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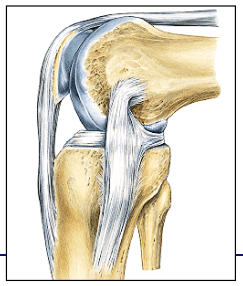
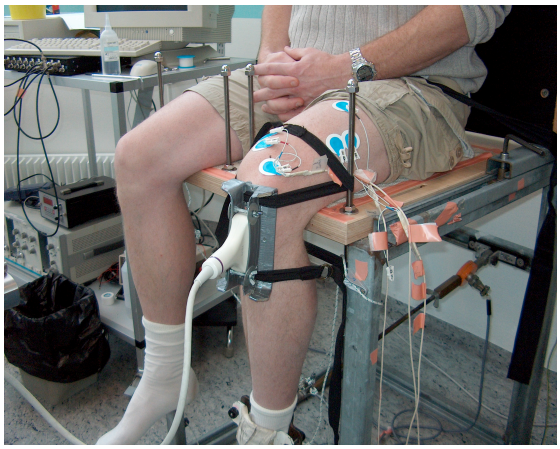
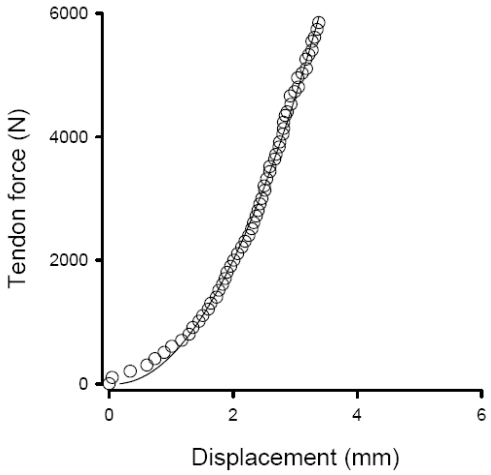


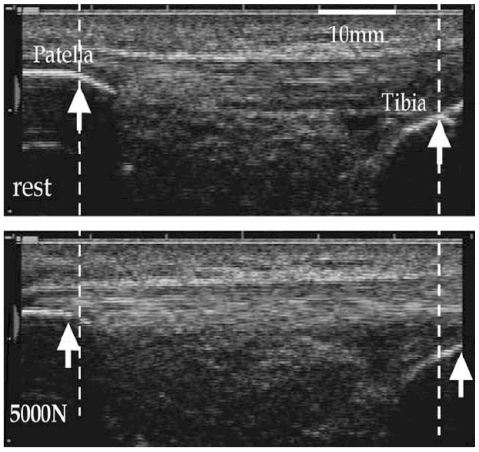
# Ph.D. thesis

Christian Couppé

## *Human tendon response to loading, unloading and aging*






Institute of Sports Medicine Copenhagen
Bispebjerg Hospital & Center for Healthy Aging
Copenhagen, Denmark, 2010

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*To my son William*

# Abbreviations:

| | |
|---|---|
| AGE | Advanced glycation endproduct |
| CSA | Cross-sectional area |
| HPLC | High pressure/performance liquid chromatography |
| HP | Hydroxylysylpyridinoline |
| LP | Lysylpyridinoline |
| MRI | Magnetic resonance imaging |
| N | Newton |
| Nm | Newton meter |
| US | Ultrasonography |
| ε | Strain |
| σ | Stress |
| E | Young´s modulus of elasticity |
| Pa | Pascal |
| PT | Patellar tendon |

## List of included papers

This thesis rests on three studies. The studies are listed below referred to in the thesis by their roman numerals.

**I. Habitual loading results in tendon hypertrophy and increased stiffness of the human patellar tendon.** Couppé C, Kongsgaard M, Aagaard P, Hansen P, Bojsen-Moller J, Kjaer M, Magnusson SP. *J Appl Physiol. 2008 Sep;105(3):805-10.*

**II. Mechanical properties and collagen cross-linking of the patellar tendon in old and young men.** Couppé C, Hansen P, Kongsgaard M, Kovanen V, Suetta C, Aagaard P, Kjær M, Magnusson SP. *J Appl Physiol. 2009 Sep;107(3):880-6.*

**III. The effects of immobilization on the mechanical properties of the patellar tendon in old men.** Couppé C, Suetta C, Kongsgaard M, Justesen L, Hansen P, Hviid L, Bojsen–Møller J, Aagaard P, Kjær M, Magnusson SP. (Unpublished).

# Introduction

Human movement is produced by the force of contracting muscles, which is transmitted to bone through aponeurosis and tendon. It is well known that mechanical properties of bone and muscle are influenced by loading and aging. Repetitive loading (exercise) improves physical function and prevents development of disease. Unloading has the opposite effect. Aging reduces the physical function and increases the rate of disease. Although tendon properties influence the function of the muscle–tendon complex, there is little human data that describe activity and age-associated changes in mechanical properties of tendon. In addition, it is largely unknown if changes in mechanical properties of tendon contribute to the decreased physical function. Therefore, it is important to understand tendon response to physical activity.

### Tendon Structure and Composition

The connective tissue leaves the muscle to be converted into the tendon that connects muscle to bone. Tendon is white, glossy and smooth upon assessment. Tendons have various sizes and forms. Some are long and flat. Others are round and thick (4). Some tendons are enclosed in connective tissue sheath to facilitate sliding (such as the digital flexor tendons) whereas others slide relative to adjacent tissues and skin (such as Achilles tendon). Some tendons wrap around connective tissue"pulleys" that function to increase functional excursion (such as the dorsiflexor retinaculum of the ankle), whereas others pull in a direct line from muscle to bone (such as the patellar tendon or triceps brachii tendon).

Tendons are made up by fibroblasts and an extracelluar tendon matrix composed of a mix of collagen and elastin fibers along with ground substance and inorganic components (5). Tendon contain 55-77% water, which to a large extend is associated with proteoglycans of the ECM (Extracellular Matrix) (6). Tendons are composed primarily of the structural protein, collagen (~90% of the total protein in tendon) or 65-75% of the dry mass of tendons (6). Collagen is the most abundant protein in animal world and constitutes more than 30% of the total protein in human body (7;8). The smallest units of collagen is made of polypeptide chains that composed of three amino acids arranged in series: glycine (~30%), proline (~10%) and hydroxyproline (~10%) (9). These chains, due to the three-dimensional nature of the repeating amino acid structure, polymerize in groups of three to create the collagen molecule.

The bonds between the collagen molecules are called cross-links. Cross-linking within and between collagen molecules provides structural integrity to the tendon fibrils in order to secure appropriate force-transduction (10-13). The tendon strength is obtained from cross-links that are assumed to be strengthen through interaction between the collagen and proteoglycan and ground substance. Cross-linking involves two different mechanisms, one precisely controlled enzymatic modifications by the enzyme lysyl oxidase (11;13;14) during maturation and one non-enzymatic mechanism that seems to increase with age and involves reactions with glucose and is generally referred to glycation (1;12;15). Please see a further description in "Age and tendon properties".

To accurately specify the type of collagen, the structure of the three component chains must be identified. There are numerous chains and thus, collagen itself may exist in one of many isoforms or types. In other words, collagen is classified according to its molecular organization as type 1, type II, type III etc. (16). At least 27 types of collagen have been reported (7). Type I collagen is found in skin, bone, tendon, ligament and cornea and is the most abundant type of collagen in the body. Adult tendons consist predominately of collagen type I and type III (6). Collagen type I accounts for about 86% of the dry weight in tendon. Type II collagen is primarily found in cartilage.

Figure 1 shows the generic structure of a tendon (17-20). Collagen molecules are generally aligned in parallel rows to form a collagen fibril. The structural hierarchy of collagen tissue is based on bundling of collagen fibrils. Fibrils are gathered into fascicles that are assembled into the whole tendons. Other extracelluar matrix proteins are present in tendons that give it some its viscoelastic properties. These materials include highly charged glycosaminoglycans and proteoglycans that attract water and elastin that presumably provides some of the elasticity of normal tendons (16;21).

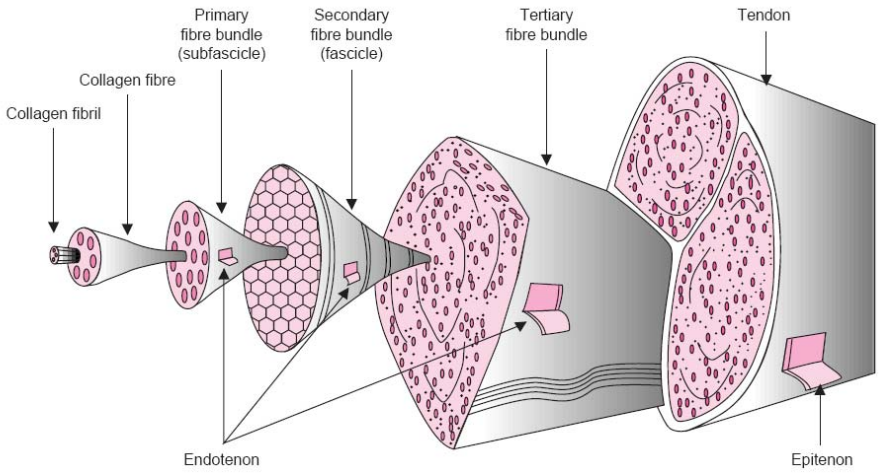


**Figure 1.** Tendon hierarchy. *From K. Khan* (3).

**Tendon biomechanics**

The tensile properties of tendons and tendon substructures can be determined by tensile testing that simultaneous record changes in force and elongation of the tendon. Traditionally, whole tendon testing has been performed *in vitro* (1), however ultrasonographic and MRI methods allow for measurement of mechanical properties, *in vivo* (6). For all measurement modalities some fundamental conventions and parameters are used to describe the mechanical properties.

When tensile force is imposed on a tendon it will elongate. The ratio of force to elongation ($\Delta F/\Delta L$) defines the tendon *stiffness*. The unit of stiffness is N/m. The shape of the force-elongation curve is curvilinear with an initial nonlinear "toe-region" and a subsequent "linear region" (figure 2). Straightening of crimps corresponds with the toe-region phenomenon (3; 4). Crimping may provide a mechanical 'buffer' against sudden elongation to prevent fibrous damage though shock absorption (7; 8). The linear region likely derives from complex microstructural mechanisms such as elongation of the collagen molecule, increase in the gap region between collagen molecules in the longitudinal direction and relative slippage of laterally neighbouring molecules along the fibril axis (10). Also, non-uniform reorganization and straightening of fibrils may contribute (9).

The structure of a tendon significantly influences the mechanical properties. If the tissue increases cross-sectional area (CSA), the stiffness of the tendon will increase, but reduce elongation for a given force level. Increased tendon length decreases stiffness and increases elongation, for a given force level (Figure 2) (1; 5). To compare material properties for tendons of different dimensions, a common approach is to normalise $\Delta L$ to the initial tendon length ($L_0$), which yields the unit-less parameter *strain*. Strain is most commonly reported as a percentage. Likewise, a change in force ($\Delta F$) is normalised to tendon CSA to provide tendon *stress*. The unit for stress is Pa. The ratio $\Delta$stress/$\Delta$strain yields the *Young´s modulus* of elasticity (unit: Pa). Stress, strain, and Young´s modulus are utilised to characterise the material properties e.g. of tendon irrespective of material dimensions.

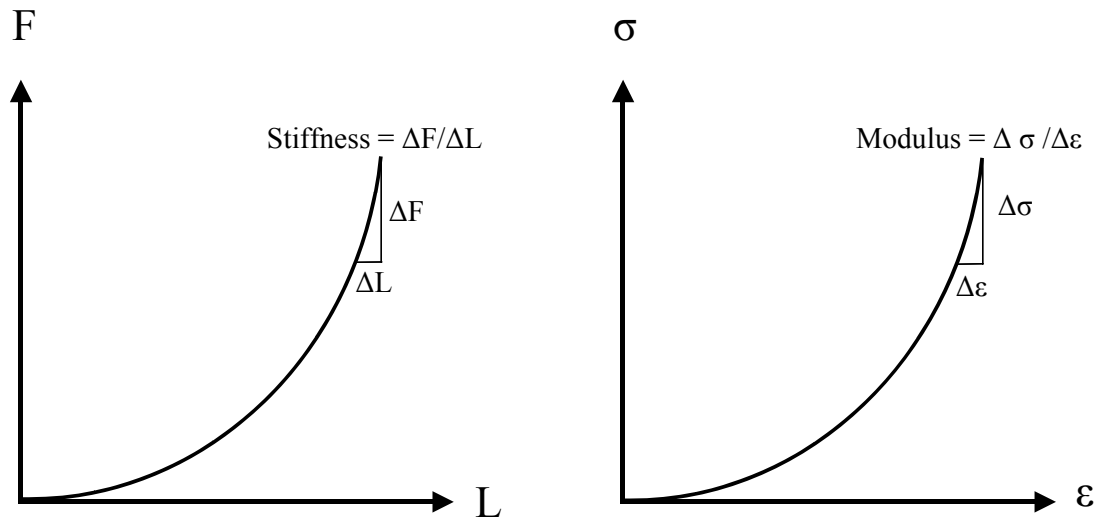


**Figure 2.** Typical force-elongation curve of a tendon on the left. F = Force (N) and L= elongation (m). Normalisation of ΔF and ΔL by tendon dimensions yields a corresponding stress-strain relation on the right. σ = Stress ($N/m^2$) and ε = Strain (%).

Tendons have viscoelastic properties i.e. they exhibit a load-dependent (*time-independent*) linear elastic component and a viscous rate-dependent (*time-dependent*) component (1). Simple spring, dashpot, and frictional elements are required to model the viscoelastic behaviour of tendon (1).

## Tendon Function

The primary role of tendon is to transmit force with minimal energy loss from the contracting muscle to the bone in order to produce movement. Tendons do have other functions than acting as "force transmitters". Tendons also work as "shock absorbers" that can store kinetic energy to be released again (22) and thereby provide considerable elastic energy to locomotion. Animal studies have reported that the tendon is capable of returning over 90% of the stored energy, but some of the stored energy is not recovered due to energy dissipation (23). Further, it appears that the strain energy recovery is larger during running than normal walking demonstrating the importance of elastic saving of energy for economy for motion (24;25). In addition, long and thin tendons (e.g. Achilles tendon) favors energy storage compared to thick and short tendon (e.g. Patellar tendon)(26;27). In sport, these features are critical, as they enhance athletic function. Further, tendons play an essential role in humans provided by evolutionary advantage for bipedal gaits such as walking and running (28). In contrast to apes, human legs have many long spring-like tendons connected to short muscle fascicle that can generate force economically (29). It was recently shown that during stance phase in human walking, the gastrocnemius muscle operates near isometric conditions while it stretches (30). These and related findings during countermovement exercise involving human jumping, support the concept that the human, *in vivo*, gastrocnemius tendon

(31;32) can store and release elastic energy, and thereby confirms previous animal studies. It has been estimated that human tendon provide 52-60% of the total work during locomotion (33). Direct measures, in human, *in vivo*, gastrocnemius tendinous tissue show that it may release~1.3 J during walking, which corresponds to 6% of the respective total external work (34), ~4 J during two leg vertical jump (32), which corresponds to 16% of the respective total external work (35) with the elastic strain energy increasing with increasing dropping height (36). However, it should be noted that thick tendon would reduce the stress across the tendon, which will raise the safety factor. Moreover, tendon also provide protection from muscle fibre tensile injury (37-39).

**Tendon strength**

The strength of the tendons is determined by its CSA and by the material properties. The material properties are determined by many factors such the intrafibrilllar cross-links and collagen type distribution (40). In this respect the fibrillar organization is also of great importance (41).

Tendons can withstand very high forces. The failure stress of tendons is generally considered to be close 100 MPa (40;42), which corresponds to 10.000 N per $cm^2$ or ~1000 Kg (43). Therefore most adult PT with a CSA around 1 $cm^2$ can withstand loads of 10.000 N, but a case report revealed that the PT of a weightlifter was able to withstand 14.500 N (or more than 17.5 times the body weight of the weightlifter) before rupturing (44).

**Tendon Loading**

The ultimate loads in tendons can be extremely large, especially in tendons such as Achilles and PT. Most studies have determined tendon loading by estimation (45-48), since it difficult to determine the tensile forces of the tendons *in vivo* (49). In the Achilles tendon peak forces can reach ~11000 $N/cm^2$ in activities such as running and jumping. The loading forces of the PT have not been investigated as much as the Achilles tendon. However, it has been estimated that forces within PT may reach 5000 N during walking, 8000 N during landing from a jump and 9000 N during fast running (47). Ishikawa et al. (50) investigated the PT forces with the fiber optique technique (49) during various types of jumps (squat jumps, countermovement jumps, submaximal drop jumps) on a sledge apparatus. They found that drop jump had the highest values ~7 kN, whereas in squat jump and counter movement jump the PT forces was within 3-6.5 kN. Others have calculated the knee moment of ~500 Nm, (Newtonmeter) which corresponds to a PT force of ~11100 N, (when the

momentarm is 4.5 cm (51)) at landing after a volleyball jump (45;46). These reported tendon forces reach or even exceed critical values if the average ultimate tensile stress is 100 MPa (52). Therefore the tendon CSA becomes paramount to reduce stress and increase the safety factor in many sport activities. Furthermore, the ultimate stress may also be questioned, since they were performed under *in vitro* conditions. However it remains unknown if the variation in tendon CSA is a contributing factor to the development of tendon injuries. Controlled lunged exercises have revealed knee extension moment of 116 Nm ~2500 N within PT (53). Lunge performance has been investigated in fencing in terms of kinematics and EMG activity associated with the movement (54-56). It was found that the most experienced fencers achieved the greatest lunge velocities suggesting that the forces in PT are considerable higher than in controlled exercises.

**Physical activity and tendon properties**

It is well known that tissues like muscle (57;58) and bone (59;60) adapt to increased loading by hypertrophy and also by improving the material properties. The changed muscle properties will increase the forces from muscle through the tendons. Consequently, this could lead to a situation where the forces on the tendons exceed the strength of the tissue with the risk of sustaining an injury. The knowledge, whether and how tendons adapt to increased loading, has until recently been quite limited. Although animal and human data indicates that muscle CSA is related to tendon CSA (61;62), and that tendons are stiffer in subjects with greater muscle strength (63). However, anthropological evidence suggests that collagen tissue adapts to increased loading with hypertrophy in order to lower stress and thereby avoid injury (28). During evolution, the adoption of bipedality resulted in fundamental anatomical restructuring of bones such as larger CSA of the calcaneal tuber relative to body mass (64). Many studies have found that compared to the early hominids, humans have substantially larger articular surface areas relative to body mass in most joint in the lower extremity (28).

Recent data in human models suggest that tendon tissue is quite metabolically responsive to tensile loading (65-67). In fact, it has been shown that both a single loading bout as well as long term habitual loading produces a markedly elevated collagen synthesis response (68-70). 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 remains ambiguous.

Studies that address the influence of exercise on the mechanical properties of tendon have until recently largely been conducted in animal models. These data show that endurance like exercise is associated with an increase (71-73), decrease (72), or unchanged (27;74) tendon size, and thus do not provide a coherent picture. It appears that tendon of immature animals are more likely to hypertrophy than tendons of mature animals (61;75). It has been suggested that endurance training results in qualitative but not quantitative changes (74;76), whereas other researches have concluded that tendon reacts to endurance training by both quantitative (hypertrophy) and qualitative ( stress-strain properties) adaptation(77). In humans, cross-sectional data suggests that endurance training is associated with a larger Achilles tendon CSA (62;78;79), which appears to be site specific (62;79). However, in a recent intervention study it was shown that 9 months of endurance training in untrained persons left the Achilles tendon CSA unchanged (80). On the other hand, animal data have shown that muscle strength/size is related to the tendon size (61), suggesting that perhaps the magnitude of loading (81) influences tendon size. 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 (82-86), but rather a markedly altered modulus, which implies that there is a change in the composition of the structure rather than the size. However, it was recently shown in humans that resistance training for 12 weeks yielded regions specific increases in PT CSA, without a change in modulus (87). Region specific hypertrophy ~5% and increased stiffness in Achilles tendon after 14 weeks of high strain loads (90%MVC), has also recently been confirmed. In this study no change was observed in paired tendon that trained with low strain magnitude (55% MVC) (88). Seynnes et al.(89) recently observed a 5-6% hypertrophy response along the PT together with increased tendon stiffness and modulus followed by 9 weeks of knee extension resistance training.

