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Perspective Article

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Metabolic activity and collagen turnover in human tendon in response to physical activity

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Abstract

Connective tissue of the human tendon plays an important role in force transmission. The extracellular matrix turnover of tendon is influenced by physical activity. Blood flow, oxygen demand, and the level of collagen synthesis and matrix metalloproteinases increase with mechanical loading. Gene transcription and especially post-translational modifications of proteins of the extracellular matrix are enhanced following exercise. Conversely, inactivity markedly decreases collagen turnover. Training leads to a chronically increased collagen turnover, and dependent on the type of collagen also to some degree of net collagen synthesis. These changes modify the biomechanical properties of the tissue (for example, viscoelastic characteristics) as well as the structural properties of the in collagen (for example, cross-sectional area). Mechanical loading of human tendon does result in a marked interstitial increase in growth factors that are known potentially to stimulate synthesis of collagen and other extracellular matrix proteins. Taken together, human tendon tissue mounts a vigorous acute and chronic response to mechanical loading in terms of metabolic-circulatory changes as well as of extracellular matrix formation. These changes may contribute to training-induced adaptation of biomechanical properties consisting of altered resistance to loading and enhanced tolerance to strenuous exercise. Understanding of such changes is a pre-requisite in the development of measures aimed at prevention of overuse tendon injuries occurring during sport, work or leisure-related activities.

Keywords: Extracellular Matrix, Mechanotransduction, Physical Training, Mechanical Properties, Intramuscular Connective Tissue

Introduction

For skeletal muscle cells to function adequately they need to be attached to each other as well as to the bone[1]. The tendon serves as an important mediator of force transmission. The fibrillar arrangement of the tendon allows for passive energy absorption and loading[2]. It is well recognized that the tensile strength of the matrix is based on intra- and intermolecular cross links, the orientation, density and length of the collagen fibrils and fibers. However, little is known about the signals triggering connective tissue cells to respond to mechanical loading, and about the subsequent expression of specific extracellular matrix proteins[3]. Thus far, the adaptation of tendon connective tissue to loading has been thought to be of limited magnitude, and requiring prolonged stimulation to create even moderate tissue changes[4]. However, it is known that metabolism of collagen and the connective tissue network responds dynamically to altered levels of physical activity For example, reduced activity leads to diminished biosynthesis of connective tissue components[5]. Conversely, exercise accelerates formation and degradation of connective tissue in both muscle and tendon, and may reflect both physiological adaptation and repair of damage of extracellular matrix structures[5,6,7].

Tendon collagen content and organization

Collagen as the predominant component of extracellular matrix constitutes about 20-25% of total body protein[8]. Type I and III collagens are the most abundant tendon collagens and are produced principally by fibroblasts on the mem-

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Content of collagen and pyridinoline in human tissue

Tissue	Collagen (nmol/g wt)	Pyridinoline (nmol/g wt)	Pyr/collagen (mmol/mol)
Tendon	677+57	370+124	547+183
Ligament	510+84	208+60	408+117
Bone	307+71	60+21	197+67
Skeletal muscle	59+17	22+5	376+91
Dermis	335+64	5+4	16+12
Liver	39+18	16+4	405+89
Lung	108+25	22+4	202+34
Heart	25+5	10+5	410+209
Intestine	161+27	64+15	396+94
Kidney	87+30	26+7	302+82
Arteries (aorta)	295+92	59+10	201+34

Table 1. Total collagen as determined from the hydroxyproline concentration (collagen(mol) = (hydroxyproline(g) x 7.5)/300.000) and pyridinoline (Pyr) as determined by extraction from collagen hydrolysates of powdered aliquots from specific tissues are expressed in nmol/g wet tissue (wt). It is evident that due to the amount of total tissue in the body, bone and dermis will contribute largely to the amount of collagen, whereas skeletal muscle will comprise a large amount of total pyridinoline content.

brane-bound ribosomes of the rough endoplasmic reticulum and secreted within the extracellular matrix[4]. Collagen synthesis and degradation are measured indirectly from determination of increases or decreases in steps of collagen biosynthesis. Procollagens contain amino terminal and carboxy terminal extension peptides at the respective ends of the collagen molecule. After secretion the amino-propeptides are cleaved by specific proteinases and the collagens self-assemble into fibrils or other supramolecular structures. The release of procollagens and collagen turnover have been studied extensively in the bone, but only recently have similar studies been initiated in the tendon[7]. Following the transcription of genes coding for type I collagen, the pro-α chains undergo marked post-translational modifications. First hydroxylation leads to converting proline residues to 4-hydroxyproline or 3-hydroxyproline by three different hydroxylases. Proline and 4-hydroxyproline promote the formation of intermolecular cross links. The 4-hydroxyproline formation is catalysed by prolyl-4-hydroxylase (PH), an enzyme with activity limited to collagens. Levels of PH activity generally increase and decrease with the rates of collagen biosynthesis, and assays of the enzyme activity have traditionally been used for estimating changes in the rate of collagen biosynthesis in various experimental and physiological conditions[9-17].

