and young men. J Appl Physiol 2009; 107(3):880-6.
50. Olesen JL, Heinemeier KM, Haddad F, et al. Expression of insulin-like growth factor I, insulin-like growth factor binding proteins, and collagen mRNA in mechanically loaded plantaris tendon. J Appl Physiol 2006;101(1):183-8.
51. Sullivan BE, Carroll CC, Jemiolo B, et al. Effect of acute resistance exercise and sex on human patellar tendon structural and regulatory mRNA expression. J Appl Physiol 2008.
52. Yang G, Crawford RC, Wang JH. Proliferation and collagen production of human patellar tendon fibroblasts in response to cyclic uniaxial stretching in serum-free conditions. J Biomech 2004;37(10):1543-50.
53. Nakama LH, King KB, Abrahamsson S, Rempel DM. VEGF, VEGFR-1, and CTGF cell densities in tendon are increased with cyclical loading: An *in vivo* tendinopathy model. J Orthop Res 2006;24(3):393-400.
54. Schild C, Trueb B. Mechanical stress is required for high-level expression of connective tissue growth factor. Exp Cell Res 2002;274(1):83-91.
55. Hansson HA, Engstrom AM, Holm S, Rosenqvist AL. Somatomedin C immunoreactivity in the Achilles tendon varies in a dynamic manner with the mechanical load. Acta Physiol Scand 1988;134(2):199-208.
56. Abrahamsson SO, Lohmander S. Differential effects of insulin-like growth factor-I on matrix and DNA synthesis in various regions and types of rabbit tendons. J Orthop Res 1996;14(3):370-6.
57. Butt RP, Bishop JE. Mechanical load enhances the stimulatory effect of serum growth factors on cardiac fibroblast procollagen synthesis. J Mol Cell Cardiol 1997; 29(4):1141-51.
58. Duncan MR, Berman B. Stimulation of collagen and glycosaminoglycan production in cultured human adult dermal fibroblasts by recombinant human interleukin 6. J Invest Dermatol 1991;97(4):686-92.
59. Ghazizadeh M, Tosa M, Shimizu H, Hyakusoku H, Kawanami O. Functional implications of the IL-6 signaling pathway in keloid pathogenesis. J Invest Dermatol 2007;127(1):98-105.
60. Langberg H, Olesen JL, Gemmer C, Kjaer M. Substantial elevation of interleukin-6 concentration in peritendinous tissue, in contrast to muscle, following prolonged exercise in humans. J Physiol 2002;542(Pt3):985-990542(Pt3):985-990.
61. Nakatani T, Marui T, Hitora T, Doita M, Nishida K, Kurosaka M. Mechanical stretching force promotes collagen synthesis by cultured cells from human ligamentum flavum via transforming growth factor-beta1. J Orthop Res 2002;20(6):1380-86.
62. Kim SG, Akaike T, Sasagaw T, Atomi Y, Kurosawa H. Gene expression of type I and type III collagen by mechanical stretch in anterior cruciate ligament cells. Cell Struct Funct 2002;27(3):139-44.
63. Heinemeier K, Langberg H, Olesen JL, Kjaer M. Role of TGF-beta1 in relation to exercise-induced type I collagen synthesis in human tendinous tissue. J Appl Physiol 2003;95(6):2390-7.
64. Grotendorst GR, Okochi H, Hayashi N. A novel transforming growth factor beta response element controls the expression of the connective tissue growth factor gene. Cell Growth Differ 1996;7(4):469-80.
65. Duncan MR, Frazier KS, Abramson S, et al. Connective tissue growth factor mediates transforming growth factor

beta-induced collagen synthesis: down-regulation by cAMP. FASEB J 1999;13(13):1774-86.
66. Asundi KR, King KB, Rempel DM. Evaluation of gene expression through qRT-PCR in cyclically loaded tendons: an *in vivo* model. Eur J Appl Physiol 2008; 102(3):265-70.
67. Legerlotz K, Schjerling P, Langberg H, Bruggemann GP, Niehoff A. The effect of running, strength, and vibration strength training on the mechanical, morphological, and biochemical properties of the Achilles tendon in rats. J Appl Physiol 2007;102(2):564-72.
68. Abrahamsson SO, Lundborg G, Lohmander LS. Recombinant human insulin-like growth factor-I stimulates *in vitro* matrix synthesis and cell proliferation in rabbit flexor tendon. J Orthop Res 1991;9(4):495-502.
69. Doessing S, Heinemeier KM, Holm L, et al. Growth hormone stimulates the collagen synthesis in human tendon and skeletal muscle without affecting myofibrillar protein synthesis. J Physiol 2010;588(Pt2):341-51.
70. Scott A, Cook JL, Hart DA, Walker DC, Duronio V, Khan KM. Tenocyte responses to mechanical loading *in vivo*: a role for local insulin-like growth factor 1 signaling in early tendinosis in rats. Arthritis Rheum 2007;56(3):871-81.
71. Heinemeier KM, Olesen JL, Schjerling P, et al. Short-term strength training and the expression of myostatin and IGF-I isoforms in rat muscle and tendon: differential effects of specific contraction types. J Appl Physiol 2007; 102(2):573-81.