Conclusions from studies of cross-sectional design are inevitably hampered by issues of training history, selection bias and inter-subject variations. Additionally, longitudinal training studies may have been of insufficient duration to produce a robust tendon hypertrophy response. Finally, existing training studies (82-86) have examined tendon size in a region that appears unresponsive to training associated adaptation. However, some of these limitations may be partially circumscribed 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.

| Reference | Design | Subject | Age (yrs) | Training mode | duration | CSA (%) | Stiffness (%) | Modulus (%) |
|---|---|---|---|---|---|---|---|---|
| Reeves 2003 (82) | Prospective RCT | 18women&men Healthy | 74 | Knee ext.+ leg press 70% 1RM | 14 weeks (3/week) | ns ** | 65 | 69 |
| Kubo 2006 (84) | Prospective | 14 men Healthy | 20 24 | Isometric leg press 70% 1RM | 12 weeks (4/week) | ns | ns | ns |
| Kongsgaard 2007 (87) | Prospecttive | 12 men Healthy | 25 | Knee ext. 70% 1RM vs. 15%1RM | 12 weeks ( 3/week) | 4-6 | 13 | ns |
| Couppé 2008 (90) | Cross-sectional | 7 elite athletes men | 23 | Habitual sports specific loading | >5 years | 20-28 | 36 | ns |
| Seynnes 2009 (89) | Prospective | 15 men Healthy | 20 | Knee ext. 80% 1RM | 9 weeks ( 3/week) | 5-6 | 20 | 24 |

**Table 1.** Patellar tendon and physical activity, human studies, *in vivo*. **CSA analysed with ultrasonography (US)

## Aging and tendon properties

Aging is associated with a decline in muscle mass, strength and physical function (91), and although tendon properties influence the overall function of the muscle–tendon complex (92;93), there is a relative lack of human data that describe possible age-associated changes in mechanical properties of tendon. Animal data show that aging yields a stronger and stiffer tendon (23;94-97), a weaker and more compliant tendon (98-100), or leaves the tendon unchanged (101) and are thus inconclusive. In contrast, data on isolated human cadaver tendon suggest that the aging process largely leaves the mechanical properties unaltered (102-104). Despite the development of the US-based methods to evaluate human tendon properties, *in vivo*, (105-107) the effect of aging on the mechanical properties of human tendon *in vivo* remains elusive. Tendon strain in humans has been shown to decrease (108-110) or increase (111-115) with aging. Yet others have shown that aging leaves the mechanical properties of the PT unchanged (116). Thus, the picture is incoherent, which may partly be related to methodological and design differences, the physical activity level of the sample population, and the type of tendon tested, i.e. the tendon-aponeurosis complex or free tendon alone.

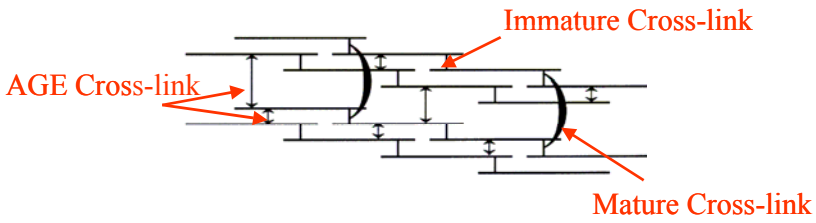


**Figure 3.** Location of cross-links. Immature, mature and AGE (Avanced Glycation Endproduct) cross-links during aging of the collagen fibril. Adapted from *Bailey et al.. 1998 (1).*

In tendon, the trivalent intermolecular pyridinoline cross-links (primarily hydroxylysyl-pyridinoline and lysyl-pyridinoline) stabilize the fibrillar structure of collagen and thus contribute to the mechanical properties of the tendon (10;12;117). These cross-links are formed from enzymatically derived covalent immature cross-links (Figure 3), which undergo a spontaneous conversion into more mature trivalent cross-links with collagen maturation (Figure 3) (10). The slow turnover of mature collagen allows further cross-linking via the adventitious non-enzymic reactions of glucose with the lysyl and arginine amino acid residues in the collagen triple helix as a true aging process (10;15;118)(Figure 4).This non-enzymatic process results in the accumulation of advanced glycation end products (AGE) in tendon tissue. The most widely studied AGE is pentosidine (119;120). AGE accumulation is known to accelerate with aging and diabetes (15;121;122) and is believed to yield a stiffer and more load-resistant tendon (123;124). It has been shown that AGE cross-link density in collagenous tissues (125-128) and in human tendons, (129;130) is markedly higher in older compared to younger individuals, and there is only sparse human data on mature cross-link density of tendon and how these are influenced by aging (130). However, recent reports of an age-associated reduction in human tendon stiffness, *in vivo* (111-115), in conjunction with the notion of simultaneously elevated cross-link density and AGE accumulation is difficult to reconcile. To the best of our knowledge there are currently no human studies examining collagen cross-linking and mechanical properties of the PT, *in vivo*, in old and young men.

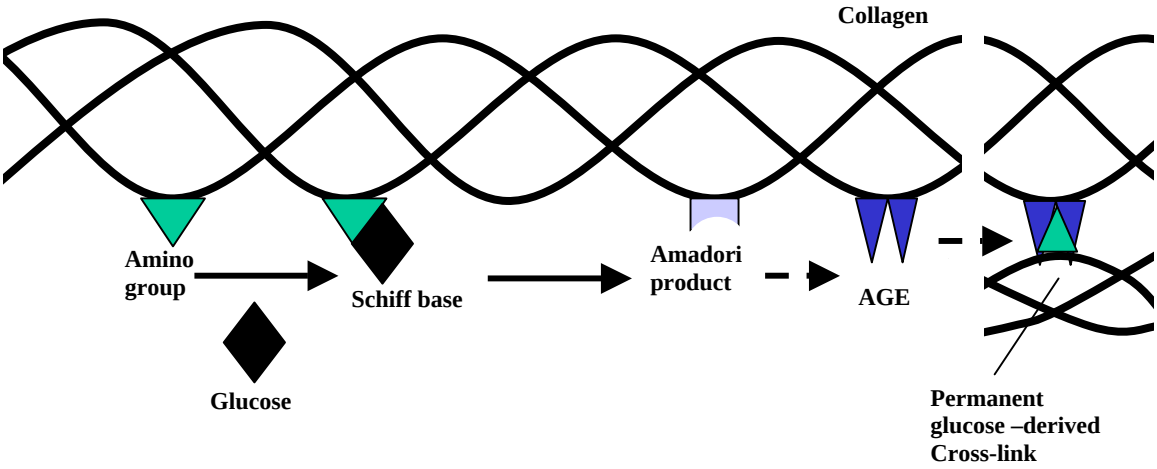


**Figure 4.** Formation of glucose-derived cross-links, shown highly schematically, begins when glucose attaches to an amino group ($NH^2$) of a protein such as collagen. The initial product, known as a Schiff base, soon transforms itself into a Amadori prouct, which can eventually pas through several incompletely understood steps (*broken arrow*) to become an AGE. In many instances AGE´s are like unsprung traps, poised to snap shut on free amino groups of any nearby protein and to form cross-links. *From Cerami et al.*1987(2).

## Immobilization and tendon properties

It is well known that immobilization results in a rapid loss of muscle volume and strength, which will negatively influence muscle function (93;131;132). The effects of immobilization on the mechanical properties of the tendon have been studied extensively in animal models (133;134), and several studies show a decline of the mechanical properties of the tendon (72;135-140), while some demonstrate the opposite (141;142). Human studies also suggest that the tendon undergoes a substantial reduction in the mechanical properties with immobilization (143-149), which has been principally attributed to changes in material rather than size (143-149). However, the aforementioned effects of immobilization on the mechanical properties of tendon have predominantly been investigated in young persons (Table 3), despite the fact that old persons are frequently exposed to periods of disuse caused by disease or injury (150;151). It remains unknown if similar magnitudes of changes occur in older persons. However, evidence suggest that quadriceps muscle function is more affected in old than young persons by unloading (132;152), indicating that the magnitude of change in tendon properties is perhaps even greater in the elderly.

| Reference | Design | Subject (age) | N | Tendon | Duration (Days) | CSA (%) | Stiffness (%) | Modulus (%) | MVC (%) | RFD (%) | EMD (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Kubo (146) 2000* | Prospective | Healthy (24) | 6 | *VL | 20 | | -33 | | -20 | -47 | 21 |
| *Kubo (153) 2004* | Prospective | Healthy (24) | 6 | *VL<br>*MG | 20 | NS<br>NS | -28<br>-14, ns | | -23 | | |
| *Kubo (147) 2004* | Prospective ** | Healthy (24) | 6<br>6 | *VL | 20 | NS | -29<br>ns | | | | 22 |
| *Reeves (143) 2005* | Prospective ** | Healthy (33) | 9<br>9 | *MG | 90 | NS | -58<br>-37 | -57<br>-38 | | | |
| *Maganaris (154) 2006* | Cross-sectional | Spinal-cord injured (36) | 6 | Patellar | (1.5-24) yrs | ↓17 | -77 | -59 | | | |
| *De Boer (145) 2007* | Prospective | Healthy (19) | 9 | Patellar | 14/23 | NS | -10/-30 | -10/-30 | -15 /-21 | -42/ | /16 |
| *Zhao (148) 2008* | Cross-sectional | Hemiparetic +women (60) | 10 | *MG | ( >1 yr) | NS | -43 | -38 | | | |
| *Shin (144) 2008* | Prospective | Healthy +1women (24) | 5 | Achilles Tendon | 44 | NS | | -17 | -54 | | |
| *Seynnes (149) 2008* | Prospective | Healthy (19) | 8 | *MG | 23 | | -36 | | -10 | | |

**Table 2** Human studies on tendon and immobilization**.** All subjects non-active men unless noted, ** 1 training group, * Tendon-aponeurosis, Medial Gastrocnemius = MG, Vastus Lateralis = VL

## Hypothesis

*Hypothesis for study 1*: A side specific increased loading will yield a increased tendon hypertrophy and stiffness, but will leave the material properties unchanged.

*Hypothesis for study 2*: Tendon of older persons is more compliant compared to young persons.

*Hypothesis for study 3*: Immobilization will produce a marked decline in muscle function and tendon properties in older persons.

## Objectives/purpose

The overall purpose of this thesis was to study the effects of habitual loading, aging and immobilization on the human PT structural and mechanical properties, *in vivo*.

*Study 1*

The specific purpose of this study was to examine patellar tendon (PT) size and mechanical properties in subjects (elite badminton players and fencers) with a side-to-side strength difference of ≥ 15 % due to sport induced loading.

*Study 2*

The purpose of this study was to examine the mechanical properties of the patellar tendon (PT), *in vivo* and collagen cross-link composition in old (OM) and young men (YM) subjects.

*Study 3*

The purpose of this study was to examine the effects of short-term unilateral immobilization on the human patellar tendon (PT) structural and mechanical properties in old men (OM), *in vivo*.

# Material and Methods

In the following section the material and methods are presented along with methodological consideration. Detailed information about material and methods can be obtained in each paper enclosed in the thesis.

### Material

Rationale for design and material in:

*Study I (Cross-sectional study):*

We included 7 elite fencers (n = 4) and badminton (n = 3) players that met the criteria of a side-to-side isometric knee extensor strength difference of ≥ 15 %. They had participated in their respective sport for more than five years at an elite level.

The main reason for choosing this design was because that there are several shortcomings in the human studies. The majority of studies are cross-sectional in design, and because there is considerable inter-subject variation (155), it may be difficult to detect difference in limited samples. Also, the longitudinal training studies are typically of relatively short duration, which may simply be insufficient for tissue hypertrophy to take place. One way to particularly avoid these issues was to examine PT properties in subjects who have trained one leg unilaterally for a long time, since the 'untrained' leg then could serve as a within subject 'control'. Both badminton and fencing involves repeatedly performing rapid forward lunges with a preferred and thus more loaded lead extremity compared to the non-lead extremity, which served as a within-subject control. Sapega (156) and Nystrom (157) found more than a 14% strength difference between the legs in elite fencers reflecting a sport specific loading (Figure 5). The sport specific loading in badminton and fencing creates a higher impact on the leg and therefore also on the PT compared with other sports without jumping and unilateral rapid lunges (158). In addition, seven healthy recreational athletes, who did not engage in fencing or badminton were examined with respect to thigh strength and tendon morphology, but not mechanical properties, was also included to ensure that there was no side to side difference between the lead extremity and non-lead extremity side as determined by preferred kicking leg. All players underwent clinical and US assessments on both the lead and non-lead extremity in order to exclude players with knee pathology or patellar tendinopathy (159;160).

**Figure 5**. Typical forward lunge in badminton and fencing, where one side (lead-extremity) is used repetitively compared to the other (non-lead extremity).

*Study II (Cross-sectional study):*

In study II, 7 old (OM) and 10 young men (YM) with similar activity level (OM 5±6 hrs/weeks, YM 5±2 hrs/weeks) were included. In order to ensure health status and to determine levels of physical activity during work and leisure time, participants were asked questions (e.g.: hours of exercise/ per week) based on previous questionnaires (161). It has been shown that physical activity such as strength training and habitual loading pattern may result in tendon hypertrophy (87;88;90), which would influence the mechanical properties of the tendon (82). In contrast to previous *in vivo* studies (108;111-116), we took this aspect into account by comparing age groups with similar activity level.

*Study III (Interventional immobilization study):*

Eight old men with a moderate physical activity level volunteered to participate in the study. They had one lower limb randomly assigned to an immobilization intervention for 2 weeks. The contra lateral limb served as control. The within –subject (paired) design thereby excluded potentially bias of individual intrinsic and extrinsic factors.

## Methods

*Muscle and tendon dimensions*

In all of the included studies Magnetic Resonance Imaging (MRI) was used for determining tendon CSA and length. MRI is a widespread technique for non-invasive visualization of internal tissue

structures. MRI provides images at a relatively high resolution that can be intuitively interpreted. With respect to tendons, the technique has been commonly applied to determine CSA and length either to measure structural changes or to normalize mechanical measurements to stress and strain. The reason for choosing MRI, rather than to other imaging modalities such as US, was that with the MRI-technique tendon hypertrophy has been observed following resistance training (87). US failed to show differences in CSA along the length of the PT (82;154), perhaps due to inaccuracy (162). Subject were asked to abstain from sport activities for 24 hours before the MRI procedure (163;164), since it has been demonstrated that the tendon increases its volume by fluid accumulation.

The anatomical cross sectional area (CSA) of the quadriceps femoris muscle was measured 20 cm proximal to the tibia plateau (mid-thigh level) by magnetic resonance imaging (MRI) (General Electric, Sigma Horizon LX 1.5 Tesla, T1 weighted SE) using a lower extremity coil. The images were obtained using the following parameters: TR/TE = 500/14 ms, FOV 18, matrix 512x512 and slice thickness = 6 mm (87;90). Subsequently, the lean muscle mass of the quadriceps muscle (subcutaneous and intermuscular non-contractile tissue were excluded in the measurement) was manually outlined using the software program Osiris 4.19 (http://www.sim.hcuge.ch/osiris/). The mean value of three measurements of the same image was used for analysis. PT CSA and length were determined with the use of MRI (General Electric, Sigma Horizon LX 1.5 Tesla, T1 weighted SE) (87;90). PT CSA was determined by axial plane MR using the following parameters: TR/TE 400/14 ms, FOV 20, matrix 256x256, slice thickness 5.0 mm and spacing 0 mm. The axial scans were performed perpendicular to the PT. As described in detail elsewhere, the tendon CSA was measured I) just distal to the patellar insertion, II) just proximal to the tibia insertion and III) midway between these two sites (87;90) . The PT length was determined from sagital plane MRI using the following parameters: TR 500, ET: 3 x (TE: 12.4 ms), FOV 16, matrix 256x192, slice thickness 4.0 mm and no spacing. The PT length was obtained by measuring the distance from the dorsal insertion at the patella apex to the dorsal insertion on the tibia.

The PT CSA and length was manually outlined using the software program Osiris 4.19 (http://www.sim.hcuge.ch/osiris/). This software was used due to its ability adjust image intensity levels. The color intensity of each image was adjusted using the National Institute Healthcare color scale mode of the software. Tendon CSA and length was measured using the gray scale image display.

In study I, the tendon CSA was measured I) distal to the patellar insertion, II) proximal to the tibia insertion and III) midway between these two sites in order to examine possible site-specific changes. It has been previously observed by Kongsgaard et al. (87) that mechanical loading result in hypertrophy at the distal and proximal portion of the PT. The increased tendon CSA will reduce tendon stress for given force level, which have implications of PT injury that usually occurs at the proximal and distal region (165;166).

In study II, tendon CSA data was normalized to body weight and raised to the power of 2/3 (167) in order to compare tendon dimensions between the subjects of various body size. In study II and III, possible site specific differences in tendon CSA were not reported, but tendon CSA was calculated as an average CSA along the tendon from the 3 levels (proximal, mid and distal CSA).

The ability to reproduce CSA measurements on MRI images were investigated in study I, where the mean value of three measurements of the same image was used for analysis. The reproducibility data showed that the typical error percent of repeated measures of tendon CSA was 2.5 % for repeated measurements at the proximal tendon level, 2.5 % for repeated measurements at the mid-tendon tendon level and 2.0 % for repeated measures at the most distal tendon level. In all the studies the MRI examiner was blinded to the subjects including the lead (study 1) or unloaded extremity (study III) in order to avoid investigator bias.

*Maximal voluntary contraction (MVC)*

In study I, II, and III, maximal voluntary strength was determined by an isometric knee extension on both legs. In study I, the subjects performed a 10-min warm-up on a stationary bike (5 min at 150 W and 5 min at 200 W) prior to testing. This was done in order to select players with a side-to-side difference in strength of ≥ 15 % was defined as: [lead extremity – non-lead extremity] / non-lead extremity * 100 ≥ 15 %.

In all studies, the subjects were seated in a custom made rigid chair with both hips and knees flexed to an angle of 90°. The reproducibility data showed that the typical error percent of repeated measures of MVC was 4.5% for repeated measurements. In study II and III the MVC was determined after testing of the mechanical properties of the tendon.

*Mechanical properties of tendon*

The subjects were seated in a custom made rigid chair with both hips and knees flexed to an angle of 90°. A leg cuff, which was connected to a strain gauge (Bofors KRG-4, Bofors, Sweden) through a rigid steel rod perpendicular to the lower leg, was mounted on the leg just above the medial malleolus. An ultrasound probe (7.5 MHz, linear array B-mode, Sonoline Sienna, Siemens, Erlangen, Germany) was fitted into a custom made rigid cast that was secured to the skin above the PT in the sagital plane. The ultrasound probe and cast was positioned so that the patella, the PT and the tibia were all visible within the viewing field throughout the ramped contractions (Figure6).

The ultrasound S-VHS video images obtained during the ramp trials were sampled at 50 Hz on a PC using frame-by-frame capturing software (Matrox Marvel G400-TV, Dorval, Canada). Force was sampled on two separate PC´s at 50 Hz via a 12-bit A/D converter (dt 2810A, Data Translation, MA, USA). The two computers were inter-connected to permit synchronous sampling of all data using a custom-built trigger device (168) . The subjects performed 4-5 slow isometric knee extensions ramps by applying gradually increasing force until maximum over a 10 s. period during which PT displacement and knee extension force were measured simultaneously. Each ramp was separated by a 2 minutes rest. All measurements were performed on one side, randomized to either the right or left knee. During the ramp contractions, force was sampled at 50 Hz and lowpass filtered at a 1.0 Hz cutoff frequency using a $4^{th}$ order zero-lag Butterworth filter.

Tendon force was calculated by dividing the estimated total knee extension moment by the internal moment arm, which was estimated from individually measured femur lengths (169). Tendon stress was calculated by dividing tendon force with the average of the 3 levels (proximal, mid and distal) of the PT CSA determined from MRI. Tendon deformation was defined as the change in distance between the patellar apex and the tibia (105;170). Tendon strain was calculated as the change in length related to the initial tendon length ($\Delta L/L_0$). Each single force-deformation curve was fitted to a $2^{nd}$ or $3^{rd}$ order polynomial fit, which yielded $R^2>0.98$. Tendon stiffness ($\Delta$force/$\Delta$deformation) and Young's modulus ($\Delta$stress/$\Delta$strain) based on common force were calculated in the final 10% or 20% of the force-deformation and stress-strain curves respectively (170).

The use of ultrasonography (B-mode) to determine mechanical properties of the tendon, *in vivo* is widely used. Fukashiro pioneered the use of ultrasnography (B-mode) for measurements of the whole tendon-aponeurosis complex in the mid 90s (106) and later it was developed to measure the isolated tendon (107). The method is easy to apply and its limitations are

widely acknowledged. From the beginning the technique was used to evaluate deformation of the intramuscular fascicular structures.

Several research groups have tried to investigate the free tendon such as the PT using the US (B-mode) methodology by recording the movement of the patella. This is however a proximal displacement of the patella relative to the skin. Structural deformation in the sagital plane is quantified in sequential US images and then manually analyzed (82;107;145;171;172). It has been shown that the US-based method of obtaining PT deformation requires that the movement of the tibia also has to be accounted for (105;173), which has not been achieved in other studies (82;107;145;171;172). Hansen et al (105) demonstrated that the tibia-movement account for 45% of the total PT deformation under the ramp contractions. Therefore, the measurement of the total PT elongation will be underestimated, if the tibia movement is omitted. For that reason we employed the method of Hansen et al in the 3 studies (I+ II +III). This method has been shown to be accurate and reliable (105). The within-day correlation coefficients and typical error percent of repeated measures were 0.95 and 9.9% for tendon stiffness, 0.97 and 5.5% for tendon strain, and 0.94 and 9.4% for Youngs Modulus.

Importantly, the method can only account for changes in 2 dimensions, when measuring structural deformation. It has been demonstrated that small changes in tendon length during sampling can occur due to the scanning-angle, e.g. medial-lateral movement of the transducer (168). In the three studies this was avoided using an external cast that fixates the transducer to the skin.

