

Chapter I

General Introduction

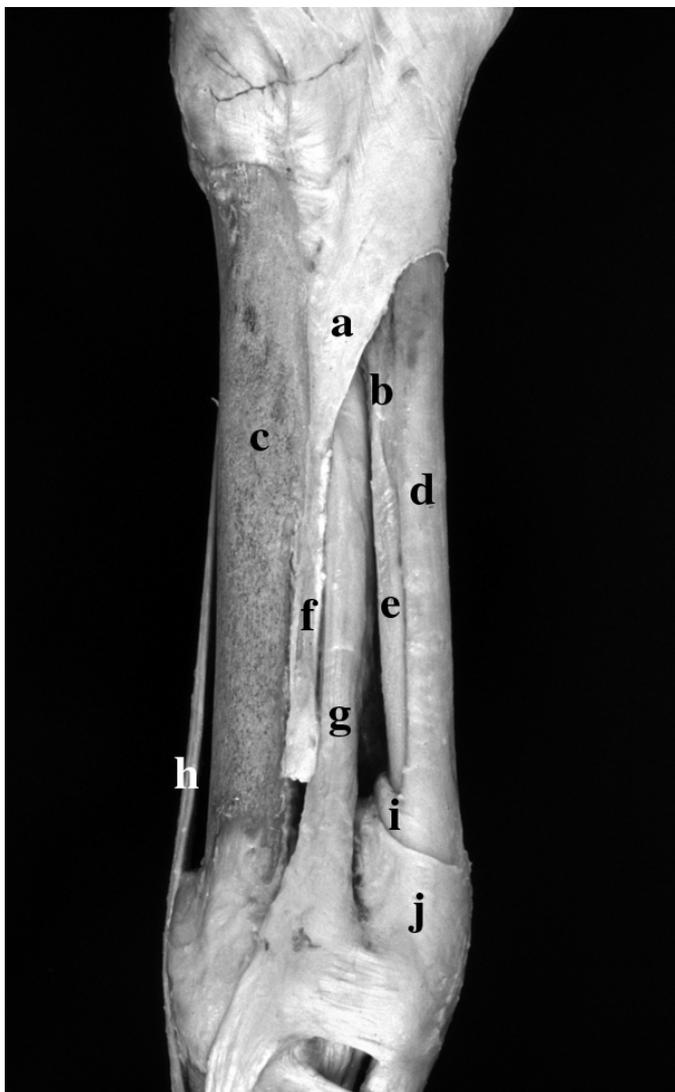


Figure 1. Medial aspect of the carpometacarpal area, showing the gross anatomy of the **superficial digital flexor (SDF)** tendon. a) flexor retinaculum, b) ligamentum accessorium of deep digital flexor (DDF) tendon, c) 3rd metacarpal bone, d) superficial digital flexor (SDF) tendon, e) deep digital flexor (DDF) tendon, f) 2nd metacarpal bone, g) m. tendo-interosseus, h) extensor digitalis communis tendon, i) manica flexoria, j) palmar annular ligament. **See Color Supplement.**

(Courtesy Jean-Marie Denoix)

In the beginning there was the equine practitioner, concerned about the prevention, diagnosis and treatment of the frequently encountered injuries of the superficial digital flexor tendon, full of frustration about their frequent relapse. Relapses, which were partly to blame on the inferior quality of the repair tissue, but partly on his own inaccurate assessment of the functional capacities of the injured tendon. Then came the clinical researcher, seeking to reduce his frustration by the use of a new promising diagnostic modality that for the first time claimed to allow an inward view into tendon tissue. Finally, emerged the scientist, fascinated by the unique ultra-structural architecture of elastic tendons and full of admiration for the enormous performances of these long and thin structures in the equine athlete, acting so close to their biomechanical limits.