Determination of pyridinoline (Pyr) has shown that the content of Pyr is especially high in tendons and ligaments, and that the Pyr to collagen ratio is high in the tendon as compared to that in bone[18] (Table 1). This is compatible with a central role of cross links in biomechanical properties in these structures.

Previous studies of properties of tendon as well as collagen metabolism have been limited to *in vitro* studies and biomechanical studies on tissues from human cadavers. They led to a better understanding of basic mechanisms, but provided only limited insight into *in vivo* changes in the human body during exercise[19] (Table 2). For example, overuse of tendon structures has been described to occur as a result of sport activities and due to occupational overloading. It is often associated with inflammation and structural changes. Only a few animal models have been established as a reliable tool to evaluate overloading of connective tissue. Because these models often undergo extreme loading resulting in severe tissue damage, they bear little similarity with real life situations in people who typically develop overuse injuries gradually, over a period of time. Another drawback inherent to many studies based on *in vitro* tissue sampling is that they do not allow for continuous measurements during exercise. Whereas this might be considered of lesser importance with regard to collagen formation and degradation, it is of the utmost importance in situations, e.g., exercise, where changes in enviromental blood flow, tissue oxygenation and metabolism may contribute to

Studies of collagen and its properties in tendon and skeletal muscle with relation to physical activity

	In vitro	*In vivo*
Collagen turnover	Cultured cells Retractory collagen gels Tissue stainings and analysis Isotope incorporation (HyPro)	Isotopes (stable) + biopsy Microdialysis
Mechanical function	Material testing (fibrils, whole tendons)	Ultrasound stress-strain Dynamic MR-scanning
Blood flow/metabolism	Microsphere	Near infrared spectroscopy PET-scanning
Inflammation	Biopsy analysis	Microdialysis

Table 2. Examples of methodologies and models used to perform *in vitro* and *in vivo* studies within collagen metabolism and properties. Several other methodologies are described, but the table emphasizes the increasing availability of *in vivo* techniques that can be utilized during exercise.

overloading during exercise or other activities.

Several *in vitro* systems for determination of cellular responses to mechanical tensile stress are available at the present time[20]. In one of them, cells are seeded upon a flexible substrate which allows for easy control of strain, however, actual forces exerted upon individual cells cannot be accurately quantified[21]. An alternative method to study cellular responses to loading is to grow fibroblasts on native three-dimensional collagen matrices to which they attach. Though this method allows determination of tensile forces developed by pulling on collagen fibrils, it inadequately defines ensuing strain. Fibroblast cell cultures subjected to biaxial mechanical loading often produce uneven strain, and thus the results obtained from cell cultures represent only an average of these ranges of movements[22]. *In vitro* systems enable localized measurements and intervention. Recently developed *in vivo* human models allow real-time determinations of metabolism, blood flow, inflammatory activity and collagen turnover in relation to specific body regions, e.g., human tendon tissue during and after exercise[7,23-25]. These methods together with real-time ultrasound determinations of mechanical properties of human tendon during muscular activity[26] and magnetic resonance imaging are promising approaches to study the coupling of tissue metabolism and viscoelastic properties of human tendons (Table 2).

The use of radioactive isotope labelling of connective tissue in live animals has been used for many years[27]. Infusion of stable isotopes (^{13}C or ^{15}N-proline) with subsequent sampling of human tendon tissue has allowed for determination of collagen synthesis in human tendon (as well as in muscle, bone and skin). Based on these studies it has been shown that tendon collagen is synthesized at the rate of 2-3% (fractional synthesis rate) per 24 hours[28].

Regulation of extracellular matrix gene expression and collagen turnover in tendon

Muscle and tendon collagen and the connective tissue network are known to respond to altered levels of physical activity[5,29,30]. In contrast to physical loading, immobilization of rat hind limb leads to a decrease in the activities of collagen synthesizing enzymes both in skeletal muscle and tendon[13]. This suggests that the biosynthesis of the collagen network decreases as a result of reduced muscular and tendinous activity[31]. The rate of the total collagen synthesis depends mostly on the overall protein balance of the tissue, but it seems to be positively affected by stretch in both muscle and tendon[13].