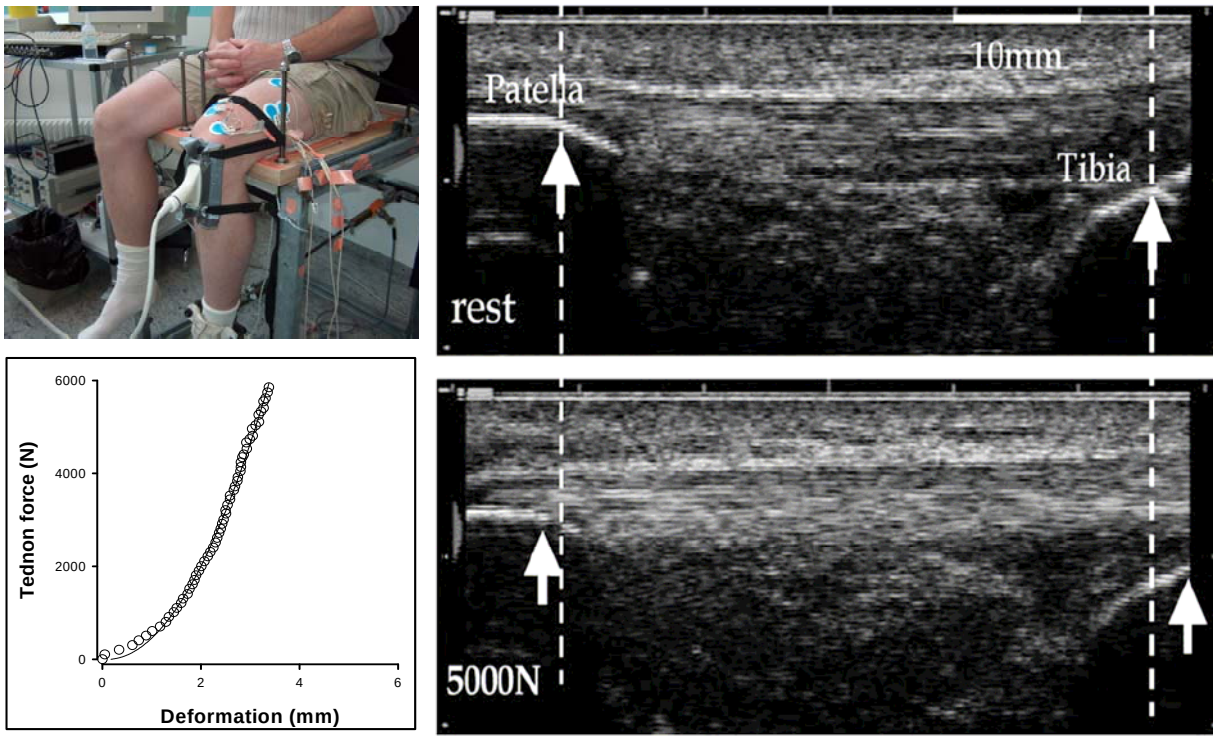


**Figure 6**.Measurements of mechanical properties of the patellar tendon. The subjects were seated in a custom made rigid chair with both hips and knees flexed 90°. A leg cuff, which was connected to a strain gauge (Bofors KRG-4, Bofors, Sweden) through a rigid steel rod perpendicular to the lower leg, was mounted on the leg just above the medial malleolus. An ultrasound probe (7.5 MHz, linear array B-mode, Sonoline Sienna, Siemens, Erlangen, Germany) was fitted into a custom made rigid cast that was secured to the skin above the PT in the sagital plane. The ultrasound probe and cast was positioned so that the patella, the PT and the tibia were all visible within the viewing field throughout the ramped contractions. Sycnhronized values of PT force and tendon deformation were used to construct force-deformation curves from which the mechanical properties were subsequently calculated based on polynomial fitting.

An automated frame-by frame tracking software based upon the Lucas Kanade/optical flow estimation method was developed by our lab, that reduces labor time and eliminates the potential examiner bias (105;168;170). Magnusson et al. showed that the software is reproducible and accurate (170). One of the limiting factors includes the sampling frequency of US and the video recording equipment when it comes to analysis of faster contraction velocities. Furthermore, the method lacks an accurate measurement of tendon force.

Tendon is highly viscoelastic tissue. The viscoelastic properties of the tendon will influence the overall function of the muscle–tendon complex (22;25;52;61;93). Therefore, the loading rate will influence the curve or slope due to creep. By definition, a slower muscle contraction produces less stiffness than a fast contraction within the same tendon. This has recently been shown by Pearson (172). In our lab and in the 3 studies (I+II+III), we used 10 s. ramps for 2

two reasons 1) it reduces the rate of force development 2) it ensures more proper tracking of tendon deformation. Subjects in all studies were tested at the same time of the day (171).

It is also well accepted that in addition to the magnitude and rate of tensile loading, the history of loading will affect the tendon properties. This history dependency is readily observed during repeated tensile loading of isolated tendon as the stress-strain curve is shifted to the right, in particular with the initial loading cycles (20;174). Such an acute response of tendon material may have significant physiological implications since it would influence the overall length-tension curve of the muscle-tendon complex. With respect to laboratory measurement procedures, such a rightward shift of the load-deformation curve demands so-called pre-conditioning to obtain reproducible data (20;174). Therefore, before each test of mechanical properties of the PT a preconditioning test were performed following a 5 min. warm-up on a stationary bike.

To avoid an underestimation of real PT force (105;155), the co-activation of the hamstrings have to be taken into account. EMG (Electromyographic) activity from the biceps femoris was therefore obtained during the ramped isometric extension. Due to an unknown noise-source during the first study, which we were not able to filter, we chose to omit the biceps femoris EMG activity in the calculations. From our previous studies we calculated that this error would underestimate the tendon force and tendon stress by 5-20%. However, studies have demonstrated that co-contraction does not change during maximal isometric contractions after 12 weeks of strength training (82;175).

*Patellar tendon biopsies*

A Bard MAGNUM® Biopsy Instrument (C.R. Bard, Inc., Covington, USA), with a disposable core biopsy needle (14G) was used. Following sterilization the skin was injected with local anaesthetic (lidocaine, 1%) and a 3-5 mm long incision was created just distal to the patella apex. The biopsy needle was inserted onto the tendon surface at a ~30° angle and fired securing a tissue sample of approximately 8 mg. Samples were snap-frozen in liquid nitrogen and stored at -80°C. Tendon biopsies were taken from the same side as tendon mechanical properties assessments were performed. No previous biopsy had been taken from that site in all subjects. All biopsy samples were analyzed in an investigator-blinded fashion.

*Biochemical analysis*

Freeze dried tendon samples were hydrolyzed in 6 M HCl, (+108 C, 24 h) and evaporated into dryness and dissolved into H2O. Hydroxyproline, the collagen specific amino acid, was measured spectrophotometrically (176) to quantify collagen protein (176). Hydroxylysyl pyridinoline (HP), lysyl pyridinoline (LP) and pentosidine (PENT) were analyzed via a single reversed phase HPLC (high performance liquid chromatography) run and detected on the basis of their natural fluorescence (177). At 0-16 minutes the wavelength for HP and LP fluorescence was 400 nm for emission and 295 nm for excitation. The wavelengths were changed at 16-60 minutes to 328/378 nm to measure the PENT. For the elution of the cross-links, a gradient was built up to contain 17% eluent B (75% acetonitrile with 0.13% HFBA) at 0 min and 25% eluent B at 30 min. Eluent A was 0.13% HFBA. Flow rate was 1ml/min. HP was eluted at 12 min, LP at 13,5 min and PENT at 23 min. The HPLC system used included Quaternary Gradient Pump unit, PU-2089 Plus, Intelligent AutosamplerAS-2057 Plus, and Intelligent Fluoresence Detector, FP-2020 by Jasco. Data processing software was Jasco Chrompass. The LiChroCART" ®125-4 column was from Merck Hitachi. The results of HP, LP and PENT are given in comparison with the standards injected at four different concentrations in each HPLC run. The intra-assay CVs based on duplicates within a run were 2.6 %, 3.7 %, and 3.9 % for HP, LP, and PENT, respectively. The detection limit for HP and LP is 0.4 pmol and 0.05 pmol for PENT.

## Statistics

The statistical analyses of all three studies (I+II+III) were performed with GraphPad Prism ® version 4.01. Non-parametric statistics were applied in all instances. An alpha level of $P<0.05$ was considered significant and all tests were two-tailed.

*Study 1*

Wilcoxon matched-pairs signed-ranks tests were used to examine if there was a side-to-side difference in measured variables. Friedman's analyses of variance with subsequent Dunn's Multiple Comparison tests were used to detect possible tendon region-specific differences along its length. Typical error percent for duplicates measures were used to analyze reproducibility. The typical error percent is calculated as ($SD_{diff}\sqrt{2}$/ /Mean overall) x 100 and provides a measure of the relative measurement error.

*Study II*

Mann-Whitney U Tests were used to examine if there were differences between the groups in the measured variables. Spearman´s rank-order correlation was used to analyze the strength of relationships between variables.

*Study III*

Wilcoxon signed rank test were used to examine if there were differences between pre and post immobilization values for the measured variables.

# Results and Discussion

In this part, the most important findings from the 3 studies will be presented and discussed. Further detailed information about the study results can be obtained in the articles later in the thesis

**Study I**

*Results*

In the recreational athletes there was no side to side difference in MVC (lead extremity 217±14 Nm, non-lead extremity 203±16 Nm)($P$>0.05). In the elite athletes MVC was significantly greater in the lead extremity (239±26 Nm) compared to the non-lead extremity (197±23 Nm)($P$<0.01).

In the recreational athletes there was no side to side differences in PT CSA for the distal (lead extremity 124±7 $mm^2$, non-lead extremity 120±10 $mm^2$), mid (lead extremity 75±8 $mm^2$, non-lead extremity 74±9 $mm^2$) or proximal (lead extremity 84±8 $mm^2$, non-lead extremity 89±7 $mm^2$) tendon. The CSA of the tendon for the elite athletes are shown in Figure 7. There was a significant side to side difference in CSA at the distal and proximal tendon ($P$<0.05), but not at the mid-tendon section ($P$=0.218). The PT CSA was greater at the distal tendon compared to mid and proximal tendon on both the lead extremity and non-lead extremity ($P$<0.05).

Mechanical properties at a common force are shown in Table 3. Tendon stiffness was higher on the lead extremity compared to the non-lead extremity *(*$P$<0.05) (Figure 8). Stress was lower on the lead extremity compared to the non-lead extremity at the proximal (Figure 9) and distal tendon level ($P$<0.05).

| | Lead extremity | Non-lead extremity |
|---|---|---|
| Deformation (mm) | 2.5 ± 0.3 | 3.0 ± 0.4 |
| Stiffness (N mm$^{-1}$) | 4766 ± 716$^{*}$ | 3494 ± 446 |
| Stress (MPa) | | |
| Proximal tendon | 52.9 ± 4.8$^{**}$ | 66.0 ± 8.0 |
| Mid tendon | 65.6 ± 5.6 | 71.2 ± 8.2 |
| Distal tendon | 40.9 ± 4.4$^{*\#}$ | 46.6 ± 4.4$^{\#}$ |
| Strain (%) | 5.0 ± 0.5 | 5.9 ± 0.6 |
| Modulus (GPa) | | |
| Proximal tendon | 2.27 ± 0.27 | 2.16 ± 0.28 |
| Mid tendon | 2.87 ± 0.39 | 2.26 ± 0.25 |
| Distal tendon | 1.79 ± 0.25$^{\#}$ | 1.54 ± 0.19$^{\#}$ |

**Table 3**. Patella tendon mechanical properties based on common force. Mean±SEM. Significantly different from non-lead extremity $^{*}$P<0.05, $^{**}$ P<0.01. Significantly different from mid- and proximal tendon $^{\#}$ P<0.01.

*Discussion*

Study 1 examined PT size and mechanical properties in subjects that had a side to side difference in knee extensor strength as a result of habitual sport specific loading. The main findings were that the lead extremity, which was on average 22 % stronger than the non-lead extremity, had a greater distal, proximal and average (24%) PT CSA, which was not evident at the mid-tendon tendon level, indicating a region specific tendon hypertrophy. Further, the lead extremity displayed greater tendon stiffness without a significant difference in modulus, suggesting that the change in mechanical properties was largely the result of a change in size.

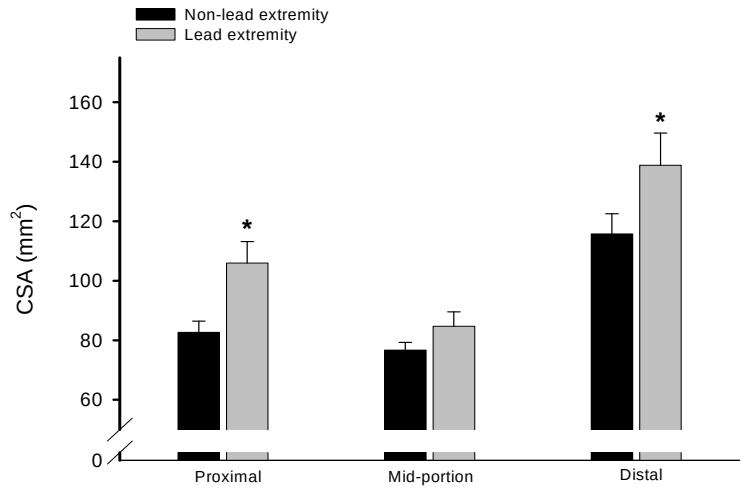


**Figure7.** Mean ± SEM. The PT CSA distribution. There was a significant side to side difference in CSA at the distal and proximal level but not at the mid-tendon section ($^{*}$P<0.05). CSA was greater at the distal level compared to mid- and proximal tendon on both the lead extremity and non-lead extremity ($^{\#}$P<0.05).

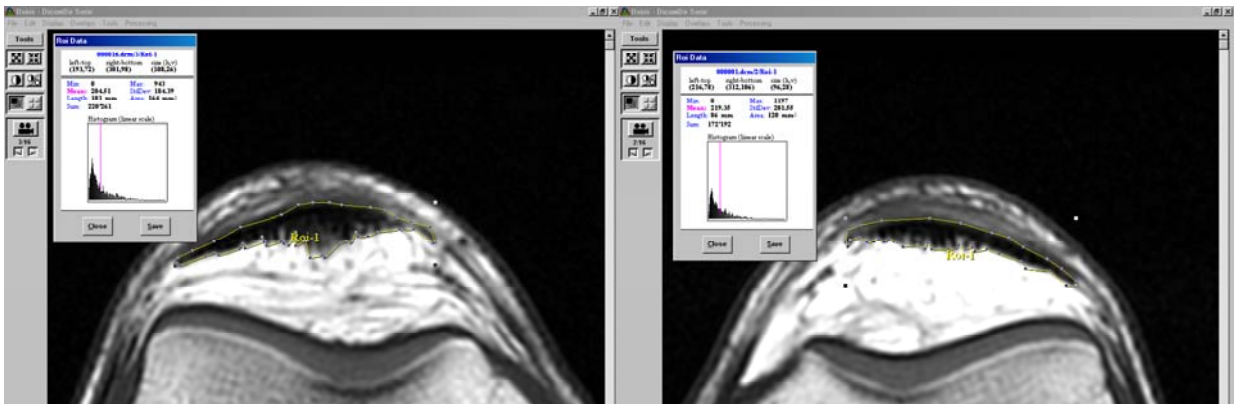

**Figure 8.** Patellar tendon CSA (just distal to the patellar insertion) on the lead (left image) and nonlead extremity in one elite badminton player determined by axial plane MRI.

It was demonstrated that the lead extremity had a greater PT CSA in the distal (20 %) proximal region (28%), but not in the mid–section of the tendon. Further, there were no PT CSA side to side differences in recreational athletes. The magnitude of this region specific human tendon hypertrophy confirms and reinforces previous cross-sectional data (62;78;79) and more recent training data (87). Furthermore, the fact that the lead extremity had a 24% greater PT CSA and was 22% stronger than the non-lead extremity, indicates that tendon size follows muscle strength, confirming previous animal data (43;61). However, it was recently shown in humans that 9 weeks of knee extension resistance training yielded a 5-6% region specific hypertrophy response along the PT together with increased tendon stiffness and modulus (89).

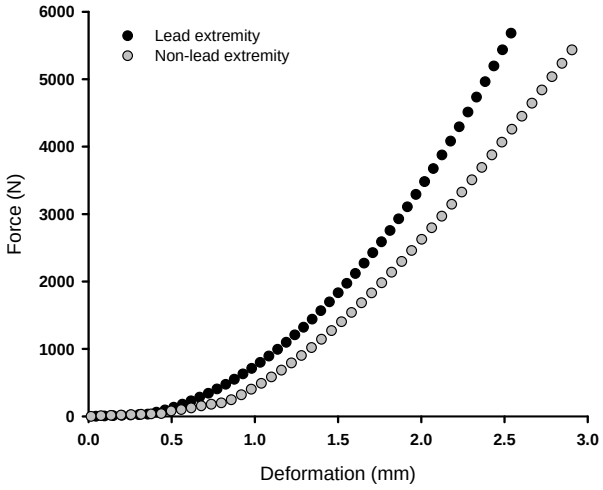


**Figure 9.** The PT force-deformation relationship to a common force. Stiffness was higher on the lead extremity compared to the non-lead extremity ($P<0.05$). Values are means of all subjects.

Like previous studies (87;90), Seynnes et al. have confirmed a substantial increase in tendon CSA at the proximal and distal region, and interestingly also finds a small increase in tendon CSA at the mid-portion of the PT. In the present study, we did not observe an increase at the mid-tendon, maybe due to the sample size was limited. However, Kongsgaard et al.(87) was not able demonstrate an increased tendon CSA at the mid-portion, indicating a limited hypertrophy response in this part of the tendon (178-180). Moreover, region specific hypertrophy (~5%) and increased stiffness in Achilles tendon after 14 weeks of high strain loads (90%MVC), has also recently been confirmed. In this study, no change was observed in paired tendon that trained with low strain magnitude (55% MVC) (88). This indicates that a firm strain or force threshold may exist before tendon adaptations occur such as increase in tendon CSA and tendon stiffness. More, recently, it has been suggested that the applied strain on the connective tissues regulates the catabolic and anabolic responses of the cells (181). Further, an external mechanical loading of the tissue above the upper limit at which the endogenous contraction of the fibroblast may maintain their tensional homeostasis should stimulate cells for remodeling, whereas a reduction of the mechanical threshold below the lower limit will lead to tissue destruction (182).

Altogether, it seem that tendon primarily adapts to increase loading by tendon hypertrophy that leads to greater tendon stiffness. In addition, the increased tendon CSA and tendon stiffness have several clinical implications; it will decrease tendon stress and strain for any given magnitude of tensile loading, and may therefore reduce the risk of overload injuries, which may be protective mechanism. Further, the greater stiffness may also improve the load transfer (e.g. RFD) and performance (e.g. running economy)(183).

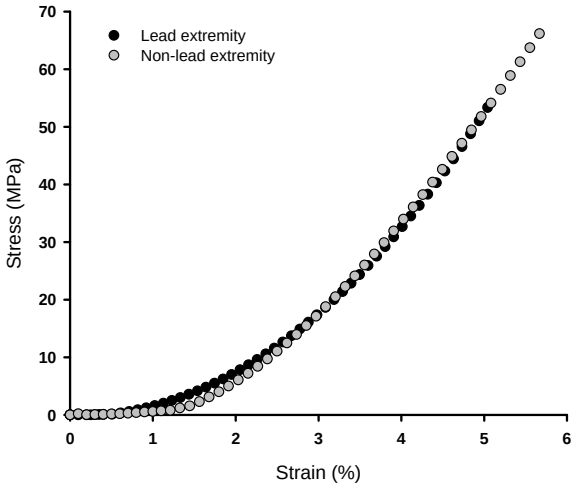


**Figure 10.** The PT stress-strain relationship based on common force. Stress was lower on the lead extremity compared to the non-lead extremity at the proximal tendon level ($P<0.05$). Values are means of all subjects.

## Study II

*Results*

Tendon dimensions are shown in Table 4. Tendon length did not differ between YM and OM. Absolute average tendon CSA did not differ between OM and YM. Similarly, there was no between group difference in average tendon CSA normalized for body weight.

| | OM (n = 7) | YM (n = 10) |
|---|---|---|
| Tendon length (mm) | 43±4 | 43±5 |
| Tendon CSA ($mm^2$) | 101±17 | 103±8 |
| Tendon CSA ($mm^2/BW^{2/3}$) | 5.34±0.48 | 5.47±0.25 |
| Q-ACSA ($mm^2$) | 6214±705** | 8431±522 |

**Table 4.** Patellar tendon dimensions and quadriceps muscle cross-sectional area (Q-ACSA) for old (OM) and young men (YM). Values are mean±SD. ** Significantly different from YM, P<0.01.

Mechanical properties determined at maximal force are shown in Table 5. Maximal tendon force was lower in OM than YM ($P$<0.05). There were no differences between OM and YM with respect to tendon deformation, stiffness, strain, stress or Young's modulus based on average

Mechanical properties at a common force are shown in Table 6. Again, there were no differences between OM and YM for any of the variables (Figure 11 & 12).

Collagen concentration and cross-link density data are shown in Table 7. Collagen concentration was lower in OM than YM ($P$<0.05). Both HP and LP ($P$<0.05) as well as PENT ($P$<0.01) concentrations in collagen were higher in OM than YM. PENT was positively related to age in YM (r = 0.74, $P$<0.01, Figure 13), but not in OM (r = 0.65, $P$ = 0.11). There were no significant correlations between the mechanical and biochemical variables.

| | OM (n = 7) | YM (n = 10) |
|---|---|---|
| Force (N) | 5161±737* | 7415±2184 |
| Deformation (mm) | 2.6±0.4 | 2.9±0.9 |
| Stiffness (N/mm) | 3926±1091 | 5546±1871 |
| Stress (MPa) | 51±8 | 65±24 |
| Strain (%) | 6.1±0.9 | 6.9±2.3 |
| Modulus (GPa) | 1.7±0.3 | 2.2±0.7 |

**Table 5.** Patellar tendon mechanical properties for old (OM) and young men (YM) based maximum force. Values are mean±SD. * Significantly different from YM, P<0.05.

