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Tendons and Ligaments

Foreword

This issue is largely devoted to a topic seldom discussed by this and many other journals dealing with orthopaedic and connective tissue research. A total of 8 papers have analyzed in the form of review, perspective or original research on basic structure and biochemistry of tendons, and the structural, biochemical and biomechanical changes tendons undergo with development, age or activity. Tendons (and ligaments) are an integral, though less studied, part of the musculoskeletal system. They are generally considered to be dull, somewhat lifeless ropes attaching muscles to bones in the case of tendons or, in the case of ligaments, connecting two bones. This notion is akin to that one held about bones some eons ago: they were thought to be rigid, unchanging supporting structures. Today we know that bones are very metabolically active undergoing constant remodeling that depends to a large extent on their mobility status. Similarly, there are constant and reciprocal interactions between tendon structure and biochemistry on one side, and tendon mechanical function on the other.

To understand the basis of tendon development and growth-dependent modeling and remodeling one has to be familiar with collagen fibrillogenesis and its regulation. The first paper by Zhang et al.[1] does just that. This group of authors (David Birk and his colleagues) summarizes the wealth of data on collagen fibrillogenesis, a lot of which comes from their laboratory. They review collagen fibrillogenesis beginning as an assembly of collagen molecules in a series of extracellular compartments, progressing through post-depositional maturation leading to thicker and longer fibrils and ending in their coalescence in the final stages of fiber production. Zhang et al. discuss data indicating that at least in chicken embryos the growth of fibrils occurring from pre-formed fibril intermediates depends on mechanical loading of tendons through active movement of the embryonal chick limb.

Tendon fibrillogenesis is regulated by several mechanisms. The initial fibril assembly is in part regulated by the heterotypic interaction between two different (I and III) fibrillar collagen types. The roles of other collagen types in fibrillogenesis are also discussed, with a focus on the so-called FACIT or fibril-associated collagens (types IX, XII, XIV, XVI, XIX, and XX).

Work with mice deficient in leucine-rich repeat proteoglycans provides us with better insight into proteoglycan regulation of fibrillogenesis. The implications of deficiency in one or two leucine-rich repeat proteoglycans (fibromodulin, lumican, decorin and biglycan) in mice in regulation of tendon fibrillogenesis are discussed here as well as in another paper by Yoon and Halper in this issue[2]. These studies established that in addition to decorin and biglycan, fibromodulin and lumican play important roles in fibrillogenesis, confirming that loss of even one of the proteoglycans alters the mechanical properties of tendons.

The following paper by Doschak and Zernicke[3] should be of particular interest to readers of this Journal. It reviews the structure and function of the types of insertion or entheses between bone-tendon and bone-ligament, and thus complements the first paper. Authors also discuss the time-dependent properties of viscoelastic materials such as tendons, ligaments and their entheses and how their transition through different zones of increasing stiffness in surrounding tissues may offer a mechanical advantage. The primary function of the enthesis is reduction of strain concentrations at the interface of compositionally distinct tissues during loading. For example, fibrocartilage (located in regions of tendons subjected to compression) interdigitates with the underlying bone and, through an increase in the cross-sectional area of interface between the more compliant tendon and the bone mediates as decrease in stress concentrations during load transmission from the tendon or ligament to the bone. Finally they summarize data, mostly from their laboratory, pertinent to pathogenesis of enthesopathies and their repair using several methods, including short-term anti-resorptive therapy with bisphosphonates and surgical procedures.