The cleavage of the carboxypeptide during collagen synthesis allows for indirect determinations of type I collagen formation. Development of assays for such markers of type I collagen synthesis include the measurement of the carboxy terminal propeptide of type I collagen (PICP) and for degradation of collagen include the carboxy terminal telopeptide region of type I collagen (ICTP). These assays have made it possible to study the effect of exercise on type I collagen

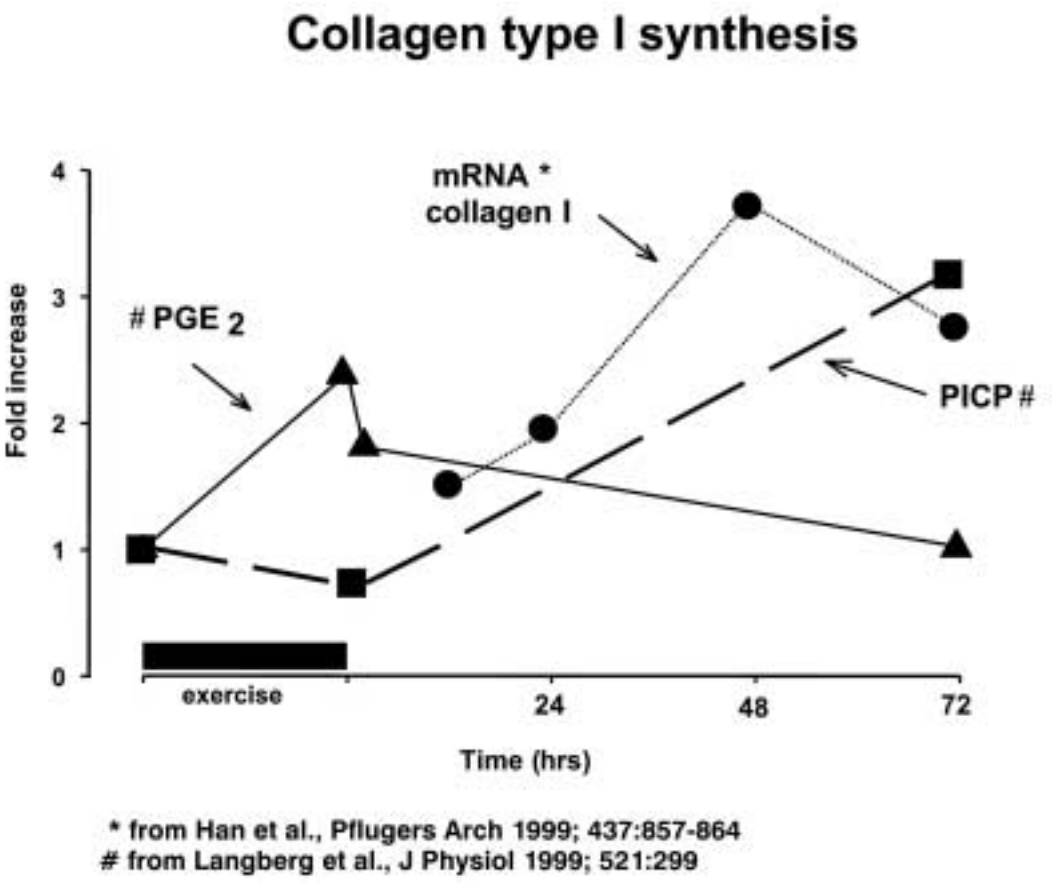

Figure 1. Changes in peritendinous interstitial concentrations of procollagen type I propeptide (PICP) and prostaglandin E2 (PGE2) in response to 3 hours of running (36 km) in healthy athletes, obtained by the use of the microdialysis technique. The relative recovery of infused radiolabeled prostaglandin and collagen was calculated. Changes in interstitial concentrations are given in fold change compared with basal levels. Data on determination of mRNA for procollagen type I in muscle samples of rats subjected to intense running is from Han et al.[9].