*Discussion*

To the best of our knowledge this is the first study that has examined both the mechanical properties of the human PT, *in vivo*, and collagen cross-links densities in YM and OM persons with a similar physical activity level. The main findings were that the collagen concentration was lower in OM than YM, while the enzymatically derived cross-links (HP and LP) were greater in OM compared to YM. At the same time, the non-enzymatically derived AGE marker (PENT) was markedly more abundant in OM compared to YM. However, despite these apparent age-related differences in the tendon collagen properties, the tendon mechanical properties in the two age separated groups did not diverge appreciably.

| | OM (n = 7) | YM (n = 10) |
|---|---|---|
| Deformation (mm) | 2.3±0.4 | 2.4±0.6 |
| Stiffness (N/mm) | 3511±837 | 3290±869 |
| Stress (MPa) | 41±7 | 37±5 |
| Strain (%) | 5.3±0.9 | 5.9±1.7 |
| Modulus (GPa) | 1.5±0.4 | 1.4±0.4 |

**Table 6.** Patellar tendon mechanical properties for old (OM) and young men (YM) based on common force. Values are mean±SD.

The present mechanical data diverges from that of others based on human *in vivo* model with one exception (116), and this may be related to methodological differences and study design. It has been shown that strength training and habitual loading pattern may result in tendon hypertrophy (87;88;90), which would influence the mechanical properties of the tendon (82). In contrast to previous *in vivo* studies (108;111-116), we have taken this aspect into account by comparing age groups with similar activity level. However, it cannot be ruled out that prior life long training history, including exercise mode and intensity, which was unaccounted for in the study may have influenced the data. It should also be recognized that the present data and that of others are based on cross-sectional designs with inherent limitations, including the striking variations in tendon mechanical properties between subjects (155), and therefore a type II error cannot be ruled out.

| | OM (n = 7) | YM (n = 10) |
|---|---|---|
| Collagen (mg/mg dry weight) | 0.49±0.27* | 0.73±0.14 |
| HP (mmol/mol collagen) | 898±172* | 645±183 |
| LP (mmol/mol collagen) | 49±38** | 16±8 |
| PENT (mmol/mol collagen) | 73±13** | 11±2 |

**Table 7.** Concentration of collagen and hydroxylysyl pyridinoline (HP), lysyl pyridinoline (LP) and pentosidine (PENT) cross-links in the patellar tendons of old (OM) and young men (YM). Values are mean±SD. *, ** Significantly different from the values of YM, ($P<0.05$ and $P<0.01$, respectively).

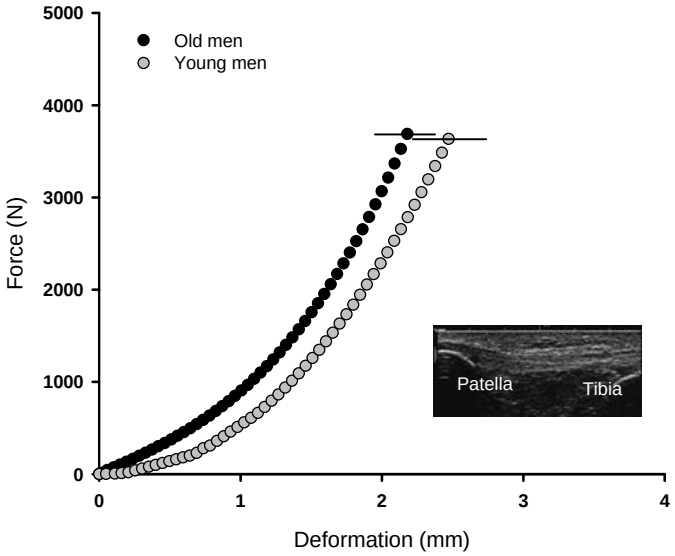


**Figure 11.** The patellar tendon force-deformation relationship based on common force. Values are mean±SD of all subjects. There were no differences between old (OM) and young men (YM) with respect to tendon deformation or stiffness (P>0.05).

The density of mature lysyl oxidase-derived intermolecular covalent cross-links, such as the HP and LP, gradually increase during tendon tissue maturation, and it is commonly believed that these cross-links are the chief contributors to the function and mechanical properties of the tendon (10;12;117;184). In animal models it has been shown that there is a positive relationship between HP cross- link density and mechanical strength of the healing medial collateral ligament tissue and ACL graft (185;186), and furthermore that changes in HP and LP density will result in altered mechanical properties in collagenous tissue (10;187). However, there is only sparse human data on mature cross-link density in tendon and how these are influenced by aging (130). A small but significant age-related increase of LP has been demonstrated in the supraspinatus tendon, *in vitro* (130). The present data on LP and HP density on YM corresponds well with that previously reported in a similar population (159). However, the present data also demonstrate that HP and LP density of the human PT was ~ 40 % and 3-fold higher, respectively, in OM compared to YM, which suggests a rather marked age associated elevation in these enzymatic cross-links. It is noteworthy that despite the rather robust difference in both HP and LP there was no difference in the mechanical properties of the tendon. However, it should be noted that fibril length (188;189), fibril diameter (190), and proteoglycans and glycosaminoglycans (6;191;192) have all been

implicated in contributing to the tendon mechanical properties, and the relative contribution of these factors and that of mature cross-links remains elusive.

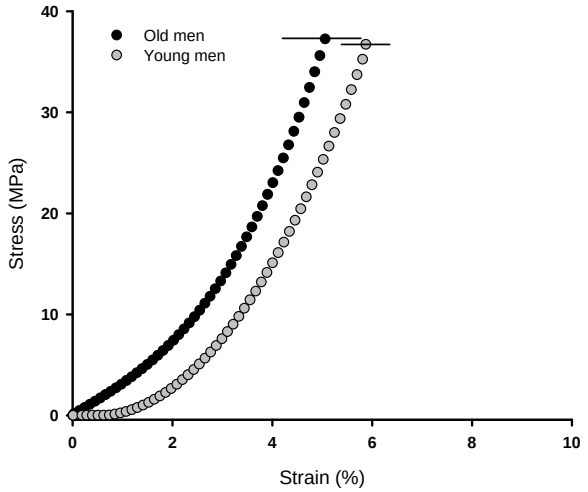


**Figure 12.** The patellar tendon stress-strain relationship based on common force. Values are mean±SD of all subjects. There were no differences between old (OM) and young men (YM) with respect to tendon strain, stress or Young´s modulus based on average tendon CSA (P>0.05).

To our knowledge these are the first data showing that AGE is markedly increased in OM compared to YM in the human PT. AGE cross-links, including PENT, are formed when lysine amino acid residues in the collagen triple helix come into contact with glucose (10;15), and the accumulation of these cross-links are known to accelerate with aging (6;10) and disease processes such as diabetes (15;121;122), atherosclerosis (193), Alzheimer (194;195) and renal failure (196). AGE products are also used as markers of tissue turnover (130). It has been shown that the difference between young and older individuals in AGE cross-link density in collagen is approximately 2-fold in human skeletal muscle (127), 5-fold in human bone (128), 7-fold in human tendon (present data), 9-fold in human ligaments (126), and 33-fold in human cadaver cartilage (125), demonstrating the tissue specific turnover. The fact that PENT density is 7-fold greater in tendon of OM compared with YM (Table 6) coupled with the fact that PENT appears to be related to age in a narrow age span (Figure 13) firmly demonstrates the positive relationship between AGE cross-linking of human PT collagen and aging, *ex vivo*. These data corroborate and extend those previously reported on cadaver tissue (129;130). From a functional standpoint, an elevated AGE cross-link density has been suggested to result in increased tensile stress and tendon stiffness in animal models (97;123;124;197-200). However, in the present study both the stiffness (Figure 11) and the Young's modulus (Figure 12) of the tendon did not differ between OM and YM despite the

7-fold difference in PENT, which suggest that factors other than AGE may also play a major role in determining the mechanical properties of human tendon.

In the present study, the total collagen concentration of the PT was ~ 34 % lower in OM compared to YM (Table 6), and this age linked reduction is in accordance with that found in the canine PT (101) and rat tail tendon (99). It is possible that the lower collagen concentration with aging may represent the reduced size and/or density of collagen fibrils that is known to occur with aging (178;184;190;201;202). It was recently reported that MRI signal intensity of the PT was reduced with aging (116), which may be a function of the reduction in collagen concentration observed in the present study. Unfortunately, the size of the obtained biopsy in the present study precluded transmission electron microscopy analysis for fibril size and density. The lower collagen concentration in OM may be an age-related change in the tendon per se, and/or a function of reduced tendon loading due to an age-related loss of muscle mass/strength. Interestingly, the average whole tendon CSA did not differ between OM and YM (Table 3), which in light of the reduced collagen concentration may be related to increased amounts of other extracelluar matrix components, such a proteoglycans and glycosaminoglycans. Alternatively, the retained tendon CSA in OM may result from tendon intrafibrillar fat that can accumulate with aging (203-205).

Albeit speculative, it is possible that the elevated enzymatic and/or non-enzymatic cross-link density in OM compared to YM served to maintain tendon stiffness and Young's modulus despite the diminished collagen concentration. Such maintenance of tendon stiffness would serve to maintain effective transfer of muscle force despite a lower absolute muscle size (Table 3) and strength (Table 4). In this context it should be noted that the present data were obtained in moderately physically active person and future studies will need to address the effect of training per se in elderly.

In conclusion, the data of present study raise the possibility that the dimensions and mechanical properties of the human PT, *in vivo*, may not differ between old and young men. On the other hand, old men displayed lower collagen concentration, but greater enzymatic (HP & LP) and non-enzymatic (PENT) collagen cross-links compared to young men. This age related increase both in enzymatic and non-enzymatic cross-linking compounds, may serve to maintain the mechanical properties of tendon with aging.

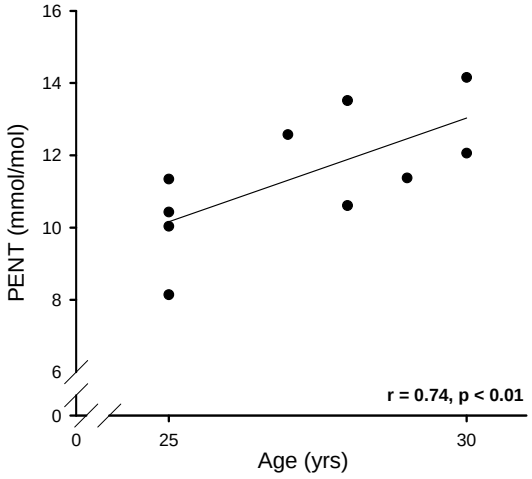


**Figure 13.** PENT was positively related to age in young men (YM) (r = 0.74, P<0.01).

## Study III

*Results*

There were no side-to-side differences for any of the measured variables prior to immobilization. Peak knee extensor moment decreased on the immobilized side (143±25 vs. 119±26 Nm, *P*<0.05). A statistical trend was observed toward a decrease in peak knee extensor moment (152±32 vs.139±39 Nm, *P*=0.078) on the control side.

| (n = 8) | Immobilized side | | Control side | |
|---|---|---|---|---|
| | Pre | Post | Pre | Post |
| Tendon length (mm) | 43±3 | 43±3 | 43±6 | 43±3 |
| Tendon CSA ($mm^2$) | 101±11 | 101±11 | 99±15 | 102±16 |

**Table 8.** Patellar tendon dimensions for the immobilized side and control side. Values are mean ± SD. There were no differences in patellar tendon dimensions between Pre and Post immobilization

Tendon dimensions are shown in Table 8. There were no changes with respect to tendon length or absolute tendon CSA on the immobilized or the control side. Mechanical properties assessed at maximum common force are shown in Table 9. Tendon stiffness decreased both on the immobilized side (*P*<0.01) (Figure 14) and on the control side (*P*<0.01) (Figure 15). Likewise, Young´s Modulus decreased on the immobilized side (*P*<0.05) (Figure 16) and on the

control side ($P$<0.05) (Figure 17). There were no other differences with respect to tendon deformation, strain or stress on either the immobilized side or the control side.

| (n = 8) | Immobilized side | | Control side | |
|---|---|---|---|---|
| | Pre | Post | Pre | Post |
| Deformation (mm) | 2.3±0.5 | 2.7±0.8 | 2.3±0.4 | 2.8±0.6 |
| Stiffness (N mm$^{-1}$) | 2949±799 | 2366±774** | 3330±839 | 2365±436** |
| Stress (MPa) | 32±7 | 33±10 | 34±8 | 32±8 |
| Strain (%) | 5.5±1.6 | 6.6±2.2 | 5.3±0.7 | 6.5±1.7 |
| Modulus (GPa) | 1.2±0.3 | 1.0±0.3* | 1.5±0.4 | 1.0±0.3* |

**Table 9.** Patellar tendon mechanical properties for the immobilized side and control side based on common force. Values are mean ± SD. Significantly different from Pre, *P<0.05, **P<0.01.

*Discussion*

To the best of our knowledge this is the first study that has examined the effects of short-term unilateral immobilization on the structural and mechanical properties of the human PT, *in vivo*, in old healthy individuals. The main findings were that the mechanical properties of the PT decreased on the immobilized side. A decline in stiffness and Young´s modulus took place without any measurable change in the size of the tendon, suggesting that the material properties of the tendon were primarily affected by the unloading.

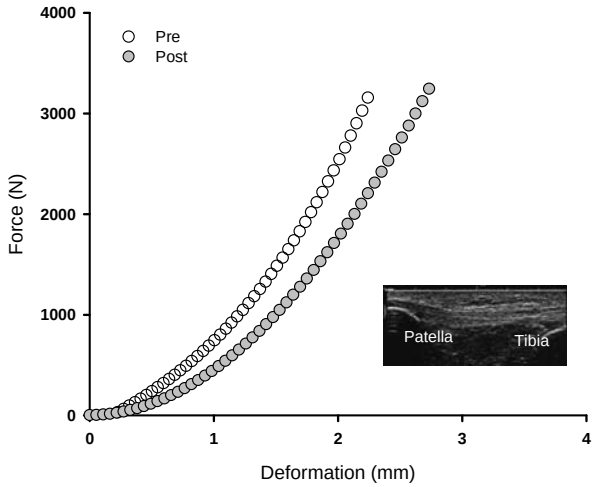


**Figure 14.** The patellar tendon force-deformation relationship based on common force for the immobilized side. Values are mean; SD values are stated in Table 2. Stiffness decreased after 14 days of immobilization (Pre vs. Post, P<0.01).

It is well known that the immobilization associated drop in muscle strength is greater than that of muscle mass, and that both contribute to a reduced muscle function (92;93;132). The larger drop in muscle strength has mainly been attributed to rapid changes in the nervous system that occur with short term unloading (92;206-208). Interestingly, recent studies report that unloading affect tendon properties to a greater extent than muscle loss, which in turn may contribute to a decline in electromechanical delay (the time lag between muscle activation and muscle force production) and reduced rate of force development (92;145;146;153;209). However, evidence suggests that quadriceps muscle function is more affected in old than in young persons by unloading (132;152), indicating that the magnitude of change in tendon properties is perhaps even greater in the elderly. Nevertheless, the aforementioned effects of immobilization on the mechanical properties of tendon have mainly been investigated in young persons, despite the fact that old persons are frequently exposed to periods of disuse caused by disease or injury (150;151), and if similar or greater magnitudes of changes occur in older persons remains unknown. However, in the present study, it was demonstrated for the first time *in vivo* for aging human individuals, that 14 days of immobilization led to decreased tendon stiffness on the immobilized side, which was accompanied by a decrease in Young´s Modulus. Furthermore, a similar effect was observed on the control side. This decline occurred without changes in tendon dimensions, indicating that the material properties of the patella tendon were altered for our old subjects, which is in line with previous reports in young persons (144-146;149;153). In the present study, we extended these findings by showing that muscle tendon mechanics are also affected by acute disuse in the elderly, and that the magnitude of change is perhaps even greater. Collectively, these data demonstrate that short-term limb unloading has considerable effects on the mechanical properties of the tendon that to some extent mirrors that of the muscle, and that these changes can be expected to occur in the early phase of unloading.

It has been proposed that the degree of tension on the PT during immobilization could influence the tendon biomechanical properties quite dramatically within one week (210). The present study did not investigate possible biochemical changes, and it should be kept in mind that the immobilized knee was positioned in a relatively shorten position (~30° knee flexion), which likely is associated with no or only very minor passive tension in the patella tendon (170). The acute lack of tensile tendon input during the immobilization period might have influenced the material properties of the tendon, since tendon stiffness and modulus decreased without tendon atrophy. Unloading tenocytes have been shown to have severe effects on collagen expression, proteoglycan

expression, growth factor secretion and metalloproteinases (182;211). Furthermore, it has recently been demonstrated that complete tension deprivation in the rabbit PT induced fibroblast apoptosis within 24 hrs (212). Thus, it is possible that the removed tension development in the PT was the initiating event for the present findings of reduced tendon stiffness and Youngs Modulus.

A number of limitations may be observed with the present study. Unfortunately we were unable to obtain tendon biopsies in the present study, which would have allowed us to address possible changes in tendon composition. Furthermore, to our surprise changes occurred in the non-immobilized limb, which rendered it inadequate as a control. Albeit non-significant, muscle strength tended (*P*=0.078) to decrease on the non-immobilized side. Furthermore, both stiffness and modulus also decreased on the non-immobilized side. Thus, although the data show that the non-immobilized side was not an ideal control in this sample, the data suggests that muscle strength changes are accompanied by changes in tendon properties. A possible explanation for the findings in the control limb is that unilateral casting and crutch walking in old persons may influence the

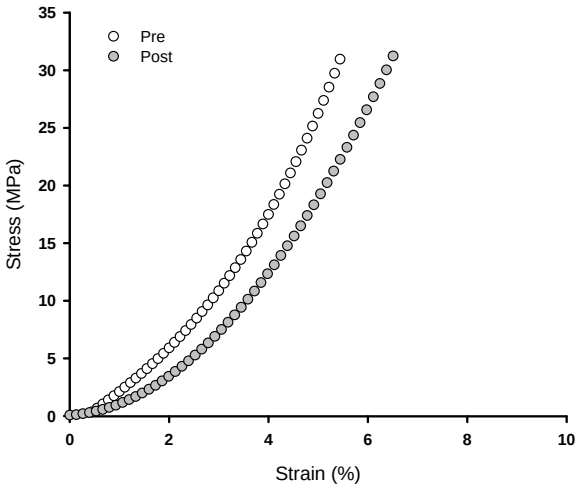


**Figure 15.** The patellar tendon stress-strain relationship based on common force for the immobilized side. Values are mean; SD values are stated in Table 2. Stiffness decreased after 14 days of immobilization (Pre vs. Post, P<0.05).

overall activity level. It has been reported that overall amount of weight-bearing support per day is deceased during unilateral limp suspension monitored by accelerometry (213;214).

In conclusion, a decline in stiffness and Young´s modulus was observed following short-term (14 days) unilateral limb immobilization in old men without any changes in tendon size, suggesting that the material properties of the tendon were affected by unloading. These findings indicate that short-term inactivity has a major negative influence on the mechanical properties of the human PT in the elderly.

# Conclusions

The main focus in this thesis was to investigate the effects of long-term habitual sport specific loading, aging and short-term immobilization on the human PT structural and mechanical properties, *in vivo*.

It was found that a habitually more loaded PT of a stronger lower extremity had a greater PT CSA compared to a less loaded PT of a weaker lower extremity. However, the greater tendon CSA was evident at the distal and proximal level and not at the mid-tendon, indicating a region specific tendon hypertrophy. Further, the habitually more loaded PT displayed greater tendon stiffness without a significant difference in modulus, suggesting that the change in mechanical properties was largely the result of a change in size. In study II, investigating the age-related changes in tendon structure and function, it was observed that the collagen concentration was lower in OM than YM with a similar physical activity level, while the enzymatically derived cross-links (HP and LP) were greater in OM compared to YM. At the same time, the non-enzymatically derived AGE marker (pentosidine) was markedly more abundant in OM compared to YM. Despite these apparent age-related differences in the tendon collagen properties, the tendon mechanical properties in the two age separated groups did not diverge appreciably. It is possible that the elevated enzymatic and/or non-enzymatic cross-link density in OM compared to YM served to maintain tendon stiffness and Young's modulus despite the diminished collagen concentration. Such maintenance of tendon stiffness would serve to maintain effective transfer of muscle force despite a lower absolute muscle size and strength. In the third study, short-term (14 days) unilateral limb immobilization in old men resulted in a decrease in the mechanical properties of the PT on both sides. A decline in stiffness and Young´s modulus without any changes in tendon size was observed, suggesting that the material properties of the tendon were affected by unloading. These findings indicate that short-term inactivity has a major negative influence on the mechanical properties of the human PT in the elderly.

# Perspective

The completed studies answered some questions, but also prompted several additional questions that may be addressed in future studies.

Based on Study I, it may be of interest to better understand the hypertrophy response of chronic habitual loading by investigating the composition of tendon through tendon biopsy (e.g., collagen cross-links, fibrils morphology, and density). Furthermore, it is still largely unknown how the tendon adapts to different types of loading. It could, therefore, be interesting to look at the effect of different loading regimes such as resistance versus endurance training. Additionally, the possible combined effects of these two, and how they influence the mechanical properties and the composition of the tendon is not understood.

From bone and muscle research, it is well established that mechanical loading and physical activity in general improves tissue properties, physical function, and prevents disease. It seems that age of initiation of physical activity effects disease. Therefore, the importance and effect of activity (training) during the growing years and how it influences the mechanical properties of the tendon can be studied in several sport populations. Furthermore, the effect of early physical training on the tendon properties, not only needs to be studied in younger persons but also in the elderly.

In line with the findings in Study II, it may also be of interest to investigate the importance of different levels of physical activity on aging tendon. For instance, it may be of interest to study whether the mechanical properties and the composition of the tendon, *in vivo*, are changed in master athletes (older persons that have trained all their life), and especially the role of AGE cross-links and how they are influenced by physical activity.