Banes et al. have documented that mechanical load stimulates expression of numerous genes in avian flexor tendon cells[4]. Two papers in this issue deal with several aspects of collagen turnover and changes in gene expression, one by Kjaer et al.[5] and the second by Halper et al.[6] Kjaer et al. provide a comprehensive review of tendon response to loading and exercise[5]. The accelerated formation and degradation of connective tissue in both muscle and tendon as a result of exercise have been well documented by many groups, including theirs. Kjaer et al. address and provide plausible explanations for apparent contradictions. For example, to explain increase both in collagen synthesis and in matrix metalloproteinases with mechanical loading, they hypothesize that this reflects both physiological adaptation and repair of damage of extracellular matrix structures during exercise. They discuss increased gene transcription and especially post-translational modifications of proteins of the extracellular matrix and growth factors following exercise as preceding stimulation of synthesis of collagen and other extracellular matrix proteins, and as pre-requisites for changes in biomechanical properties as well as the structural properties modifications in collagen. They highlight the differences between the *in vitro* and *in vivo* models of properties of tendons. The main advantage of *in vivo* studies on humans is that they allow continuous measurements in environmental blood flow, tissue oxy-

genation and metabolism during and after exercise. The authors speculate that training-induced increased turnover of collagen type I facilitates tissue reorganization, and that prolonged training results in a net increase in tendon tissue and thus probably in increased tissue strength. They also raise two other interesting points. The first is that the increased cross-sectional area of tendons in the elderly, who are less active and have smaller maximal muscle strength than younger people, might be a compensatory mechanism to counteract reduced tendon quality. The second point draws attention to increased local production of prostaglandins with mechanical loading, which may sensitize tendons to overuse injuries.

Halper et al. took an opposite approach by looking at gene expression in cultured tendon explants. They attributed the decrease in the level of collagen and in expression of mRNAs for collagen regulators, such as TGFβ and HSP47 over the period of several days to lack of mechanical loading[6].

Though collagen represents 90% of tendon dry weight, proteoglycans are essential to maintain collagen fibril organization and thus biomechanical function. Kathryn Vogel's pioneering work showed that the nature of mechanical loading (tensional vs. compression) determines, at least as far as the most abundant tendon proteoglycans are concerned, the proteoglycan composition, and, to a certain extent, the structure, of the tendon[7,8]. Two papers are devoted exclusively to this topic. While the paper by Yoon and Halper[2] is mostly a review of biochemistry and physiology of proteoglycans in the tendon it, together with the paper by Vogel and Peters[9], brings new insights on the role of proteoglycans in the tendon. Yoon and Halper studied changes occurring in proteoglycans in gastrocnemius tendons from very young, rapidly growing chickens. The increasing concentrations of glycosaminoglycans (especially in the form of chondroitin sulfate, keratin sulfate and hyaluronan) and proteoglycans (presumably mostly in the form of decorin) in growing tendons correlated well with a rapid increase in diameter of collagen fibrils at this stage. Most importantly, Vogel and Peters suggest a novel function for proteoglycans besides regulation of collagen fibrillogenesis and providing tissues with resiliency. They share with us their finding of proteoglycans (aggrecan and hyaluronan) and type VI collagen in distinct layers of Alcian blue-stained material surrounding vascular elements at the point where several fiber bundles come together in the area adjacent to fibrocartilage. Vogel and Peters hypothesize that proteoglycans accumulated in such a peculiar location protect the integrity of vasculature in tendon subjected to bending and shear in addition to facilitating the movement of collagen fiber bundles relative to one another and to separating and lubricating the collagen bundles during normal loading.

Tendons are adapted to respond to mechanical load to which they are, under ordinary circumstances, constantly subjected. How load is recognized by cells, transmitted and translated into biochemical signaling and responses has become less mysterious in recent years. Wall and Banes summarize current knowledge of mechanisms of immediate responses to loading[10]. Fluid flow, strain, shear and their combinations stimuli activate early mechanotransduction pathways, such as Ca^{2+} signaling and intercellular communication, in tenocytes. It is likely that extracellular Ca^{2+} mediates initial tenocyte response to mechanical load perceived as changes in fluid flow at least in some species. These events are further mediated through stretch- and voltage-activated channels (e.g., purinoceptors, adrenoceptors, ryanodine receptor-mediated Ca^{2+} release, gap junctions and connexin hemichannels). The roles of signaling molecules (e.g., calcium, diacylglycerol, inositol (1,4,5)-trisphosphate, nucleotides and nucleosides) in intracellular and/or extracellular signaling roles are discussed as well. Because the Ca^{2+}-mediated response was observed only in some species, e.g., humans, the authors propose the existence of another class of mechanosensitive channels responsive to mechanical load through a Ca^{2+}-independent pathway.