turnover. Whereas a single episode of exercise has little effect on the levels of these two markers in circulating blood, prolonged exercise or weeks of training lead to increased type I collagen turnover and net formation[7]. Assays determining the serum levels of PICP and ICTP are unable to determine the tissue or organ origin of these markers though it is known that most of the type I procollagen markers in the blood originate in the bone. The use of the microdialysis technique allows *in vivo* determination of biochemical substances in a variety of tissues, and it has recently been applied to the peritendinous space of the Achilles tendon in runners before, immediately after, and 72hours after 36 km of running[7] (Figure 1). With this technique it was demonstrated that acute exercise induces changes in metabolic and inflammatory activity of the peritendinous region[23]. In addition, acute exercise stimulated synthesis of type I collagen during the recovery process, suggesting that acute physical loading leads to adaptations in non-bone related collagen in humans[7]. Furthermore, when type I collagen synthesis and degradation in connective tissue of the human Achilles peritendinous space was studied before and after 4 and 11 weeks of intense physical training, an adaptive response of the collagen type I metabolism of the peritendinous tissue around the human Achilles tendon was found in response to physical training[32]. The increase in interstitial concentrations of PICP rose within 4 weeks of training and remained elevated thereafter for the entire training period, indicating that collagen type I synthesis was chronically elevated in response to training. As blood values for PICP did not change significantly over the training period, it is likely that the increased collagen type I synthesis occurred locally in non-bone tendon connective tissue rather than throughout the body. Tissue ICTP concentrations also rose in response to training, but only transiently, and interstitial levels of ICTP returned to basal levels with more prolonged training[32]. Taken together, the findings indicate that the initial response to training is an increase in turnover of collagen type I, and that this is followed by mostly anabolic processes resulting in an increased net synthesis of collagen type I in non-bone connective tissue such as tendon. The pattern of stimulation of both synthesis and degradation

in the anabolic process in response to exercise in tendon-related connective tissue is in accordance with events occurring in muscles and muscle proteins in response to loading[10]. Stretch-induced hypertrophy of chicken skeletal muscle has been shown to increase muscle collagen turnover using tracer methods[27]. Such data correlate with findings in humans where collagen synthesis increased markedly at the beginning of training. Laurent et al. concluded that a large amount of newly synthesized collagen was wasted, resulting in disproportionately high collagen turnover rate compared with the magnitude of net synthesis of collagen[27].

Acute exercise has been shown to cause an increase in collagen catabolism as measured during exercise in humans[33]. Furthermore, in the peritendinous space of the Achilles tendon collagen formation was depressed immediately in response to acute exercise and followed by a rise in synthesis rate[7]. As individuals in the latter training study were training on a daily basis, it is difficult to differentiate effects of each round of acute exercise from the chronic training adaptation. It has been demonstrated that acute exercise elevates collagen type I formation for at least 3-4 days post-exercise[7,28]. Therefore, previous acute bouts of exercise will influence the outcome of each subsequent one. This probably explains why highly trained runners (training up to 12 hours per week) in one study had high basal levels of PICP[7,32]. Thus, it cannot be excluded that the effect on collagen metabolism found during a program with daily training, simply reflects an effect on collagen formation from the last training bout, rather than from the chronic effect of training. Whether a net synthesis of collagen type I is transformed into measurable increases in tendon size is far from clear. However, in accordance with this view it has been demonstrated in animal models that training results in enlargement of the tendon diameter[4]. Furthermore, recent MRI observations of the Achilles tendon in trained runners versus sedentary humans have shown that the tendon cross-sectional area was enlarged in trained individuals compared to untrained controls[34]. Based on these data one can speculate that training initially results in an increased turnover of collagen type I to allow for reorganization of the tissue, and that more prolonged training results in a net increase in tendon tissue and thus, probably alterations in tissue strength. Interestingly, the amount of tendon tissue in the elderly is increased despite the fact that they are less active and have a smaller maximal muscle strength that their younger counterparts[35]. The increased cross-sectional area in elderly individuals might be a compensatory mechanism to counteract a reduced tendon quality, and thus to increase the safety margin to avoid a tendon rupture[35].