From Study III and from a rehabilitation point of view, it would be essential to investigate if the mechanical and the material properties can be restored after a period of immobilization in the elderly. Lastly, it is of interest to study if the effects of immobilization on the mechanical properties of the tendon can be prevented by passive range of motion or by putting the tendon into elongated position during immobilization.

# Abstract

The overall purpose of this thesis was to study the effects of habitual loading, aging and immobilization on the human PT structural and mechanical properties, *in vivo*.

*Study 1*

The specific purpose of this study was to examine PT (PT) size and mechanical properties in subjects (elite badminton players and fencers) with a side-to-side strength difference of ≥ 15 % due to sport induced loading.

The main findings were that the lead extremity, which was on average 22 % stronger than the non-lead extremity, had a greater distal and proximal PT CSA, which was not evident at the mid-tendon tendon level, indicating a region specific tendon hypertrophy. Further, the lead extremity displayed greater tendon stiffness without a significant difference in modulus, suggesting that the change in mechanical properties was largely the result of a change in size.

*Study II*

The purpose of this study was to examine the mechanical properties and pyridinoline and pentosidine cross-link, and collagen concentrations of the PT, *in vivo*, in old (OM) and young men (YM) with a similar physical activity level.

The main findings were that the collagen concentration was lower in OM than YM, while the enzymatically derived cross-links (HP and LP) were greater in OM compared to YM. At the same time, the non-enzymatically derived AGE marker (PENT) was markedly more abundant in OM compared to YM. However, despite these apparent age-related differences in the tendon collagen properties, the tendon mechanical properties in the two age separated groups did not diverge appreciably.

*Study III*

The purpose of this study was to examine the effects of short-term (14 days) unilateral immobilization on the human PT structural and mechanical properties in old men (OM), *in vivo*.

The main findings were that the mechanical properties of the PT decreased on the immobilized side. Furthermore, a similar effect was observed on the control side. A decline in stiffness and Young´s modulus took place without any measurable change in the size of the tendon, suggesting that the material properties of the tendon were primarily affected by the unloading.

# Dansk abstract

Hovedformålet med denne afhandling var at undersøge effekterne af langtidstræning, aldring og immobilisering på den humane patellasenes strukturelle og mekaniske egenskaber, *in vivo*. I afhandlingen indgår tre separate studier.

*Studie I*

Formålet med dette studie var at undersøge patellasenens størrelse og mekaniske egenskaber hos personer (elite badmintonspillere og fægtere) med en side til side styrke ≥ 15 % som følge af langvarig idrætsspecifik belastning.

Hovedfundene i studiet var, at patellasenen på fremfaldsbenet, som gennemsnitligt var 22% stærkere end ikke-fremfaldbenet, havde et større tværsnitsareal i den distale og proximale del men ikke i midt-sene delen, hvilket typer på en regionsspecifik hypertrofi. Yderligere havde fremfaldsbenet en stivere patellasene, uden at der var forskel i Modulus, hvilket indikerer at ændringer i de mekaniske egenskaber var et resultat af ændret sene størrelse.

*Studie II*

Formålet med dette studie var at undersøge de mekaniske egenkaber, pyridinolin og pentosidine tværbindinger samt kollagen koncentrationen i patellasenen, *in vivo*, hos unge og ældre mænd med samme aktivitetsniveau.

Hovedfundene i studiet var, at kollagen koncentrationen var lavere hos ældre mænd sammenlignet med unge mænd, mens enzymatisk-dannede tværbindinger var højere hos ældre mænd end unge mænd. Ligeledes var den non-enzymatiske AGE tværbindings markør (pentosidine) væsentligt forhøjet hos ældre sammenlignet med unge mænd. På trods af de markante alders-relaterede forskelle i senens koncentration af kollagen og tværbindinger, var der ingen forskel i de mekaniske egenskaber mellem de to grupper.

*Studie III*

Formålet med dette studie var at undersøge effekterne af kort-tids (14 dage) unilateral immobilisering på den humane patellasenes strukturelle og mekaniske egenkaber hos ældre mænd, *in vivo.*

Hovedfundene i studiet var en nedgang i sene stivhed og Youngs Modulus efter immobilisering uden en samtidig ændring af patellasenes dimensioner , hvilket tyder på, at patellasenens materiale egenskaber var ændret efter immobiliseringen, som følge af mindre aktivitet.

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# Habitual loading results in tendon hypertrophy and increased stiffness of the human patellar tendon

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**Couppé C, Kongsgaard M, Aagaard P, Hansen P, Bojsen-Moller J, Kjaer M, Magnusson SP.** Habitual loading results in tendon hypertrophy and increased stiffness of the human patellar tendon. *J Appl Physiol* 105: 805–810, 2008. First published June 12, 2008; doi:10.1152/japplphysiol.90361.2008.—The purpose of this study was to examine patellar tendon (PT) size and mechanical properties in subjects with a side-to-side strength difference of ≥15% due to sport-induced loading. Seven elite fencers and badminton players were included. Cross-sectional area (CSA) of the PT obtained from MRI and ultrasonography-based measurement of tibial and patellar movement together with PT force during isometric contractions were used to estimate mechanical properties of the PT bilaterally. We found that distal tendon and PT, but not mid-tendon, CSA were greater on the lead extremity compared with the nonlead extremity (distal: 139 ± 11 vs. 116 ± 7 mm$^2$; mid-tendon: 85 ± 5 vs. 77 ± 3 mm$^2$; proximal: 106 ± 7 vs. 83 ± 4 mm$^2$; $P < 0.05$). Distal tendon CSA was greater than proximal and mid-tendon CSA on both the lead and nonlead extremity ($P < 0.05$). For a given common force, stress was lower on the lead extremity (52.9 ± 4.8 MPa) compared with the nonlead extremity (66.0 ± 8.0 MPa; $P < 0.05$). PT stiffness was also higher in the lead extremity (4,766 ± 716 N/mm) compared with the nonlead extremity (3,494 ± 446 N/mm) ($P < 0.05$), whereas the modulus did not differ (lead 2.27 ± 0.27 GPa vs. nonlead 2.16 ± 0.28 GPa) at a common force. These data show that a habitual loading is associated with a significant increase in PT size and mechanical properties.

unilateral; strength; tendon size

HUMAN MOVEMENT COMES ABOUT from the force created by contracting muscles, which is transmitted to bone via aponeurosis and tendon. Recent data in human models suggest that tendon tissue is quite metabolically responsive to tensile loading (4, 12, 16). In fact, it has been shown that both a single loading bout as well as long-term habitual loading produce a markedly elevated collagen synthesis response (20, 21, 26). 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, remains ambiguous.

Studies that address the influence of exercise on the mechanical properties of tendon have until recently largely been conducted in animal models. These data show that endurance-like exercise is associated with an increase (1, 35, 39), decrease (39), or unchanged (5, 36) tendon size, and thus they do not provide a coherent picture. In humans, cross-sectional data suggest that endurance training is associated with a larger Achilles tendon cross-sectional area (CSA) (17, 24, 33), which appears to be site specific (17, 24). However, in a recent intervention study it was shown that 9 mo of endurance training in untrained persons left the Achilles tendon CSA unchanged (13). On the other hand, animal data have shown that muscle size is related to the tendon size (8), suggesting that perhaps the magnitude of loading influences tendon size. Several human studies have shown that resistance training over 12–14 wk that produces increases in muscle strength of up to 21% does not result in an accompanying increase in tendon CSA (19, 30, 31), but rather a markedly altered modulus, which implies that there is a change in the composition of the structure rather than the size. However, it was recently shown in humans that resistance training for 12 wk yielded region-specific increases in patellar tendon (PT) CSA, without a change in modulus (18).

Conclusions from studies of cross-sectional design are inevitably hampered by issues of training history, selection bias, and intersubject variations. Additionally, longitudinal training studies may have been of insufficient duration to produce a robust tendon hypertrophy response. Finally, existing training studies (19, 30, 31) have examined tendon size in a region that appears unresponsive to training-associated adaptation. However, some of these limitations may be partially circumscribed 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. Therefore, the purpose of the present study was to examine region-specific PT size and mechanical properties in subjects who display a side-to-side strength difference of ≥15% due to persistent sport-induced loading over several years.

## MATERIALS AND METHODS

*Subjects.* Both badminton and fencing involve repeatedly performing rapid forward lunges with a preferred and thus more loaded lead extremity compared with the nonlead extremity, which in this study served as a within-subject control. Sapega et al. (34) and Nystrom et al. (28) found a >14% strength difference between the legs in elite fencers, reflecting a sport-specific loading. The sport-specific loading in badminton and fencing creates a higher impact on the leg and therefore also on the PT compared with other sports without jumping and unilateral rapid lunges (27). A total of 22 athletes volunteered to participate in the study. They were recruited from the Danish National Fencing Center and the International Badminton Academy in Copenhagen, Denmark. Seven elite fencers ($n = 4$) and badminton ($n = 3$) players met the criteria of a side-to-side isometric knee extensor

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strength difference of ≥15% (23 ± 2 yr, 74 ± 3 kg, 184 ± 3 cm). They had participated in their respective sport for >5 yr at an elite level. In addition, seven recreational athletes (24 ± 1 yr, 77 ± 4 kg, 182 ± 2 cm) who did not engage in fencing or badminton were examined with respect to thigh strength and tendon morphology, but not mechanical properties, to ensure that there was no side-to-side difference between the dominant and nondominant side as determined by preferred kicking leg. All were healthy and without knee pathology. The study complied with the Declaration of Helsinki and was approved by the local ethics committee. All subjects gave their informed consent before the experiment.

*Maximal voluntary contraction.* Maximal voluntary strength was determined by an isometric knee extension. Subjects performed a 10-min warm-up on a stationary bike (5 min at 150 W and 5 min at 200 W) before testing. The subjects were seated in a custom-made rigid chair with both hips and knees flexed to an angle of 90°. A leg cuff was connected to a strain gauge (model KRG-4, Bofors, Bofors, Sweden) through a rigid steel rod perpendicular to the lower leg and was mounted on the leg just above the medial malleolus. The tibia moment arm was measured (from the point of fixation to the lateral epicondyle of the knee) to calculate the knee extensor moment. Force was sampled on a personal computer (PC) at 50 Hz via a 12-bit analog-to-digital converter (model dt 2810A, Data Translation, Marlboro, MA). The subjects performed four to five maximal voluntary isometric knee extension contractions (MVC) separated by 1 min (sampled at 1,000 Hz) (3). The tests were conducted on both legs. A side-to-side difference in strength of ≥15% was defined as follows: (lead extremity − nonlead extremity)/nonlead extremity ·100 ≥ 15%.

*Muscle and tendon morphological measurements.* The anatomic CSA of the quadriceps femoris muscle was measured 20 cm proximal from the tibia plateau (mid-thigh level) by magnetic resonance imaging (MRI) [Signa Horizon LX 1.5 T, General Electric, longitudinal relaxation time (T1)-weighted spin echo (SE)] using a lower extremity coil. The images were obtained using the following parameters: transverse relaxation time (TR)/echo time (TE) = 500/14 ms, field of view (FOV) 18, matrix 512 × 512, and slice thickness 6 mm (18). Subsequently, the lean muscle mass of the quadriceps muscle (subcutaneous and intermuscular noncontractile tissue were not included in the measurement) was manually outlined using the software program Osiris 4.19 (http://www.sim.hcuge.ch/osiris/). The mean value of three measurements of the same image was used for analysis. PT CSA and PT length were determined with the use of MRI and the lower extremity coil (Signa Horizon LX 1.5 T, General Electric, T1-weighted SE) (18). PT CSA was determined by axial plane magnetic resonance using the following parameters: TR/TE 400/14 ms, FOV 20, matrix 256 × 256, slice thickness 5.0 mm, and spacing 0 mm. The axial scans were performed perpendicular to the PT. The tendon CSA was measured *1*) just distal to the patellar insertion, *2*) just proximal to the tibia insertion, and *3*) midway between these two sites (Fig. 1). The PT length was determined from sagittal plane MRI using the following parameters: TR 500, echo train 3× (TE 12.4 ms), FOV 16, matrix 256 × 192, slice thickness 4.0 mm, and no spacing. The PT length was obtained by measuring the distance from the dorsal insertion at the patella apex to the dorsal insertion on the tibia. The PT CSA and PT length were manually outlined using the software program Osiris 4.19 (http://www.sim.hcuge.ch/osiris/). The color intensity of each image was adjusted using the National Institutes of Health color scale mode of the software. Tendon CSA and length were measured using the gray-scale image display. The mean value of three measurements of the same image was used for analysis. The reproducibility data showed that the typical error percentage of repeated measures of tendon CSA was 2.5% for repeated measurements at the proximal tendon level, 2.5% for repeated measurements at the mid-tendon tendon level, and 2.0% for repeated measures at the most distal tendon level. The MRI investigator was blinded with regards to the subjects and side.

*Assessment of PT mechanical properties.* The details of the measurement, including the reliability of the method in our laboratory, has been reported previously (14). The within-day correlation coefficients and typical error percent of repeated measures were 0.95 and 9.9% for stiffness, 0.97 and 5.5% for strain, and 0.94 and 9.4% for modulus. Subjects performed a 5-min warm-up on a stationary bike to secure

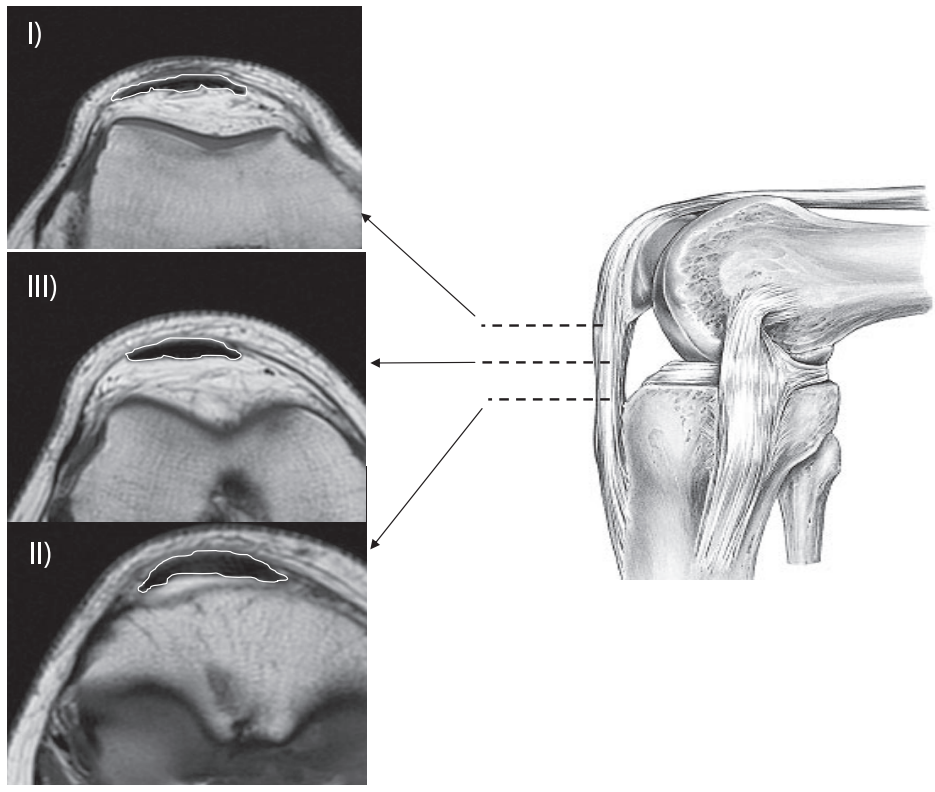


Fig. 1. Axial scans were performed perpendicular to the patellar tendon. Tendon cross-sectional area (CSA) was measured just distal to the patellar insertion (*I*), just proximal to the tibia insertion (*II*), and midway between these 2 sites (*III*).

proper preconditioning of the tendon before testing. Thereafter, the subjects were seated in a custom made rigid chair with both hips and knees flexed to an angle of 90°. A leg cuff, which was connected to a strain gauge (model KRG-4, Bofors) through a rigid steel rod perpendicular to the lower leg, was mounted on the leg just above the medial malleolus. An ultrasound probe (7.5 MHz, linear array B-mode, Sonoline Sienna, Siemens, Erlangen, Germany) was fitted into a custom-made rigid cast that was secured to the skin above the PT in the sagittal plane. The ultrasound probe and cast was positioned so that the patella, the PT and the tibia were all visible within the viewing field throughout the ramped contractions (Fig. 3).

The ultrasound S-VHS video images obtained during the ramp trials were sampled at 50 Hz on a PC using frame-by-frame capturing software (model G400-TV, Matrox Marvel, Dorval, Canada). Force was sampled on two separate PCs at 50 Hz via a 12-bit analog-to-digital converter (model dt 2810A, Data Translation). The two computers were interconnected to permit synchronous sampling of all data using a custom-built trigger device (3) . The subjects performed four to five slow isometric ramp contractions by applying gradually increasing force on the cuff over a 10-s period while PT displacement and knee extension force were measured simultaneously. Each ramp was separated by a 2-min rest. All measurements were performed on both legs. During the ramp contractions, force was sampled at 50 Hz and filtered at a 1.0-Hz cutoff frequency using a fourth-order zero-log Butterworth filter.

PT force was calculated by dividing the estimated total knee extension moment by the internal moment arm, which was estimated from individually measured femur lengths (37). PT stress was calculated by dividing tendon force by the proximal, mid-, and distal tendon CSA determined from the MRI. PT deformation was defined as the change in distance between the patellar apex and the tibia (14, 23). Tendon strain was calculated as the change in length related to the original length. Each force-deformation curve was fitted to a second- or third-order polynomial fit, which yielded $R^2 > 0.97$. Tendon stiffness (Δforce/Δdeformation) and modulus (Δstress/Δstrain) were calculated in the final 10% of the force-deformation and stress-strain curves, respectively (22, 23).

*Statistical analysis.* The two isometric ramp contractions that yielded the greatest force were used for further analysis. To make side-to-side comparisons and thereby account for differences in isometric ramp contractions from side to side, the trials for both sides were subsequently analyzed to the lowest common force for each individual subject. Wilcoxon matched-pairs signed-ranks tests were used to examine whether there was a side-to-side difference in measured variables. Friedman's analysis of variance, including the Dunn's multiple comparison test, was used to detect possible tendon region-specific differences along its length. An alpha level of $P < 0.05$ was considered significant. Results are reported as means ± SE.

## RESULTS

*Quadriceps strength and CSA.* In the recreational athletes, there was no side-to-side difference in MVC (dominant 217 ± 14 N·m, nondominant 203 ± 16 N·m)($P > 0.05$), or quadriceps CSA (dominant 7,159 ± 323 mm$^2$, nondominant 6,951 ± 261 mm$^2$)($P > 0.05$). In the elite athletes, MVC was significantly greater in the lead extremity (239 ± 26 N·m) compared with the nonlead extremity (197 ± 23 N·m) ($P < 0.01$). Similarly quadriceps femoris CSA was significantly greater in the lead extremity (7,907 ± 656 mm$^2$) compared with the nonlead extremity (7,410 ± 561 mm$^{2)}$ ($P < 0.05$).

*PT CSA.* In the recreational athletes there was no side-to-side differences in PT CSA for the distal (dominant 124 ± 7 mm$^2$, nondominant 120 ± 10 mm$^2$), mid- (dominant 75 ± 8 mm$^2$, nondominant 74 ± 9 mm$^2$), or proximal (dominant 84 ± 8 mm$^2$, nondominant 89 ± 7 mm$^2$) tendon. The CSA of the tendon for the elite athletes are shown in Fig. 2. There was a significant side-to-side difference in CSA at the distal (lead 139 ± 11 mm$^2$, nonlead 116 ± 7 mm$^2$) and proximal tendon (lead 106 ± 7 mm$^2$, nonlead 83 ± 4 mm$^2$) ($P < 0.05$) but not at the mid-tendon section (lead 85 ± 5 mm$^2$, nonlead 77 ± 3 mm$^2$) ($P = 0.218$). The PT CSA was greater at the distal tendon compared with mid- and proximal tendon on both the lead extremity and nonlead extremity ($P < 0.05$).

*PT mechanical properties.* Mechanical properties determined at maximal force are shown in Table 1. Tendon force and stiffness were higher on the lead extremity compared with the nonlead extremity ($P < 0.05$). In addition, maximal stress was lower on the lead extremity compared with the nonlead extremity at the proximal tendon level ($P < 0.05$). There was no side-to-side difference for deformation, strain, or Young's modulus based on proximal, mid-, or distal tendon CSA. Stress and modulus were lower at the distal tendon compared with mid- and proximal tendon ($P < 0.01$) on both sides.

Mechanical properties at a common force are shown in Table 2. Tendon stiffness was higher on the lead extremity compared with the nonlead extremity ($P < 0.05$) (Fig. 3). Stress was lower on the lead extremity compared with the nonlead extremity at the proximal (Fig. 4) and distal tendon level ($P < 0.05$). There was no side-to-side difference for deformation, strain, or Young's modulus based on proximal, mid-, or distal tendon CSA. Tendon stress and modulus were lower at the distal level compared with mid- and proximal tendon on both sides ($P < 0.01$). There was no difference in modulus when an average of the three levels of CSA was used (lead extremity 2.31 ± 0.29 GPa, nonlead extremity 1.99 ± 0.23 GPa).