Gross Anatomy of the Superficial Digital Flexor Tendon and Clinical Relevance

The superficial flexor muscle originates from the medial epicondyle of the humerus and runs distally along the palmar side of the radius, converting to the **superficial digital flexor (SDF)** tendon just proximal to the carpus. The tendon then runs distally along the metacarpus and over the proximal sesamoids in the fetlock area and splits at the level of the first phalanx into lateral and medial parts. These insert on the lateral and medial tubercles on the proximal aspect of the second phalanx and also with a minor branch on the latero-distal aspect of the first phalanx.¹

Injuries of tendons and ligaments are amongst the most frequent disorders of the locomotory apparatus in the horse.^{2,3} In the Thoroughbred racehorse, in training in the United Kingdom and in continental Europe, the incidence of tendon injuries is approximately 30%, mostly in the superficial digital flexor tendon of the forelimbs.⁴ By far the most frequently injured area is the metacarpal section, palmar to the 3rd metacarpal bone (**Figure 1**).^{5,6}

Micro-Structure of Tendons and Biochemical Composition

Tendons consist of highly specialized connective tissue, which is characterized by hierarchically arranged filaments embedded in a hydrophilic matrix with a relatively small volume of cells. The extracellular matrix (ECM) consists of collagen, elastin, proteoglycans and glycoproteins.⁷

Main structural protein is tropocollagen (65-80% of the dry mass), that basically consists of three helically arranged polypeptide chains. Five tropocollagen molecules are twisted to filaments, which are organized into larger longitudinal bundles, the structural units of the tendon. According to their size these are called microfibrils, subfibrils, fibrils, and fibers (subfascicle or primary tendon bundle), fascicles (secondary tendon bundle), and tertiary tendon bundles (**Figure 2**).^{8,9} If not loaded,

collagen fibrils and fibres are not straight when visualized microscopically, but have a specific undulating appearance, called crimp waves. The crimp angle is the angle the waves make with the long axis of the tendon fibril. The tertiary bundles are surrounded by loose connective tissue, called the endotendon or interfascicular connective tissue (IFCT), which provides vascular supply and probably also circular support.¹⁰

Proteoglycans and glycosaminoglycans form other important constituents of the ECM. Glycosaminoglycans (GAG 's) are unbranched polysaccharide chains composed of repeating disaccharide units. Based on composition four main groups of glycosaminoglycans can be distinguished: (1) hyaluronan or hyaluronic acid, (2) chondroitin sulphate and dermatan sulphate, (3) heparan sulphate and heparin, and (4) keratan sulphate, of which only group (1) is non-sulphated (=HA), and groups (2), (3), and (4) are (poly)sulphated (= PSGAG's). Except for HA, all GAG's are covalently attached to protein, forming proteoglycans of almost limitless diversity. They form a gel of varying pore size and charge density and may thus serve as selective sieves to regulate the traffic of molecules and cells according to their size and/or charge. In vitro proteoglycans bind various secreted molecules, and also in vivo proteoglycans are thought to play a major role in the chemical signaling between cells. Furthermore, proteoglycans bind and regulate the activities of proteolytic enzymes (proteases).¹¹

The prevalent cell type in tendon tissue is the fibroblast. These cells belong to a family of connective tissue cells, whose members are not only related but can also to an unusual extent convert into each other. Fibroblasts are the least specialized cells of this family, but may develop into the more specialized chondrocytes (in cartilage), osteoblasts (in bone), and tenocytes (in tendon). All these cells are specialized in the secretion of collagenous extracellular matrix and are jointly responsible for the architectural framework of the body.¹¹ When loaded, fibroblasts adjust shape and their metabolic activity with respect to the production of extracellular matrix components.¹¹⁻¹³ Different types of loading (compression, tension) elicit different types of metabolic response.