An important step in collagen type I formation is the enzymatic regulation by procollagen C-proteinase (PCP) of the cleavage of PICP and PINP from procollagen to form insoluble collagen[36]. It has been demonstrated that mechanical load *in vitro* can increase the expression of the PCP gene, but not of the procollagen C-proteinase that enhances protein (PCPE) in dermal fibroblasts[22]. In addition to this, it was shown that both the synthesis and processing of procollagen was enhanced by loading *in vitro*. This effect was specific as non-collagen protein synthesis in that study was not elevated[22]. Furthermore, an enhanced processing of procollagen to insoluble collagen was found, as a larger increase in the actual amount of insoluble collagen produced compared to the increase in procollagen synthesis. Interestingly, in that study it was demonstrated that the above described changes only occurred if cells were in tissue cultures containing transforming growth factor-beta (TGF-beta) or serum[22], indicating that mechanical loading in itself was not able to cause changes unless certain (growth) factors were present. The role of growth factors in collagen synthesis fits well with the presence of serum growth factors such as plasma-derived growth factor (PDGF), TGF-beta, interleukin-1 and their effect on fibroblast activation *in vitro*, but those studies were generally performed with no attempt to investigate mechanical loading. In one study the impact of mechanical strain was shown to be associated with PDGF. Insulin-like growth factors (IGF-I and II) are known to have multiple effects on fibroblasts *in vitro*[37-41], but their exact role for collagen synthesis of human tendon and muscle in relation to loading remains to be elucidated. It was shown that mechanical loading of human tendon results in a rise in interstitial concentration of TGF-beta[42], IGF-1 and IL-6[43]. The effect of specific growth factors or serum components in combination with mechanical loading on procollagen synthesis and PCP gene regulation indicate a synergy between signaling pathways in regards to procollagen gene expression and processing, similar to what has been documented also in cardiac fibroblasts[44]. At the present time the role of growth factors in regulation of collagen synthesis and of mechanotransduction in humans during and after exercise and loading of the tendon connective tissue is incompletely understood.

The signaling pathways responsible for mechanotransduction responses are yet to be elucidated, but several candidates have been suggested[45,46]. Integrin molecules are major structural components of adhesion complexes at the cell membrane linking the extracellular matrix to the cytoskeleton[47,48]. In this way integrins establish a mechanical continuum along which forces can be transmitted from the outside to the inside of the cell and vice versa[49]. At the myotendinous junction, especially, lack of integrin expression will lead to structural damage during muscle contraction[50]. Although not yet confirmed, integrins are likely candidates for sensing tensile stress at the cell surface[49,51-53]. Some evidence has been presented that integrin-associated proteins are involved in the signalling adaptive cellular responses upon mechanical loading of the tissue[54]. This fits also with the finding that gene expression for several extracellular components is regulated at the transcriptional or pre-translational level by mechanical stress.

Collagen degradation is initiated mainly by matrix metalloproteinases (MMPs), which are present in tissues mostly as latent proMMPs[55]. Tissue inhibitors of MMPs (TIMPs) inhib-

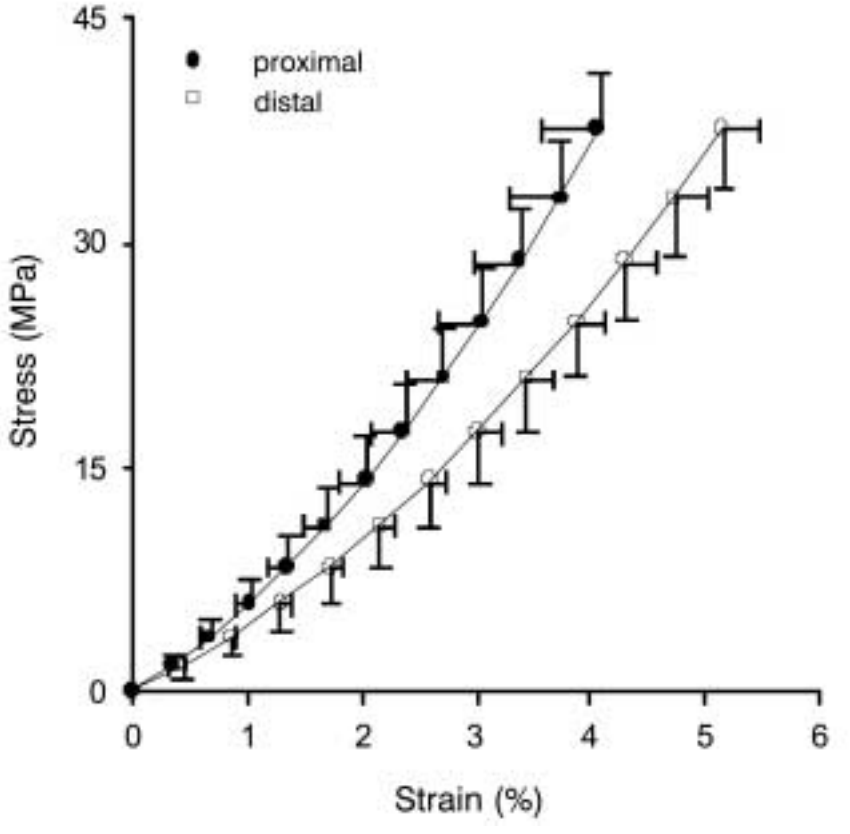

Figure 2. The estimated stress-strain curve for the human proximal and distal triceps surae aponeurosis and tendon after correction for ankle joint rotation and antagonist co-activation during graded voluntary 10 s isometric plantarflextion efforts (n=5). From Magnusson et al.[26].