## DISCUSSION

The present study examined PT size and mechanical properties in subjects who had a side-to-side difference in knee extensor strength as a result of habitual sport-specific loading. The main findings were that the lead extremity, which was on average 22% stronger than the nonlead extremity, had a greater distal and proximal PT CSA, which was not evident at the mid-tendon tendon level, indicating a region-specific tendon

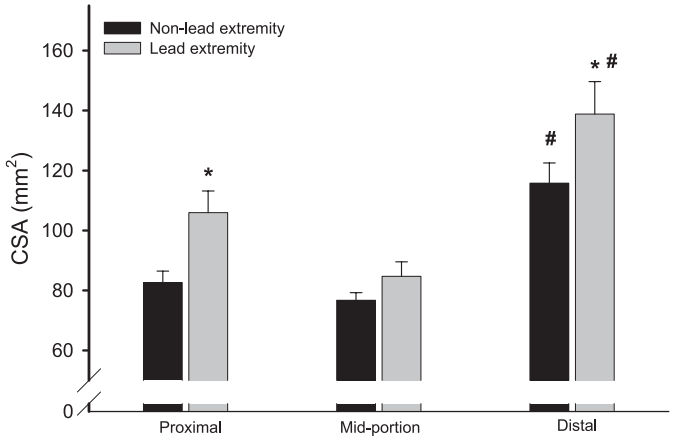


Fig. 2. Patellar tendon CSA distribution. Values are means ± SE. *There was a significant side-to-side difference in CSA at the distal and proximal level but not at the mid-tendon section, $P < 0.05$. #CSA was greater at the distal level compared with mid- and proximal tendon on both the lead extremity and nonlead extremity, $P < 0.05$.

Table 1. *Patella tendon mechanical properties based on maximum force*

| | Lead Extremity | Nonlead Extremity |
|---|---|---|
| Force, N | 6,886±833* | 5,832±631 |
| Deformation, mm | 2.8±0.3 | 3.1±0.4 |
| Stiffness, N/mm | 6,011±906* | 4,436±570 |
| Stress, MPa | | |
| Proximal tendon | 62.1±5.1* | 73.0±8.4 |
| Mid-tendon | 77.0±6.4 | 77.1±8.7 |
| Distal tendon | 47.9±4.8† | 50.8±3.9† |
| Strain, % | 5.5±0.5 | 6.1±0.6 |
| Modulus, GPa | | |
| Proximal tendon | 2.88±0.35 | 2.76±0.33 |
| Mid-tendon | 3.65±0.53 | 2.92±0.34 |
| Distal tendon | 2.22±0.35† | 1.98±0.28† |

Values are means ± SE. *Significantly different from nonlead extremity, $P < 0.05$. †Significantly different from mid- and proximal tendon, $P < 0.01$.

hypertrophy. Furthermore, the lead extremity displayed greater tendon stiffness without a significant difference in modulus, suggesting that the change in mechanical properties was largely the result of a change in size.

The present data show a tendon hypertrophy response associated with chronic loading as evidenced by the greater tendon CSA (20–28%) on the lead extremity. Although tendon tissue until recently has been considered basically inert, human in vivo data persuasively demonstrate that human tendon tissue is very responsive to single bouts of loading and chronic mechanical loading (4, 20, 21, 26). However, whether the elevated synthesis generates an increase in tendon size (hypertrophy) or an altered composition and a change in mechanical function remains largely unknown. Data from animal models show increased (1, 35, 39), decreased (39), or unchanged (5, 36) tendon size in response to endurance exercise. In humans, cross-sectional data show that endurance-trained persons have a larger Achilles tendon CSA compared with that of untrained persons (17, 24, 33). On the other hand, it has been shown that 9 mo of endurance training did not produce any measurable increase in the Achilles tendon CSA or mechanical properties (13). It is possible that duration of the endurance training may explain this apparent discrepancy.

Animal studies have shown a positive relationship between muscle strength/size and tendon CSA (8), which suggests that

Table 2. *Patella tendon mechanical properties based on common force*

| | Lead Extremity | Nonlead Extremity |
|---|---|---|
| Deformation, mm | 2.5±0.3 | 3.0±0.4 |
| Stiffness, N/mm | 4,766±716* | 3,494±446 |
| Stress, MPa | | |
| Proximal tendon | 52.9±4.8† | 66.0±8.0 |
| Mid-tendon | 65.6±5.6 | 71.2±8.2 |
| Distal tendon | 40.9±4.4*‡ | 46.6±4.4‡ |
| Strain, % | 5.0±0.5 | 5.9±0.6 |
| Modulus, GPa | | |
| Proximal tendon | 2.27±0.27 | 2.16±0.28 |
| Mid-tendon | 2.87±0.39 | 2.26±0.25 |
| Distal tendon | 1.79±0.25‡ | 1.54±0.19‡ |

Values are means ± SE. *Significantly different from nonlead extremity, $P < 0.05$. †Significantly different from nonlead extremity, $P < 0.01$. ‡Significantly different from mid- and proximal tendon, $P < 0.01$.

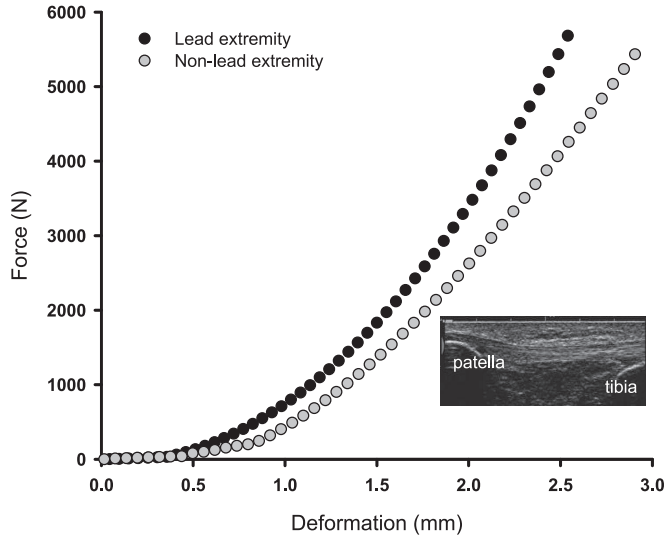


Fig. 3. Patellar tendon force-deformation relationship to a common force. Values are means of all subjects. Stiffness was higher on the lead extremity compared with the nonlead extremity ($P < 0.05$).

the magnitude of loading may also be an important variable with respect to tendon response and adaptation. It was recently shown (30, 31) that 14 wk of resistance training in elderly individuals that produced increases in muscle strength of up to 21% did not result in an increase in tendon CSA measured at the mid-tendon level. In contrast, a recent investigation showed that young men who engaged in resistance training for 12 wk that produced a 15% increase in strength and a 6% increase in muscle CSA had a significant increase (6%) in PT CSA. It is important to note that this tendon hypertrophy was observed in the distal and proximal portion but not in the middle of the tendon (18). Thus possible factors for the inconsistency between these studies include the age of the subjects and the location and method of tendon CSA measurement. The subjects of the present study were elite badminton players and

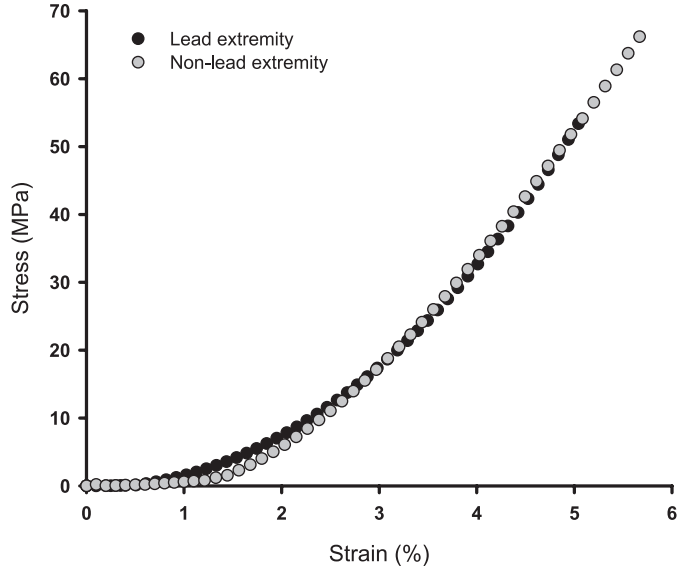


Fig. 4. Patellar tendon stress-strain relationship based on common force. Values are means of all subjects. Stress was lower on the lead extremity compared with the nonlead extremity at the proximal tendon level ($P < 0.05$).

fencers who had participated in their respective sport for several years. These sports involve a sport-specific loading whereby the athletes frequently perform rapid forward lunges with the preferred lead extremity, which places a considerable eccentric load on the knee extensors. In an elite badminton match, >70 such forward lunges may be performed on the lead extremity (C. Couppē, unpublished data). The sport-specific loading was substantiated by the sizeable side-to-side difference in strength (22%) and quadriceps CSA (7%), which was accompanied by a markedly greater tendon CSA (20–28%) of the lead extremity. The magnitude of the side-specific PT CSA (20–28%), and the lack thereof in recreational athletes, strengthens the notion that mechanical loading is associated with tendon hypertrophy as observed by Kongsgaard et al. (18). The magnitude of the CSA in the present study was similar to previous cross-sectional observations, suggesting that perhaps months to years of loading is required for tendon to hypertrophy, because only a smaller magnitude of tendon hypertrophy was observed after 12 wk of strength training in the Kongsgaard et al. study.

The data of the present study show that the PT on the lead extremity had a greater stiffness (36%) compared with the nonlead extremity (see Tables 1 and 2), whereas there was no significant difference in modulus. The load-associated increased in stiffness, and lack thereof in modulus, is in accordance with that recently found in young men following resistance training (18). However, others have demonstrated a marked increased in stiffness and in modulus after strength training in elderly individuals (30, 31). Although these diverging findings are difficult to reconcile, they may partly be related to age and the method of determining mechanical properties (14). The present findings and those of Kongsgaard et al. (18) suggest that the change in mechanical properties in young men is largely a function of increased in size, rather than a material change, although this needs to be confirmed.

The present data show that the lead extremity had a greater PT CSA in the distal (20%) and proximal region (28%) but not in the midsection of the tendon. Furthermore, there were no PT CSA side-to-side differences in recreational athletes. The magnitude of this region-specific human tendon hypertrophy confirms and reinforces previous cross-sectional data (17, 24, 33) and more recent training data (18). Albeit speculative, perhaps compression in these regions contributes to the synthesis of extracellular matrix proteins in tendinous tissue (25, 32). The midsection of the tendon had the narrowest CSA and thus a greater average stress (~25%) for a given load. Yet, in contrast to the proximal and distal tendon, the middle part of the tendon did not significantly increase in CSA as a result of the sport-specific loading. Interestingly, many PT clinical conditions occur at the insertions (9, 10) where the tendon CSA is larger and where it appears most likely to hypertrophy, and therefore it is doubtful whether whole tendon region specific average stress (and "safety factor") per se is the most important variable with respect to injury and adaptation. In contrast, the mid-tendon, which is rarely injured, did not appear to display a pronounced hypertrophy response, and it is therefore likely well designed for the imposed loads.

The knee extensor strength and tendon force of the nonlead extremity in the elite athletes in the present study are comparable to that of the recreational athletes examined herein and that reported by others (7, 18, 19). The PT deformation and strain in the present study (~3.1 mm, 5–6%) are also similar to previous reports (18, 38), but markedly different from that reported by others (~4.3 mm, ~9%) (7), which is likely related to dissimilarities in measurement methodology (14). In the present study, the average stress of the PT was 53–73 MPa. However, similar to previous reports (7, 29), the data show that the calculation of tendon stress will largely depend on where along the length of the tendon CSA is obtained. Nevertheless, the considerable variation in PT stress (30–91 MPa) reported in earlier studies (2, 14, 18, 29, 38) in the presence of reasonably comparable tendon forces (5,600–7,000 N) is most likely a function of both measurement methodology (ultrasonography and MRI) and analysis method when MRI is used. The modulus values reported herein are comparable to previous animal data on isolated tendon (6, 11, 15), but they are noticeably higher than previously reported human in vivo data (2, 18, 38). This discrepancy can most likely also be explained by the combination of differences in measuring deformation and CSA. It is noteworthy that the modulus at the proximal insertions is strikingly similar between the lead and nonlead extremity (Tables 1 and 2), which suggests that the sport-specific loading has not altered the material properties of the tendon, although this needs to be confirmed with other investigative techniques.

There are inherent limitations associated with the present study. The sample size was limited, which was in part a function of the study design and its inclusion criteria. Furthermore, the ultrasonography-based measurement technique does not permit assessment of region-specific deformation. This means that, although average stress can be calculated for specific location along the tendon, based on CSA, which varies considerably, modulus can only be estimated assuming heterogonous deformation and strain along the tendon, which remains unknown in humans, in vivo. Estimating regional modulus for the tendon yielded similar values for the proximal and distal tendon, although the values for mid-tendon approached significance ($P = 0.11$), and they may have attained significance with a larger sample. Furthermore, albeit not possible with this particular group of athletes, a study design, including tendon biopsies, may have provided some more insight into the composition of the tendons.

In summary, the present study examined region-specific PT size and mechanical properties in subjects who display a side-to-side strength difference of ≥15% due to persistent sport-induced loading over several years. These subjects were examined in an attempt to partly circumscribe issues such as *1*) training history, selection bias, and intersubject variations in cross-sectionally designed studies; *2*) the relatively short duration of earlier training studies; and *3*) the lack of assessment of region specificity in existing training studies. The data showed a regional variation in CSA along the PT, which markedly influenced average tendon stress. The habitually more loaded PT of the stronger extremity had a greater CSA compared with the contralateral side. The lead extremity also displayed greater stiffness than the contralateral side, whereas the modulus did not differ significantly. In sum, these data show that a habitual loading is associated with a robust change of the PT size and mechanical properties.

**GRANTS**

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# Mechanical properties and collagen cross-linking of the patellar tendon in old and young men

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[1]*Institute of Sports Medicine, Bispebjerg Hospital and Center for Healthy Aging, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark;* [2]*Department of Physical Therapy, Bispebjerg Hospital, Copenhagen, Denmark;* [3]*Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland; and* [4]*The University of Southern Denmark, Odense, Denmark*

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**Couppé C, Hansen P, Kongsgaard M, Kovanen V, Suetta C, Aagaard P, Kjær M, Magnusson SP.** Mechanical properties and collagen cross-linking of the patellar tendon in old and young men. *J Appl Physiol* 107: 880–886, 2009. First published June 25, 2009; doi:10.1152/japplphysiol.00291.2009.—Age-related loss in muscle mass and strength impairs daily life function in the elderly. However, it remains unknown whether tendon properties also deteriorate with age. Cross-linking of collagen molecules provides structural integrity to the tendon fibrils and has been shown to change with age in animals but has never been examined in humans in vivo. In this study, we examined the mechanical properties and pyridinoline and pentosidine cross-link and collagen concentrations of the patellar tendon in vivo in old (OM) and young men (YM). Seven OM (67 ± 3 years, 86 ± 10 kg) and 10 YM (27 ± 2 years, 81 ± 8 kg) with a similar physical activity level (OM 5 ± 6 h/wk, YM 5 ± 2 h/wk) were examined. MRI was used to assess whole tendon dimensions. Tendon mechanical properties were assessed with the use of simultaneous force and ultrasonographic measurements during ramped isometric contractions. Percutaneous tendon biopsies were taken and analyzed for hydroxylysyl pyridinoline (HP), lysyl pyridinoline (LP), pentosidine, and collagen concentrations. We found no significant differences in the dimensions or mechanical properties of the tendon between OM and YM. Collagen concentrations were lower in OM than in YM (0.49 ± 0.27 vs. 0.73 ± 0.14 mg/mg dry wt; $P < 0.05$). HP concentrations were higher in OM than in YM (898 ± 172 vs. 645 ± 183 mmol/mol; $P < 0.05$). LP concentrations were higher in OM than in YM (49 ± 38 vs. 16 ± 8 mmol/mol; $P < 0.01$), and pentosidine concentrations were higher in OM than in YM (73 ± 13 vs. 11 ± 2 mmol/mol; $P < 0.01$). These cross-sectional data raise the possibility that age may not appreciably influence the dimensions or mechanical properties of the human patellar tendon in vivo. Collagen concentration was reduced, whereas both enzymatic and nonenzymatic cross-linking of concentration was elevated in OM vs. in YM, which may be a mechanism to maintain the mechanical properties of tendon with aging.

tendon dimension; tendon mechanical properties; aging; collagen; hydroxylysyl pyridinoline; lysyl pyridinoline; advanced glycation end products

FORCE GENERATED BY MUSCLE is transferred to bones via tendons to produce movement. However, tendons are not entirely inextensible, but exhibit elastic and time-dependant properties that serve to influence the overall function of the muscle-tendon complex (3, 13, 17, 29, 58). Tendons have traditionally been considered relatively inert structures, but several recent reports have demonstrated that human tendons respond directly to physical activity by increased metabolic activity (15, 35, 42) and increased collagen synthesis (50, 51). Furthermore, strength training and habitual loading of tendons appear to be associated with increased tendon size (6, 21, 46), confirming that the aforementioned response to elevated loading results in a net increase of tendon tissue. These recent findings show that tendons respond and adapt to their specific loading history.

Aging is associated with a decline in muscle mass, strength, and physical function (66). However, although tendon properties influence the overall function of the muscle-tendon complex (58, 67), there is a relative lack of human data that describe possible age-associated changes in mechanical properties of tendon. Animal data show that aging yields a stronger and stiffer tendon (34, 69, 83), a weaker and more compliant tendon (27, 90, 91), or leaves the tendon unchanged (39); thus these data are inconclusive. In contrast, data on isolated human cadaver tendon suggest that the aging process largely leaves the mechanical properties unaltered (31, 40, 41). Despite the development of ultrasonography-based methods to evaluate human tendon properties in vivo (33, 37, 53), the effect of aging on the mechanical properties of human tendon in vivo remains elusive. Tendon strain in humans has been shown to decrease (47–49) or increase (43, 52, 61, 63, 70) with aging. Yet others have shown that aging leaves the mechanical properties of the patellar tendon unchanged (18). Thus the picture is incoherent, which may partly be related to methodological and design differences, the physical activity level of the sample population, and the type of tendon tested, i.e., the tendon-aponeurosis complex or the free tendon alone.

In tendon, the trivalent intermolecular pyridinoline cross-links [primarily hydroxylysyl pyridinoline (HP) and lysyl pyridinoline (LP)] stabilize the fibrillar structure of collagen and thus contribute to the mechanical properties of the tendon (7, 9, 12). These cross-links are formed from enzymatically derived covalent immature cross-links, which undergo a spontaneous conversion into more mature trivalent cross-links with collagen maturation (7). The slow turnover of mature collagen allows further cross-linking via the adventitious nonenzymatic reactions of glucose with the lysyl and arginine amino acid residues in the collagen triple helix as a true aging process (7, 59, 62). This nonenzymatic process results in the accumulation of advanced glycation end products (AGE) in tendon tissue. The most widely studied AGE is pentosidine. AGE accumulation is known to accelerate with aging and diabetes (16, 28, 62) and is believed to yield a stiffer and more load-resistant tendon (78, 79). It has been shown that AGE cross-link density in collag-

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enous tissues (19, 38, 81, 86) and in human tendons (11, 84) is markedly higher in older than in younger individuals, and there are only sparse human data on mature cross-link density of tendon and how these are influenced by aging (11). However, recent reports of an age-associated reduction in human tendon stiffness in vivo (43, 52, 61, 63, 70) in conjunction with the notion of simultaneously elevated cross-link density and AGE accumulation are difficult to reconcile. To the best of our knowledge, there are currently no human studies examining collagen cross-linking and mechanical properties of the patellar tendon in vivo in old and young men. Therefore, the purpose of this study was to examine both the mechanical properties of human patellar tendon in vivo and collagen cross-link composition in old (OM) and young men (YM) subjects.

## MATERIALS AND METHODS

*Subjects.* Seven OM (67 ± 3 years, 86 ± 10 kg) and 10 YM (27 ± 2 years, 81 ± 8 kg) with similar activity levels volunteered for the study (Table 1). With the use of a standardized form, participants were interviewed regarding how many hours per week they were performing organized sport or exercise on a regular basis (OM: 5 ± 6 h/wk, YM: 5 ± 2 h/wk). There were no differences in physical characteristics between OM and YM. All were healthy, did not take prescription medicine, and had no overt signs or symptoms of diabetes. In addition, the subjects had no known knee or tendon pathology. The study complied with the Declaration of Helsinki and was approved by the local ethics committee. All subjects gave their informed consent before the experiments.

*Muscle and tendon dimensions.* The anatomic cross-sectional area (CSA) of the quadriceps femoris muscle was measured 20 cm proximal to the tibia plateau (mid-thigh level) by magnetic resonance imaging (MRI) (General Electric, Sigma Horizon LX 1.5-Tesla, T1 weighted SE) using a lower extremity coil. The images were obtained using the following parameters: TR/TE = 500/14 ms, field of view (FOV) = 18, matrix = 512 × 512, and slice thickness = 6 mm (21, 46). Subsequently, the lean muscle mass of the quadriceps muscle (subcutaneous and intermuscular noncontractile tissues were excluded from the measurement) was manually outlined using the software program Osiris 4.19 (http://www.sim.hcuge.ch/osiris/). The mean value of three measurements of the same image was used for analysis. Patellar tendon CSA and length were determined with the use of MRI (General Electric, Sigma Horizon LX, 1.5-Tesla, T1 weighted SE) (21, 46). Patellar tendon CSA was determined by axial plane MRI using the following parameters: TR/TE = 400/14 ms, FOV = 20, matrix = 256 × 256, slice thickness = 5.0 mm, and spacing = 0 mm. The axial scans were performed perpendicular to the patellar tendon. As described in detail previously, the tendon CSA was measured *1*) just distal to the patellar insertion, *2*) just proximal to the tibia insertion, and *3*) midway between these two sites (21, 46). The patellar tendon length was determined from sagittal plane MRI using the following parameters: TR = 500, echo time (ET) = 3 × (TE: 12.4 ms), FOV = 16, matrix = 256 × 192, slice thickness = 4.0 mm, and no spacing. The patellar tendon length was obtained by measuring the distance from the dorsal insertion at the patella apex to the dorsal insertion on the tibia. Patellar tendon CSA and length were manually outlined using the software program Osiris 4.19 (http://www.sim.hcuge.ch/osiris/). The color intensity of each image was adjusted using the National Institutes of Health color scale mode of the software. Tendon CSA and length were measured using the grayscale image display. The average tendon CSA was calculated from the three levels (proximal, mid, and distal CSA) and used for analysis. The typical error percent of repeated measures of site-specific tendon CSA was 2–2.5% (21).