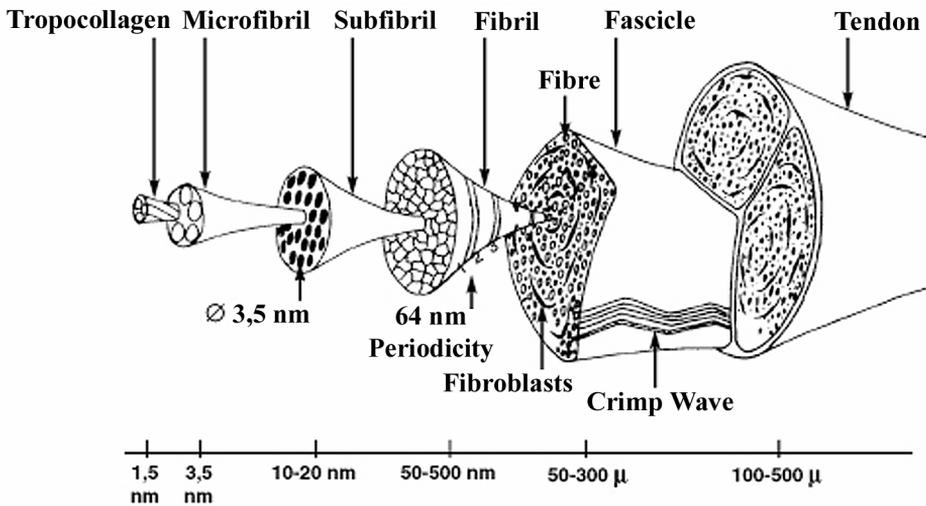


Figure 2. Architecture of the tendon (after Kastelic et al. 1978).

Tendon Biomechanics (“Biorheology”)

Tendons link muscles to bone. Flexor tendons flex the digit during the swing phase, while during the stance phase they are loaded under tensile stress with loads up to two times body weight.¹⁴ During this stance phase flexor tendons store and release elastic energy⁷, in fact acting as energy saving springs.

The response of elastic structures such as flexor tendons to load can be graphically depicted by a so-called force-elongation or stress-strain curve ($E = \frac{\text{stress}}{\text{strain}}$), in which strain is the deformation of a structure to an external load, and stress is the internal resistance to such deformation (**Figure 3a**).¹⁵ In this curve, four distinct regions can be discerned. In the “toe region” minor loading results in considerable elongation, until about 3% strain. This non-linear behavior of the tendon is the consequence of the straightening out of the crimp waves in collagen fibrils and fibers, which is completely reversible.¹⁶ Crimp appears to act as a “shock absorber” protecting the fibrils against suddenly applied load. The second part in the curve is the “linear region”, in which fibers in the tendon become arranged in a more parallel fashion. In this elastic phase the degree of deformation is dictated by the collagen’s structures and the gradient of this part of the curve represents the tensile stiffness or modulus of elasticity (**Figure 3b**). Strains until approximately 4-5% are still completely reversible. In the third phase, higher strains may result in slippage of the interfibrillar cross-links and subsequent dissociation of fibrils.^{17,18} This part of the curve starts with a so-called “primary failure” phase, lasting until approximately 8% strain, during which plastic deformation takes place. With further increasing strains,

also larger tendon bundles start to fail, the so-called “secondary failure” phase during which the curve deflects to the “yield point”. Normal SDF tendons from mature warmblood horses ultimately rupture at strains of about 12.5%, at a load of approximately $12,000 \pm 1300$ N.^{15,19}