it MMP activities[56]. Pro-MMP-2 is upregulated both at the pre- and post-translational level after a single exercise session suggesting an increase in collagen degradation. Interestingly, TIMP is often activated concomitantly with MMPs in response to physical activity[6] indicating a simultaneous activation of stimulation and inhibition of degradation. Rather than considering this as a competitive action, it is likely that MMP activity precedes TIMP activity, and thus TIMPs serve as regulators of collagen degradation termination to ensure controlled process. In human peritendinous tissue it has been shown that MMP-9 increases early and MMP-2 late after an acute session of 60 minutes running exercise[57] but the exact cellular source of their production remains to be identified, although both fibroblasts and leucocytes are likely candidates.

Immobilization leads to an increase in matrix metalloproteinase expression both at pre- and post-translational levels suggesting accelerated collagen breakdown that can be partially prevented by stretching exercises. The regulation of MMPs in relation to exercise remains to be fully understood, but it is known that specific integrins ($\alpha 2\beta 1$) regulate MMP-1 gene expression in fibroblasts cultured in contracting-retracting collagen gel[58].

Structure of collagen in tendon

The pattern of animal tendon stress-strain curves appears similar to curves obtained from human cadavers[3,59,60], but the curves do not necessarily represent tendon movement *in vivo*. Real-time ultrasonography allows for the non-invasive evaluation of fascicle movement during muscle contraction thereby providing a tool for studying human aponeurosis and tendon tissue behavior during isometric contraction *in vivo* both in tibialis anterior and the triceps surae complex including the Achilles tendon[61,62]. Recent advances with the technique correct for tendon displacement during joint angular motion or tendon load alteration resulting from co-activation of the antagonist muscles during isometric contraction[26]. It was demonstrated that the absolute displacement and the calculated stiffness of the proximal and distal aponeurosis did not differ during isometric calf muscle contraction indicating similar mechanical properties and uniform strain distribution along the length of aponeurosis[63] (Figure 2). This fits well with findings obtained previously on a variety of human and mammalian tendons during isolated biomechanical testing procedures. Trestik and Lieber

showed that frog aponeurosis had similar mechanical properties throughout its length during passive loading[64]. Thus, collagen structures in the aponeurosis may operate as a functional unit during both passive loading and active muscle contraction. However, it is clear that the aponeurosis strain properties are influenced by the type of loading. Thus, it has been shown that strain is lower during active contraction than during passive loading[65]. In that study it was furthermore shown that the strain rate was very high at the onset of contraction, and subsequently underwent an almost abrupt reduction in strain with an accompanying dramatic rise in force and thus in tendon stress. This sudden increase in stiffness could play an important role in the load interplay between tendon and muscle. It can be hypothesized that at the onset of contraction, rapid elongation of tendon allows for only moderate force development in the muscle according to the force-velocity relationship. Later in the process, strain is reduced and muscle force development increases. Depending upon the safety margin of tendon and muscle resistance to breakage, this phenomenon may result in marked force changes in the tissue and be related to development of muscle (or tendon) rupture. Although the stress-strain curve determined in humans *in vivo* was curve-linear it did not exactly match the curve obtained in animal models, indicating the existence of quantitative differences between humans and other species in the intimate interplay between mechanical function of tendon and muscle. To what extent aponeurosis and free tendon possess similar mechanical properties has not firmly been established. Whereas some findings indicate a similarity[64,66], other data indicate that the stiffness of the aponeurosis is less than that of free tendon[67,68]. Ultrasonographic studies in humans showed that during active isometric muscle contraction of the thigh the aponeurosis was displaced by only 1-2%, whereas the free tendon was displaced by 6-10%[63]. The loading upon the tendon obtained during the non-invasive ultrasonography determination of displacement with maximal isometric calf muscle contraction is around 3200 N or equivalent to 4-5 times body weight[63]. With biomechanical models it has been estimated that the human Achilles tendon is subjected to 2600 N during walking and 3100-5300 N with running[69]. When using a fiber optic technique with a buckle transducer, forces of 1400 N have been determined during walking[70] and likewise tendon forces of 1900-3800 N have been demonstrated during various jumping activities[71]. These results indicate that the load-displacement data that were obtained with ultrasonography were representative of those achieved during locomotion and jumping.