Table 1. *Physical characteristics of OM and YM*

| | OM ($n$ = 7) | YM ($n$ = 10) |
|---|---|---|
| Age, years | 67±3 | 27±2 |
| Height, cm | 178±9 | 183±4 |
| Weight, kg | 86±10 | 81±8 |
| Activity level, h/wk | 5±6 | 5±2 |

Values are means ± SD. There were no differences in physical characteristics between old men (OM) and young men (YM).

*Mechanical properties of tendon.* The details of the measurement, including the reliability of the method in our laboratory, has been reported previously (37). The within-day correlation coefficient and typical error percent results for repeated measures were 0.95 and 9.9% for tendon stiffness, 0.97 and 5.5% for tendon strain, and 0.94 and 9.4% for Young's modulus. Subjects performed a 5-min warm-up on a stationary bike to secure proper preconditioning of the tendon before testing. Thereafter, the subjects were seated in a custom-made rigid chair with both hips and knees flexed to an angle of 90°. A leg cuff, which was connected to a strain gauge (Bofors KRG-4, Bofors, Sweden) through a rigid steel rod perpendicular to the lower leg, was mounted on the leg just above the medial malleolus. An ultrasound probe (7.5 MHz, linear array B-mode; Sonoline Sienna, Siemens, Erlangen, Germany) was fitted into a custom-made rigid cast that was secured to the skin above the patellar tendon in the sagittal plane. The ultrasound probe and cast were positioned so that the patella, the patellar tendon, and the tibia were all visible within the viewing field throughout the ramped contractions (Fig. 1).

The ultrasound S-VHS video images obtained during the ramp trials were sampled at 50 Hz on a personal computer using frame-by-frame capturing software (Matrox Marvel G400-TV, Dorval, Quebec, Canada). Force was sampled on two separate personal computers at 50 Hz via a 12-bit analog-to-digital converter (dt2810A; Data Translation). The two computers were interconnected to permit synchronous sampling of all data using a custom-built trigger device (14). The subjects performed four to five slow isometric knee extensions ramps by applying gradually increasing force until maximum over a 10-s period during which patellar tendon displacement and knee extension force were measured simultaneously. Each ramp was separated by a 2-min rest period. All measurements were performed on one side, randomized to either the right or left knee. During the ramp contractions, force was sampled at 50 Hz and low-pass filtered at a 1.0-Hz cutoff frequency using a fourth-order zero-lag Butterworth filter.

Tendon force was calculated by dividing the estimated total knee extension moment by the internal moment arm, which was estimated from individually measured femur lengths (87). Tendon stress was calculated by dividing tendon force with the average of the three levels (proximal, mid, and distal) of the patellar tendon CSA determined from MRI. Tendon deformation was defined as the change in distance between the patellar apex and the tibia (37, 57). Tendon strain was calculated as the change in length related to the initial tendon length. Each single force-deformation curve was fitted to a second- or third-order polynomial fit, which yielded $R^2 > 0.98$. Tendon stiffness (Δforce/Δdeformation) and Young's modulus (Δstress/Δstrain) based on common force were calculated in the final 20% of the force-deformation and stress-strain curves, respectively (57). To compare tendon dimensions between the subjects of various body size, tendon CSA data were normalized to body weight and raised to the power of 2/3 (60).

*Patellar tendon biopsies.* A Bard MAGNUM biopsy instrument (C.R. Bard, Covington, GA) with a disposable core biopsy needle (14 gauge) was used. After sterilization, the skin was injected with local anesthetic (1% lidocaine), and a 3- to 5-mm-long incision was created just distal to the patella apex. The biopsy needle was inserted into the tendon surface at an ~30° angle and fired, securing a tissue sample of ~8 mg. Samples were snap-frozen in liquid nitrogen and stored at −80°C. Tendon biopsies were taken from the same side as tendon mechanical properties assessments were performed. No previous bi-

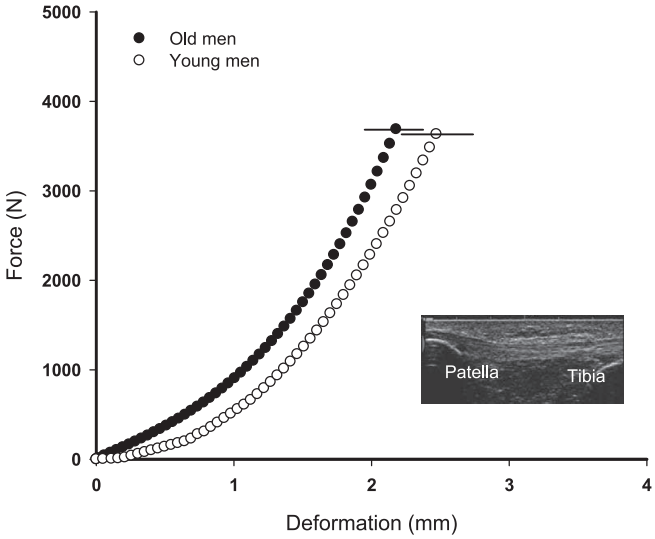


Fig. 1. The patellar tendon force-deformation relationship based on common force. Values are means ± SD of all subjects. There were no differences between old (OM) and young men (YM) with respect to tendon deformation or stiffness ($P > 0.05$).

opsy had been taken from that site in all subjects. All biopsy samples were analyzed in an investigator-blinded fashion.

*Biochemical analysis.* Freeze-dried tendon samples were hydrolyzed in 6 M HCl (+108 C, 24 h) and evaporated into dryness and dissolved in $H_2O$. Hydroxyproline, the collagen-specific amino acid, was measured spectrophotometrically (23) to quantify collagen protein (24). HP, LP, and pentosidine were analyzed via a single reversed-phase high-performance liquid chromatography (HPLC) run and detected on the basis of their natural fluorescence (10). At 0–16 min, the wavelength for HP and LP fluorescence was 400 nm for emission and 295 nm for excitation. The wavelengths were changed at 16–60 min to 328/378 nm to measure pentosidine. For the elution of the cross-links, a gradient was built up to contain 17% eluent B (75% acetonitrile with 0.13% heptafluorobutyric anhydride) at 0 min and 25% eluent B at 30 min. Eluent A was 0.13% heptafluorobutyric anhydride. Flow rate was 1 ml/min. HP was eluted at 12 min, LP at 13.5 min, and pentosidine at 23 min. The HPLC system used included Quaternary Gradient Pump unit, PU-2089 Plus, Intelligent AutosamplerAS-2057 Plus, and Intelligent Fluorescence Detector FP-2020 by Jasco. Data processing software was Jasco Chrompass. The LiChroCART 125-4 column was from Merck Hitachi. The results for HP, LP, and pentosidine are given compared with the standards injected at four different concentrations in each HPLC run. The intra-assay coefficient of variations based on duplicates within a run were 2.6%, 3.7%, and 3.9% for HP, LP, and pentosidine, respectively. The detection limit for HP and LP is 0.4 pmol and 0.05 pmol for pentosidine.

*Data reduction and analysis.* The two isometric ramp contractions that yielded the greatest maximum force were selected for further analysis. To make group comparisons and thereby account for differences in magnitude in isometric ramp contraction force, the trials for all subjects were subsequently analyzed to the lowest common force, as determined by the weakest subject (3953 N). Mann-Whitney *U*-tests were used to examine whether there were differences between the groups in the measured variables. Spearman rank-order correlation was used to analyze the strength of relationships between variables. $P < 0.05$ was considered significant. Results are reported as means ± SD.

## RESULTS

Peak knee extensor moment was lower in OM (154 ± 41 N·m) than in YM (216 ± 62 N·m) ($P < 0.05$). Similarly, quadriceps femoris CSA was lower in OM than in YM (Table 2) ($P < 0.01$).

Table 2. *Patellar tendon dimensions and quadriceps muscle CSA for OM and YM*

| | OM ($n$ = 7) | YM ($n$ = 10) |
|---|---|---|
| Tendon length, mm | 43±4 | 43±5 |
| Tendon CSA, mm$^2$ | 101±17 | 103±8 |
| Tendon CSA, mm$^2$/body wt$^{2/3}$ | 5.34±0.48 | 5.47±0.25 |
| Quadriceps muscle CSA, mm$^2$ | 6,214±705* | 8,431±522 |

Values are means ± SD. CSA, cross-sectional area. *Significantly different from YM, $P < 0.01$.

Tendon dimensions are shown in Table 2. Tendon length did not differ between YM and OM. Absolute average tendon CSA did not differ between OM and YM. Similarly, there was no between group difference in average tendon CSA normalized for body weight.

Mechanical properties determined at maximal force are shown in Table 3. Maximal tendon force was lower in OM than in YM ($P < 0.05$). There were no differences between OM and YM with respect to tendon deformation, stiffness, strain, stress, or Young's modulus based on average tendon CSA. Mechanical properties at a common force are shown in Table 4. Again, there were no differences between OM and YM for any of the variables (Figs. 1 and 2).

Collagen concentration and cross-link density data are shown in Table 5. Collagen concentration was lower in OM than in YM ($P < 0.05$). Both HP and LP ($P < 0.05$) as well as pentosidine ($P < 0.01$) concentrations in collagen were higher in OM than in YM. Pentosidine was positively related to age in YM ($r = 0.74$, $P < 0.01$, Fig. 3) but not in OM ($r = 0.65$, $P = 0.11$). There were no significant correlations between the mechanical and biochemical variables.

## DISCUSSION

To the best of our knowledge, this is the first study that has examined both the mechanical properties of the human patellar tendon in vivo and collagen cross-link densities in YM and OM with similar physical activity levels. The main findings were that the collagen concentration was lower in OM than in YM, whereas the enzymatically derived cross-links (HP and LP) were greater in OM than in YM. At the same time, the nonenzymatically derived AGE marker (pentosidine) was markedly more abundant in OM than in YM. However, despite these apparent age-related differences in the tendon collagen properties, the tendon mechanical properties in the two age groups did not diverge appreciably.

It is well known that age is associated with a loss in muscle mass and consequently a reduction in muscle function (66). It

Table 3. *Patellar tendon mechanical properties for OM and YM based on maximum force*

| | OM ($n$ = 7) | YM ($n$ = 10) |
|---|---|---|
| Force, N | 5,161±737* | 7,415±2184 |
| Deformation, mm | 2.6±0.4 | 2.9±0.9 |
| Stiffness, N/mm | 3,926±1091 | 5,546±1871 |
| Stress, MPa | 51±8 | 65±24 |
| Strain, % | 6.1±0.9 | 6.9±2.3 |
| Modulus, GPa | 1.7±0.3 | 2.2±0.7 |

Values are means ± SD. *Significantly different from YM, $P < 0.05$.

Table 4. *Patellar tendon mechanical properties for OM and YM based on common force*

| | OM ($n$ = 7) | YM ($n$ = 10) |
|---|---|---|
| Deformation, mm | 2.3±0.4 | 2.4±0.6 |
| Stiffness, N/mm | 3,511±837 | 3,290±869 |
| Stress, MPa | 41±7 | 37±5 |
| Strain, % | 5.3±0.9 | 5.9±1.7 |
| Modulus, GPa | 1.5±0.4 | 1.4±0.4 |

Values are means ± SD.

Table 5. *Concentration of collagen and hydroxylysyl pyridinoline, lysyl pyridinoline, and pentosidine cross-links in the patellar tendons of OM and YM*

| | OM ($n$ = 7) | YM ($n$ = 10) |
|---|---|---|
| Collagen, mg/mg dry wt | 0.49±0.27* | 0.73±0.14 |
| Hydroxylysyl pyridinoline, mmol/mol collagen | 898±172* | 645±183 |
| Lysyl pyridinoline, mmol/mol collagen | 49±38† | 16±8 |
| Pentosidine, mmol/mol collagen | 73±13† | 11±2 |

Values are means ± SD. *,†Significantly different from the values of YM ($P < 0.05$ and $P < 0.01$, respectively).

has also been suggested that tendon compliance and electromechanical delay increase with aging (80), which would reduce efficient transfer of contractile force and therefore amplify the age-associated decline in muscle function. However, to what extent the mechanical properties of human tendon change with aging remains unclear. Data based on animal models are disjointed as they suggest that aging reduces (27, 90, 91), augments (34, 69, 83), or leaves the mechanical properties unchanged (39). Similarly, data based on human in vivo models are inconclusive. These studies show that, with aging, the tendon becomes more compliant (43, 52, 61, 63, 70), less compliant (47–49), or remains unchanged (18, 43). In contrast, data on isolated human cadaver preparations consistently suggest that aging does not influence the mechanical properties (31, 40, 41), which is in agreement with the present data and that of Carroll et al. (18) on human patellar tendon in vivo.

The present mechanical data diverge from those of others based on the human in vivo model with one exception (18), and this may be related to methodological differences and study design. It has been shown that strength training and habitual loading pattern may result in tendon hypertrophy (6, 21, 46), which would influence the mechanical properties of the tendon (80). In contrast to previous in vivo studies (18, 43, 47, 52, 61, 63, 70), we have taken this aspect into account by comparing age groups with similar activity levels. However, it cannot be ruled out that prior life-long training history, including exercise mode and intensity, which was unaccounted for in the study, may have influenced the data. Furthermore, it has been shown that the ultrasonography-based method of obtaining patellar tendon deformation requires that the movement of the tibia also has to be considered (37, 71), which was achieved in the present study and in the study by Carroll et al. (18). Notably, the present data and that of Carroll et al. (18) cannot demonstrate any age-associated difference in mechanical properties of the patellar tendon. Moreover, several studies have investigated the effect of aging on the mechanical properties based on a composite measure of deformation that includes both that of the tendon and aponeurosis, rather than the tendon per se (43, 52, 61, 63, 70). This makes direct comparisons of results difficult since the free tendon and aponeurosis have dissimilar mechanical properties and because the stiffness of the aponeurosis can be modulated during contraction (30, 57). It should also be recognized that the present data and that of others are based on cross-sectional designs with inherent limitations, including the striking variations in tendon mechanical properties between subjects (54); therefore, a type II error cannot be ruled out.

The densities of mature lysyl oxidase-derived intermolecular covalent cross-links, such as HP and LP, gradually increase during tendon tissue maturation, and it is commonly believed that these cross-links are the chief contributors to the function and mechanical properties of the tendon (7, 9, 12, 76). In animal models, it has been shown that there is a positive

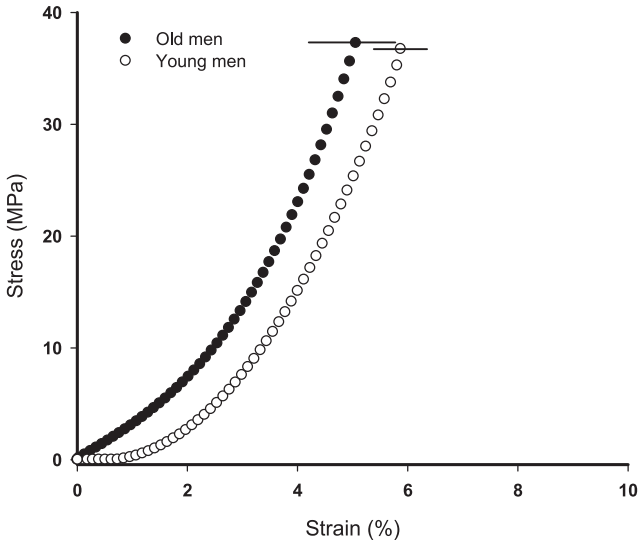


Fig. 2. The patellar tendon stress-strain relationship based on common force. Values are means ± SD of all subjects. There were no differences between OM and YM with respect to tendon strain, stress, or Young's modulus based on average tendon cross-sectional area ($P > 0.05$).

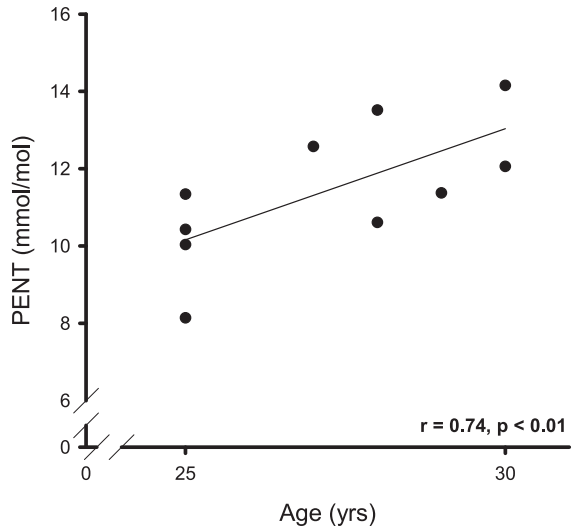


Fig. 3. Pentosidine was positively related to age in YM ($r = 0.74$, $P < 0.01$).

relationship between HP cross-link density and mechanical strength of the healing medial collateral ligament tissue and anterior cruciate ligament graft (32, 68) and furthermore that changes in HP and LP density will result in altered mechanical properties in collagenous tissue (7, 72). However, there are only sparse human data on mature cross-link density in tendon and how these are influenced by aging (11). A small but significant age-related increase of LP has been demonstrated in the supraspinatus tendon in vitro (11). The present data on LP and HP densities of YM correspond well with previously reported data in a similar population (45). However, the present data also demonstrate that HP and LP densities of the human patellar tendon were ~40% and threefold higher, respectively, in OM than in YM, suggesting a rather marked age-associated elevation in these enzymatic cross-links. It is noteworthy that, despite the rather robust difference in both HP and LP, there was no difference in the mechanical properties of the tendon. However, it should be noted that fibril length (22, 77), fibril diameter (74), and proteoglycans and glycosaminoglycans (44, 73, 75) have all been implicated in contributing to the tendon mechanical properties, and the relative contribution of these factors and that of mature cross-links remains elusive.

To our knowledge, these are the first data showing that AGE is markedly increased in the human patellar tendon of OM vs. in that of YM. AGE cross-links, including pentosidine, are formed when lysine amino acid residues in the collagen triple helix come into contact with glucose (7, 62), and the accumulation of these cross-links is known to accelerate with aging (7, 44) and disease processes such as diabetes (16, 28, 62), atherosclerosis (88), Alzheimer (20, 64), and renal failure (89). AGE products are also used as markers of tissue turnover (11). It has been shown that the difference between young and older individuals in AGE cross-link density in collagen is ~2-fold in human skeletal muscle (38), 5-fold in human bone (81), 7-fold in human tendon (present data), 9-fold in human ligaments (19), and 33-fold in human cadaver cartilage (86), demonstrating the tissue-specific turnover. The fact that pentosidine density is sevenfold greater in tendon of OM than in YM (Table 5), coupled with the fact that pentosidine appears to be related to age in a narrow age span (Fig. 3), firmly demonstrates the positive relationship between AGE cross-linking of human patellar tendon collagen and aging ex vivo. These data corroborate and extend those previously reported on cadaver tissue (11, 84). From a functional standpoint, an elevated AGE cross-link density has been suggested to result in increased tensile stress and tendon stiffness in animal models (4, 5, 8, 34, 78, 79, 85). However, in the present study, both the stiffness (Fig. 1) and the Young's modulus (Fig. 2) of the tendon did not differ between OM and YM despite the sevenfold difference in pentosidine, suggesting that factors other than AGE may also play a major role in determining the mechanical properties of human tendon.

In the present study, the total collagen concentration of the patellar tendon was ~34% lower in OM than in YM (Table 5), and this age-linked reduction is in accordance with that found in the canine patellar tendon (39) and rat tail tendon (90). It is possible that the lower collagen concentration with aging may represent the reduced size and/or density of collagen fibrils that is known to occur with aging (26, 65, 74, 76, 82). It was recently reported that MRI signal intensity of the patellar tendon was reduced with aging (18), which may be a function of the reduction in collagen concentration observed in the present study. Unfortunately, the size of the obtained biopsy in the present study precluded transmission electron microscopy analysis for fibril size and density. The lower collagen concentration in OM may be an age-related change in the tendon per se and/or a function of reduced tendon loading due to an age-related loss of muscle mass/strength. Interestingly, the average whole tendon CSA did not differ between OM and YM (Table 2), which in light of the reduced collagen concentration may be related to increased amounts of other extracellular matrix components, such a proteoglycans and glycosaminoglycans. Alternatively, the retained tendon CSA in OM may result from tendon intrafibrillar fat that can accumulate with aging (1, 2, 25). It has previously been demonstrated that the Achilles tendon CSA is larger in postmenopausal women than in young women (55), but it should be noted that female hormones may significantly impact collagen synthesis (36, 56). This potential sex-dependent factor is the reason that we have undertaken the present study in men only.

Albeit speculative, it is possible that the elevated enzymatic and/or nonenzymatic cross-link density in OM vs. that shown in YM served to maintain tendon stiffness and Young's modulus despite the diminished collagen concentration. Such maintenance of tendon stiffness would serve to maintain effective transfer of muscle force despite a lower absolute muscle size (Table 2) and strength (Table 3). In this context, it should be noted that the present data were obtained in moderately physically active individuals, and future studies will need to address the effect of training per se in elderly.