Many methods (e.g., stress-relaxation and quasi-static tests) have been developed to evaluate biomechanical function of tendons; however most of them provide only partial information about the viscoelastic behavior of these tissues. The main drawback of the dynamic test is that the material has to be characterized at each frequency over a wide range of frequencies. The quasi-linear viscoelastic method requires not only a priori assumptions about the reduced relaxation function and continuous spectrum of relaxation but laborious calculations as well. To circumvent these problems the author developed a novel approach to biomechanical evaluation[11]. The method, based on direct measurement of the complex modulus of the testing material, allows for the mechanical properties of biological tissues to be measured in real time without the need to make a priori assumptions regarding their structures. Moreover, its application to both linear and nonlinear viscoelastic materials would enable biomechanical evaluation of most biological tissues.

Our hope is that these papers will provide a stimulus not only for further discussion on the pages of the Journal, but also for research to be conducted by its readers.

References

1. Zhang G, Young BB, Ezura Y, Favata M, Soslowsky LJ, Chakravarti S, Birk DE. Development of tendon structure and function: regulation of collagen fibrillogenesis. J Musculoskelet Neuronal Interact 2005; 5:5-21.
2. Yoon JH, Halper J. Tendon proteoglycans: biochemistry and function. J Musculoskelet Neuronal Interact 2005; 5:22-34.
3. Doschak MR, Zernicke RF. Structure, function and adaptation of bone-tendon and bone-ligament complexes. J Musculoskelet Neuronal Interact 2005; 5:35-40.
4. Banes AJ, Horesovsky G, Larson C, Tsuzaki M, Judex S, Archambault J, Zernicke R, Herzog W, Kelley S, Miller L. Mechanical load stimulates expression of novel genes *in vivo* and *in vitro* in avian flexor tendon cells. Os-

teoarthritis Cartil 1999; 7:141-153.
5. Kjaer M, Langberg H, Miller BF, Boushel R, Crameri R, Koskinen S, Heinemeier K, Olesen JL, Døssing S, Hansen M, Pedersen SG, Rennie MJ, Magnusson P. Metabolic activity and collagen turnover in human tendon in response to physical activity. J Musculoskelet Neuronal Interact 2005; 5:41-52.
6. Halper J, Griffin A, Hu W, Jung C, Zhang J, Pan H, Kisaalita WS, Foutz TL, Frazier KS. *In vitro* culture decreases the expression of TGFβ, Hsp47 and type I procollagen and increases the expression of CTGF in avian tendon explants. J Musculoskelet Neuronal Interact 2005; 5:53-63.
7. Robbins JR, Evanko SP, Vogel KG. Mechanical loading and TGF-β regulate proteoglycan synthesis in tendon. Arch Biochem Biophys 1997; 342:203-211.
8. Robbins JR, Vogel KG. Regional expression of mRNA for proteoglycans and collagen in tendon. Eur J Cell Biol 1994; 64:264-270.
9. Vogel KG, Peters JA. Histochemistry defines a proteoglycan-rich layer in bovine flexor tendon subjected to bending. J Musculoskelet Neuronal Interact 2005; 5:64-69.
10. Wall ME, Banes AJ. Early responses to mechanical load in tendon: role for calcium signaling, gap junctions and intercellular communication. J Musculoskelet Neuronal Interact 2005; 5:70-84.
11. Zhang G. Evaluating the viscoelastic properties of biological tissues in a new way. J Musculoskelet Neuronal Interact 2005; 5:85-90.

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