Collagen forms the linkage between muscle and its associated collagen muscle-tendon unit. Although stretching procedures place tensile load on several structures it remains unknown what their relative contributions are to the stress relaxation response. The transmission of tension in passively stretched muscle is complex and may engage several structures, including titin, intramuscular connective tissue, and

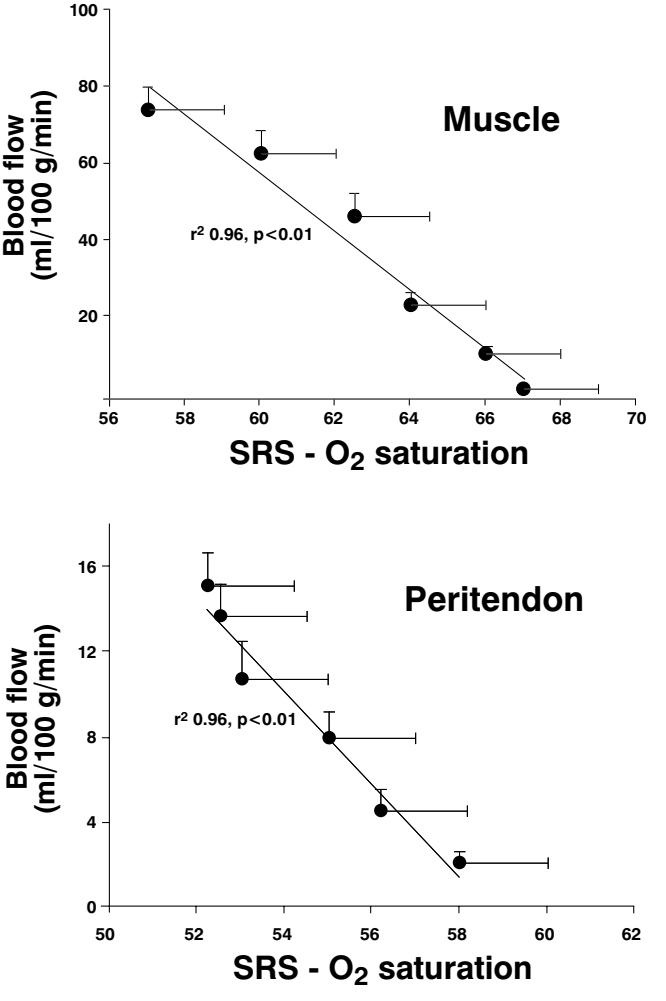

Figure 3. Correlation between spatially resolved near infrared spectroscopy (SRS) oxygen saturation and blood flow in both calf muscle (top) and in the peritendinous Achilles region (bottom) at rest and during graded dynamic plantar flexion exercise to exhaustion (n=8). Spearmann test was used to determine correlation significance. Data from Boushel et al.[24,25].

tendon. Passive stretch in a physiological range results only in 2% strain of tendon, but in 8% strain in the muscle-tendon junction[68], demonstrating differential viscoelastic properties in various regions of the muscle-tendon unit, and that tendon is less likely to deform during loading. In a recent study static stretching exercises for the human hamstring muscle group increased flexibility, maximal range of motion, which was the result of an increased tolerance to tensile load, rather than a change in the viscoelastic properties of the muscle[72,73]. Based on these results, and because the change in the viscoelastic behavior is transient in nature and resets within 1 hour, it is questionable if stretching, as it is com-

monly performed by athletes, can permanently change the passive properties of a muscle. Potentially, peripheral mechanisms, including afferent information from muscle, tendon and joint receptors, may play a role.

Loading, training and overloading of tendon structures: Potential role of blood supply, metabolic activity and inflammation

It has been possible to determine peritendinous blood flow in humans during muscular contraction by the use of radiolabeled ^{133}Xe-washout placed immediately ventral to the Achilles tendon. It was demonstrated that blood flow in the peritendon region increased 3-4 fold during heel raising exercise or during static intermittent calf muscle exercise. Lately, a dynamic ergometer model has been developed by which the Achilles tendon can be studied during standardized dynamic exercise loading. Using this model, blood flow in the Achilles peritendinous region has been shown to rise up to 7 fold during intense plantar flexion exercise compared to values obtained at rest[24,25]. Taken together, these findings indicate that blood flow in the peritendinous region increases with exercise in an exercise dependent manner, and in parallel with muscle perfusion during calf muscle contraction. The simultaneous use of near infrared spectroscopy (NIRS)[24] and arterio-venous blood sampling indicated that oxygen extraction and total hemoglobin volume increase with exercise in the peritendinous and muscle region, while there is a concomitant drop in oxygen saturation. Taken together this data suggest that vasodilation during intense exercise is coupled to tissue oxygenation of both the muscle and peritendinous region (Figure 3). It is clear from these experiments that although the increase in tendon blood flow is somehat restricted, there is no indication of any major ischemia in the tendon region during exercise.