In conclusion, results from the present study raise the possibility that the dimensions and mechanical properties of the human patellar tendon in vivo may not differ between OM and YM. On the other hand, the OM group displayed lower collagen concentration, but greater enzymatic (HP and LP) and nonenzymatic (pentosidine) collagen cross-links, than YM. This age-related increase in both enzymatic and nonenzymatic cross-linking compounds may serve to maintain the mechanical properties of tendon with aging.

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# The effects of immobilization on the mechanical properties of the patellar tendon in old men

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## Abstract

It remains unknown if inactivity changes the mechanical properties of the human patellar tendon in old healthy persons. **Purpose**: To examine the effects of short-term unilateral immobilization on the structural and mechanical properties of the patellar tendon in old men (OM), *in vivo*. **Methods**: Eight OM (67±4 yrs, 87±10 kg) with a physical activity level (5±6 hrs/wk) underwent 14 days of unilateral immobilization. They were assessed on the immobilized and control side before and after the intervention. Peak knee extensor moment was assessed during isometric MVC. MRI was used to assess whole patellar tendon dimensions. The mechanical properties of the patellar tendon were assessed using simultaneous force and ultrasonographic measurements during isometric ramp contractions. **Results:** Peak knee extensor moment decreased on the immobilized side ($P<0.05$), while a trend ($P=0.078$) was observed for the control side. On the immobilized side, tendon stiffness (Pre: 2949±799 vs. Post: 2366±774 N mm$^{-1}$, $P<0.01$) and Young´s Modulus (Pre: 1.2±0.3 vs. Post: 1.0±0.3 GPa, $P<0.05$) declined with immobilization. On the control side, tendon stiffness (Pre: 3340±1209 vs. Post: 2230±503, $P<0.01$) and Young´s Modulus (Pre: 1.5±0.4 vs. Post: 0.9±0.3, GPa, $P<0.05$) also decreased with immobilization. **Conclusion:** The data show that the mechanical properties of the patellar tendon decreased on both sides. A decline in stiffness and Young´s modulus were observed without any sizable changes of the tendon, suggesting that the material properties of the tendon were affected by unloading. These findings indicate that short-term inactivity has a substantial negative influence on the mechanical properties of the human patellar tendon in old men.

## Introduction

Human locomotion comes about when force created by contracting muscle is transmitted to the bone via aponeurosis and tendon, and the viscoelastic properties of the tendon will influence the overall function of the muscle–tendon complex (2; 8; 12; 21; 44). In contrast to previous belief, recent data show that in vivo human tendon tissue is not inert but rather responsive to both single bouts as well as habitual mechanical loading (10; 36; 37; 49). In fact, strength training and habitual exercise loading of tendons appear to be associated with an increase in tendon size and stiffness, respectively (4; 16; 32; 58). These data demonstrate that tendons respond and adapt to loading history.

It is well known that immobilization results in a rapid loss of muscle volume and strength, which will negatively influence muscle function (44; 52; 62). The effects of immobilization on the mechanical tendon properties of the tendon have been studied extensively in animal models (11; 63), and several studies show a decline of the mechanical properties of the tendon (22; 39; 47; 48; 56; 67; 68), while some demonstrate the opposite (6; 20). Human studies also suggest that the tendon undergoes a substantial reduction in the mechanical properties with immobilization (17; 33; 35; 54; 59; 60; 69), which have been principally attributed to changes in material rather than size (17; 33; 35; 54; 59; 60; 69). However, the aforementioned effects of immobilization on the mechanical properties of tendon have mainly been investigated in young persons, and thus it remains unknown if similar or more dramatic changes may occur in older healthy persons.

The purpose of the present study was therefore to examine the effects of short-term unilateral immobilization on the human patellar tendon structural and mechanical properties in old men (OM), in vivo.

## Materials & methods

*Subjects and design*

Eight old men (OM) (67±4 yrs, 87±10 kg, 178±8) with an activity level of 5±6 hrs/wk volunteered for the study. All were healthy, did not take prescription medication, had no overt signs or symptoms of diabetes, and had no known knee or tendon pathology. The study complied with the Declaration of Helsinki and was approved by the local Ethics Committee. All subjects gave their informed consent prior to the experiment.

*Immobilization*

All subjects had one lower limb randomly assigned to an immobilization intervention that consisted a 30 degree knee flexion full lightweight fiber leg cast from above the malleoli to the groin for 2 weeks. This model has earlier been shown to effectively prevent walking ability and induce significant muscle atrophy in short-term immobilization studies (26-28; 62). The contralateral limb served as a control. The subjects were instructed to use crutches, avoid ground contact with the casted leg and not to activate the muscles of their casted side during the 2 week study period. All subjects were carefully instructed to actively perform plantar flexor and extensor contractions several times a day in order to prevent potential venous thrombosis. All measurements were performed before and after the immobilization period on both sides.

*Tendon dimensions*

Patellar tendon cross-sectional area (CSA) and length were determined with the use of MRI (General Electric, Sigma Horizon LX 1.5 Tesla, T1 weighted SE) (16; 32). Patellar tendon CSA was determined by axial plane MR using the following parameters: TR/TE 400/14 ms, FOV 20, matrix 256x256, slice thickness 5.0 mm and spacing 0 mm. The axial scans were performed perpendicular to the patellar tendon. As described in detail elsewhere, the tendon CSA was measured I) just distal to the patellar insertion, II) just proximal to the tibia insertion and III) midway between these two sites (16; 32) . The patellar tendon length was determined from sagital plane MRI using the following parameters: TR 500, ET: 3 x (TE: 12.4 ms), FOV 16, matrix 256x192, slice thickness 4.0 mm and no spacing. Patellar tendon length was obtained by measuring the distance from the dorsal insertion at the patella apex to the dorsal insertion on the tibia. Patellar tendon CSA and length were manually outlined using the software program Osiris 4.19 (http://www.sim.hcuge.ch/osiris/). The color intensity of each image was adjusted using the NIH color scale mode of the software. Tendon CSA and length was measured using the gray scale image display. Average tendon CSA was calculated from the 3 sites (proximal, mid and distal CSA) and was used for analysis (15). The typical error percent of repeated measures of site-specific tendon CSA was 2-2.5 % (16). The MRI investigator was blinded with regards to side.

*Maximal voluntary contraction (MVC)*

Maximal voluntary strength was determined by isometric knee extension efforts. As described in detail elsewhere (9) the subjects were seated in a custom made rigid chair with both hips and knees flexed to an angle of 90°. A leg cuff was connected to a strain gauge (Bofors KRG-4, Bofors, Sweden) through a rigid steel rod perpendicular to the lower leg and was mounted on the leg just above the medial malleolus. The tibia moment arm was measured (from the point of fixation to the lateral epicondyle of the knee) to calculate the knee extensor moment. Force was sampled on a PC at 50 Hz via a 12-bit A/D converter (dt 2810A, Data Translation, MA, USA). The subjects performed 4-5 maximal voluntary isometric knee

extension contractions (MVC) separated by one min (9). The tests were conducted on both sides.

*Mechanical properties of tendon*

The details of the measurement, including the reliability of the method in our laboratory, have been reported previously (23). The within-day correlation coefficients and typical error percent for repeated measures were 0.95 and 9.9% for tendon stiffness, 0.97 and 5.5% for tendon strain, and 0.94 and 9.4% for Youngs Modulus, respectively. Subjects performed a 5-min warm-up on a stationary bike (~50 W) in order to secure proper preconditioning of the tendon prior to testing. Thereafter the subjects were seated in a custom made rigid chair with both hips and knees flexed 90°. A leg cuff, which was connected to a strain gauge (Bofors KRG-4, Bofors, Sweden) through a rigid steel rod perpendicular to the lower leg, was mounted on the leg just above the medial malleolus. An ultrasound probe (7.5 MHz, linear array B-mode, Sonoline Sienna, Siemens, Erlangen, Germany) was fitted into a custom made rigid cast that was secured to the skin above the patellar tendon in the sagital plane. The ultrasound probe and cast was positioned so that the patella, the patellar tendon and the tibia were all visible within the viewing field throughout the ramped contractions (Figures 1 & 2).

Ultrasound S-VHS video images of the patella tendon and its bony attachments were sampled during the ramp trials at 50 Hz on a PC using frame-by-frame capturing software (Matrox Marvel G400-TV, Dorval, Canada). Deformation images and force signals were sampled (50 Hz) on two separate PC´s, for the latter using a 12-bit A/D converter (dt 2810A, Data Translation, MA, USA). The two computers were inter-connected to permit synchronous sampling of all data using a custom-built trigger device (9) . The subjects performed 4-5 slow isometric knee extensions ramps by applying gradually increasing force until maximum over a 10 s period during which patellar tendon displacement and knee extension force were measured simultaneously. Each ramp contraction was separated by a 2-minute rest period. All measurements were performed on both sides, before and after the period of immobilization. All force signals were low-pass filtered at a 1.0 Hz cutoff frequency using a $4^{th}$ order zero-lag Butterworth filter.

Patella tendon force was calculated by dividing the estimated total knee extension moment by the internal moment arm, which was estimated from individually measured femur lengths (66). Tendon stress was calculated by dividing tendon force with the average patellar tendon CSA determined from MRI (proximal, mid, distal sites). Tendon deformation was defined as the change in distance between the patellar apex and the tibia (23; 43). Tendon strain was calculated as the change in length related to the initial tendon length ($\Delta L/L_0$). Polynomial functions ($2^{nd}$ or $3^{rd}$ order) were fitted to each single force-deformation curve, which yielded $r^2>0.96$. Tendon stiffness ($\Delta$force/$\Delta$deformation) and Young's modulus ($\Delta$stress/$\Delta$strain) were calculated for each side within each person in the final 10% (maximum common force of both legs) of the force-deformation and stress-strain curves respectively (43).

*Data reduction and analysis*

The two isometric ramp contractions that yielded the greatest maximum force were selected for further analysis. In order to make comparisons before and after immobilization for the same subject and thereby account for differences in magnitude in isometric ramp contraction force, the trials (before and after immobilization) were subsequently analyzed to the maximum common force. Wilcoxon signed rank test were used to examine if there were differences between pre and post immobilization values for the measured variables. An alpha level of $P<0.05$ was considered significant. Results are reported as mean±SD.

**Results**

There were no side-to-side differences for any of the measured variables prior to immobilization. Peak knee extensor moment decreased on the immobilized side (143±25 vs. 119±26 Nm, $P<0.05$). A statistical trend was observed toward a decrease in peak knee extensor moment (152±32 vs.139±39 Nm, $P=0.078$) on the control side.

Tendon dimensions are shown in Table 1. There were no changes with respect to tendon length or absolute tendon CSA on the immobilized or the control side.

| (n = 8) | Immobilized side | | Control side | |
|---|---|---|---|---|
| | Pre | Post | Pre | Post |
| Tendon length (mm) | 43±3 | 43±3 | 43±6 | 43±3 |
| Tendon CSA ($mm^2$) | 101±11 | 101±11 | 99±15 | 102±16 |

**Table 1.** Patellar tendon dimensions for the immobilized side and control side. Values are mean ± SD. There were no differences in patellar tendon dimensions between Pre and Post immobilization.

Mechanical properties assessed at maximum common force are shown in Table 2. Tendon stiffness decreased both on the immobilized side ($P<0.01$) (Figure 1) and on the control side ($P<0.01$) (Figure 2). Likewise, Young´s Modulus decreased on the immobilized side ($P<0.05$) (Figure 3) and on the control side ($P<0.05$) (Figure 4). There were no other differences with respect to tendon deformation, strain or stress on either the immobilized side or the control side.

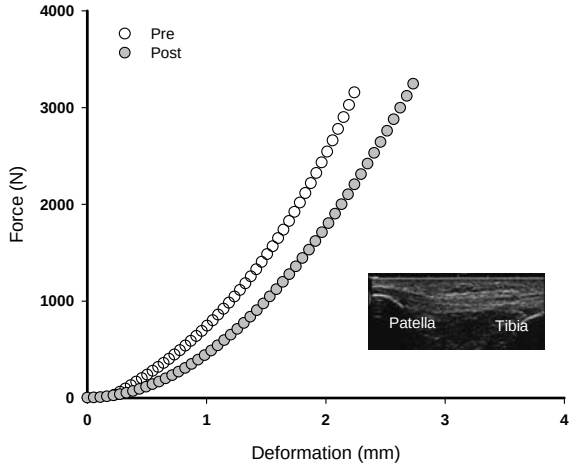


**Figure 1.** *The patellar tendon force-deformation relationship based on common force for the immobilized side. Values are means; SD values are stated in Table 2. Stiffness decreased after 14 days of immobilization (Pre vs. Post, P<0.01).*

## Discussion

To the best of our knowledge this is the first study that has examined the effects of short-term unilateral immobilization on the structural and mechanical properties of the human patellar tendon, *in vivo*, in old healthy individuals. The main findings were that the mechanical properties of the patellar tendon decreased on the immobilized side. A decline in stiffness and Young´s modulus took place without any measurable change in the size of the tendon, suggesting that the material properties of the tendon were primarily affected by the unloading.

It is well known that the immobilization associated drop in muscle strength is greater than that of muscle mass, and that both contribute to a reduced muscle function (44; 52; 62). The larger drop in muscle strength has mainly been attributed to rapid changes in the nervous system that occur with short term unloading (19; 40; 41; 52). These changes seem more accelerated in older than young persons (61). Interestingly, recent studies report that unloading affect tendon properties to a greater extent than muscle loss, which in turn may contribute to a decline in electromechanical delay (the time lag between muscle activation and muscle force production) and reduced rate of force development (7; 17; 33; 34; 52). Nordez et al. (53) reported that the tendon stretching contributed with ~30% of the total electromechanical delay (44; 52). Thus, it appears that tendon properties play a major role in the function of the muscle–tendon complex. The present data corroborate with these earlier findings and further show that the functional effect on the muscle-tendon complex is likely similar in old persons.

Most animal immobilization studies show reduced tendon stiffness without a change in tendon size indicating that the material properties of the tendon are affected by disuse (22; 39; 47; 48; 56; 67). However, some have shown opposite results (6; 20), which may be related to the use of different models, tendons and durations of immobilization. With a single exception (42), human data in young individuals show that mechanical properties of the tendon decrease with both short-term (17; 34; 35; 54; 59; 60) and long-term (54; 69) unloading in the absence of tendon atrophy, suggesting that the material properties of the tendon were primarily affected. In the present study similar findings were demonstrated for the first time in vivo for aging human individuals. Thus, 14 days of immobilization led to decreased tendon stiffness on the immobilized side, which was accompanied by a decrease in Young´s Modulus. This decline occurred without changes in tendon dimensions, indicating that the material properties of the patella tendon were altered for our old subjects, which is in line with previous reports in young persons (17; 33; 34; 59; 60).

| (n = 8) | Immobilized side | | Control side | |
|---|---|---|---|---|
| | Pre | Post | Pre | Post |
| Deformation (mm) | 2.3±0.5 | 2.7±0.8 | 2.3±0.4 | 2.8±0.6 |
| Stiffness (N mm$^{-1}$) | 2949±799 | 2366±774** | 3330±839 | 2365±436** |
| Stress (MPa) | 32±7 | 33±10 | 34±8 | 32±8 |
| Strain (%) | 5.5±1.6 | 6.6±2.2 | 5.3±0.7 | 6.5±1.7 |
| Modulus (GPa) | 1.2±0.3 | 1.0±0.3* | 1.5±0.4 | 1.0±0.3* |

**Table 2.** Patellar tendon mechanical properties for the immobilized side and control side based on common force. Values are mean ± SD. Significantly different from Pre, *P<0.05, **P<0.01.

De Boer et al. (17) found that quadriceps strength dropped ~15% and that the mechanical properties of the patella tendon declined ~10% after 2 weeks of immobilization in young men. After an additional 9 days of unloading quadriceps strength declined with a total ~20% and the mechanical properties of the patellar tendon with a total ~30%. There was an associated ~40% reduction in the rate of force development after the 14 days of unloading, which remained unchanged after 23 days. Furthermore, it has been shown in young and middle –aged men that the mechanical properties of the achilles tendon decreased up to ~58% with 90 days of immobilization (54). Collectively these data demonstrate that short-term limb unloading has considerable effects on the mechancial properties of the tendon that to some extent mirrors that of the muscle, and that these changes can be expected to occur in the early phase of unloading. In the present study we extended these findings by showing that muscle tendon mechanics are also affected by acute disuse in the elderly, and that the magnitude of change is perhaps even greater.

The biochemical changes within the tendon due to immobilization are still largely unknown (31; 55). In animal studies unloading causes a decrease

in collagen synthesis (29; 57), collagen concentration (24; 65) and the ultrastructure (fibril size/ density (50; 51). However, others have found that collagen concentration (1; 3; 57), ultrastructure (70) and tendon tissue mass (25) remain unchanged by unloading. In humans, ~2 weeks (14) and ~7 weeks of unloading (13) did not seem to affect collagen synthesis in the achilles tendon, whereas collagen synthesis rate decreased in the patellar tendon after 10-23 days of immobilization (18), suggesting that differentiated responses may be observed between anatomically distinct tendons. Interestingly, in the latter case the decreased synthesis rate was accompanied by an almost similar reduction in tendon stiffness, Young´s Modulus while tendon CSA remained unchanged, indicating that the material properties of the tendon was altered, which is line with the present findings. It should be noted that degradation and catabolic enzymes was not evaluated in the study of De Boer et al(18), and these parameters have been shown to increase with loss of tendon tension (5; 38). The observed decrease in tendon stiffness could well be associated with increased collagen degradation. The results obtained in our study, together with those of De Boer et al. (18), suggests, that the detoriating effects of unloading are rapidly evoked both in old and young individuals.

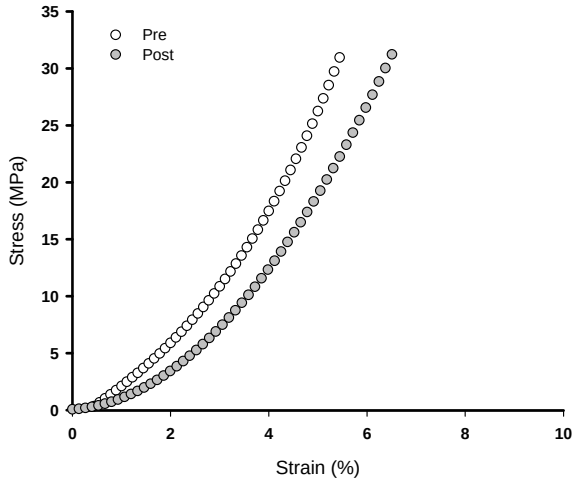


***Figure 3.*** *The patellar tendon stress-strain relationship based on common force for the immobilized side. Values are means; SD values are stated in Table 2.* Young´s *Modulus decreased after 14 days of immobilization (Pre vs. Post, P<0.05).*

It has been proposed that the degree of tension on the patellar tendon during immobilization could influence the tendon biomechanical properties quite dramatically within one week (45). Animal studies show that if the tendon is left in a unloaded (shortened) position during 7 days of immobilization, enzyme activity of the collagen synthesis is decreased (29; 57), potentially leading to altered tendon composition and weakened mechanical properties (3). In contrast, if the tendon was kept in a neutral or chronically loaded (elongated) position while immobilized, the decrease in collagen synthesis enzyme activity was only minor or even fully abolished (29; 57). The present study did not investigate possible biochemical changes, and it should be kept in mind that the immobilized knee was positioned in a relatively shorten position (~30° knee flexion), which likely is associated with no or only very minor passive tension in the patella tendon (43). The acute lack of tensile tendon input during the immobilization period might have influenced the material properties of the tendon, since tendon stiffness and modulus decreased without tendon atrophy. Unloading tenocytes have been shown to have severe effects on collagen expression, proteoglycan expression, growth factor secretion and metalloproteinases (5; 38). Furthermore, it has recently been demonstrated that complete tension deprivation in the rabbit patellar tendon induced fibroblast apoptosis within 24 hrs (30). Thus, it is possible that the removed tension development in the patella tendon was the initiating event for the present findings of reduced tendon stiffness and Youngs Modulus.

The unchanged tendon size due to immobilization is in agreement with numerous other studies (14; 17; 33; 34; 54; 60; 69). It has been reported that there is a decrease in the number and in average diameter of collagen fibrils as result of immobilization (46; 50; 51), but it has also been shown that the fibril to fibril distance increases with immobilization compared to control tendons, suggesting a less fibril dense tendon (51). It is possible that the increased fibril to fibril distance represents greater relative content of intersitial water and/or other extracellular matrix components while whole tendon CSA remains unchanged (64; 68) as proposed by others (18). Such a mechanism would explain the altered material properties but unchanged CSA in the present study.

A number of limitations may be observed with the present study. Unfortunately, we were unable to obtain tendon biopsies in the present study, which would have allowed us to address possible changes in tendon composition. Furthermore, to our surprise changes occurred in the non-immobilized limb, which rendered it inadequate as a control. Albeit non-significant, muscle strength tended ($P$=0.078) to decrease on the non-immobilized side. Furthermore, both stiffness and modulus also decreased on the non-immobilized side. Thus, although the data show that the non-immobilized side was not an ideal control in this sample, the data suggests that muscle strength changes are accompanied by changes in tendon properties. A possible explanation for the findings in the control limb is that unilateral casting and crutch walking in old persons may influence the overall activity level.

In conclusion, a decline in stiffness and Young´s modulus was observed following short-term (14 days) unilateral limb immobilization in old men without any changes in tendon size, suggesting that the material properties of the tendon were affected

by unloading. These findings indicate that short-term inactivity has a major negative influence on the mechanical properties of the human patellar tendon in the elderly.

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