The question remains how blood flow to the tendon region is regulated. Several candidates as regulators of blood flow in skeletal muscle have been proposed, and it is possible that similar substances and metabolites are vasoactive also in the tendon region[74]. Bradykinin is a particularly attractive candidate because of its simultaneous vasodilatatory and nociceptive properties. As it is known to activate prostaglandin and nitric oxide dependent pathways, it may be important not only in relation to regulation of flow to the normal tendon but also in overused, sore and hyperperfused tendons. With the use of microdialysis it has been demonstrated both in muscle and tendon tissue that there is detectable bradykinin present in those tissues, and that the interstitial concentration of bradykinin (as well as that of adenosin) increases with exercise[75]. To what extent this plays a role in the vasodilatory response has not been established. The presence of other substances of potential relevance for nociception in tendon-like substance-P and glutamine have been demostrated in animal tendon and human tendon, respectively[76,77].

Somewhat surprisingly, it was demonstrated that the pressure in the epitenon and peritendon of the Achilles tendon decreased markedly during exercise, and that a negative pressure of up to 150 mmHg can be found during intense dynamic exercise[78]. This can be explained by the fact that the Achilles tendon has been shown to move in a posterior direction when m.triceps surae is activated. The significance of these changes cannot be established from the experiment, but it can be hypothesized that this negative pressure – that most likely also occurs to some degree during stretching – allows for fluid shifts into the peritendinous region and thus lowers the local concentration of released substances that potentially could stimulate inflammation. Whether this plays a role in the development of injury or during treatment is, however, not known. Furthermore, it has been demonstrated in tendons of growing animals, that compression induces a change in extracellular matrix gene expression[79]. This effect is seen as being independent of any tensile activity in the region, and leads to increased expression of genes for both proteoglycans and type II collagen[80,81]. Such altered expression depends upon the type of mechanical loading. For example, dermal fibroblasts that differentiate into myofibroblasts express α-smooth muscle actin when subjected to repeated mechanical stress[82].

In vitro models have demonstrated an increased production of prostaglandins during repetitive motion of human mesenchymal tendon cells in cultures[83]. This response was blocked by indomethacin and was not associated with any microscopically visible damage of repeated stretching. This indicates that inflammatory mediators are secreted in response to normal loading of connective tissue. In line with this release of prostaglandin and tromboxane both in muscle and in peritendinous tissue has been demonstrated in response to exercise using the microdialysis technique[23,84]. In the resting tendon exhibiting long-term symptoms of overuse, no elevated levels of prostaglandins could be detected, whereas an exaggerated response of interstitial prostaglandin concentration could be detected in association with exercise in overused versus healthy tendon (unpublished observation). This points to a more vulnerable condition associated with chronic injury, that results in a more pronounced inflammatory response upon stimulation. The exact location of the prostaglandin production could not be determined. In muscle static contraction was not sufficient to induce it, but dynamic contraction resulted in prostaglandin release. In contrast, only moderate isometric contraction was needed to elicit a response in tendon-associated tissues. The inflammatory response was only transient and it remains unclear to what extent this response is coupled to collagen synthesis and degradation[85,86]. It was shown that exercise-induced increase in tendon flow was inhibited 40% by blockade of prostaglandin secretion with cyclooxygenase blockers, indicating that prostanoids play an important role in vasodilation in tendon tissue during exercise[87]. It remains to be seen to what extent this does play a role in inflamed tendons, and recovery after overuse.

Conclusion

The investigation of connective tissue and extracellular matrix components in relation to physical activity and tendon is at its initial phases and several questions remain unanswered. Recent findings do, however, suggest potential signaling pathways converting mechanical stimulus to gene expression and collagen synthesis. These pathways are likely to involve interactions of locally released growth factors with inflammatory and vasoactive/angiogenetic substances that lead to cytoskeletal tissue damage. With advancements in *in vitro* and *in vivo* methodologies, it is now possible to study tissue adaptation and its function in relation to varying degrees of physical activity.

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