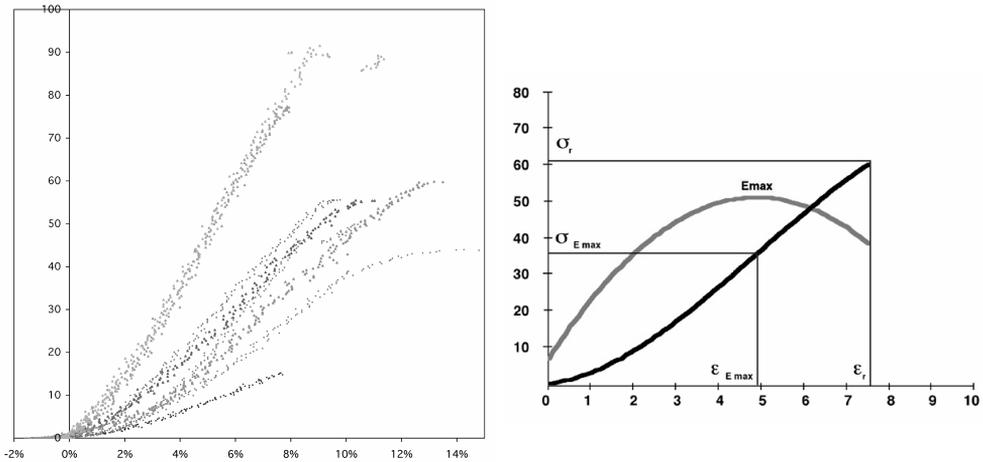


Figure 3a Plots of the biomechanical testings, recorded during loadings to failure, of tendons showing various histopathological stages. Notice remarkable differences in rheological characteristics. X: strain (%), Y: stress (MPa).

(Courtesy Nathalie Crevier-Denoix)

Figure 3b Stress-Strain curve.

- $\sigma_{E\max}$ and $\epsilon_{E\max}$ are stress and strain at point of inflexion of the curve
- E_{\max} is the maximal modulus of elasticity; with higher stresses plastic deformation starts (“primary failure phase”)
- σ_r and ϵ_r are stress and strain at rupture

(Courtesy Jean-Marie Denoix)

Relationship between Ultra-Structure of Tendons and their Biorheological Characteristics

The main structural protein that withstands tensile stress is collagen and major factors in the tensile strength and stiffness of the fibrils are the covalent intra- and intermolecular crosslinks. While the tensile strength of the tendon depends on collagen content and arrangement, stiffness is more influenced by the fibril diameter: Also viscous elements play a vital role as tendon ECM has “visco-elastic” properties. These are the result of the composite ultra-structure, consisting of inextensible collagen fibrils surrounded by a hydrated gel of strongly hydrophilic glycosaminoglycans, providing turgor and cohesion.^{8,20-22} The hydrated gel, which fills the interfibrillar space, plays a crucial role in the mechanical functioning of tendons. Pressure is transmitted equally in all directions to other structures. The fluid flow dissipates compressive energy¹¹, as described in articular cartilage and intervertebral disks, and transmits pressure to the collagen fibrils.^{23,24} Thus, a weak, although “fibre-reinforced”, gel can in fact strengthen the tendon structure.

Tendon Injury and Healing

Tendon injury can be provoked either by macrotrauma, as a consequence of a single exceeding of maximal weightbearing capacity, or, more often, by microtrauma as the result of repetitive sub-maximal overloading. Both single macrotrauma and repetitive microtrauma lead to tissue overload, eliciting an initial cell-matrix stress response. This response may result in either (a) “physiological adaptation to imposed demand”^{25,26}, (b) “degenerative changes” without major structural changes (yet ultra-structural alterations like splitting of fibrils are described) and vascular disruption²⁷, or (c) irreversible tissue damage and cell necrosis with major structural and vascular disruption, thus arousing a classical inflammatory reaction.²⁸

If irreversible tissue damage has occurred, a sequence of events takes place. At first, the remnants of cells and disrupted or necrotic tendon bundles are removed by phagocytotic and lytic activities during the inflammatory phase (until approximately 10 days post-injury), resulting in the demarcation of the lesion. Subsequently, the proliferative phase (from 4 to 45 days post-injury) starts with the formation of a fibrin clot and the proliferation of fibroblasts and synthesis of collagen fibrils, leading to the formation of a fibro-proliferative/fibro-vascular callus. This occurs both in the lesion and around the tendon. Finally, the immature collagenous structure is replaced by type I collagen fibrils which progressively crosslink and organize into larger tendon bundles which gradually become oriented along the lines of axial tension during the maturation- or remodeling- phase (45 to 120 days post-injury). By 24 weeks post-injury, the repair tissue has developed into a mature scar, although further remodeling continues for many months.²⁹

While most acute injuries result in a sudden crisis, most chronic injuries have a slow, insidious, onset. These injuries last for months or years with persistence of symptoms, resulting in chronic inflammatory responses as a consequence of cumulative ultrastructural microtrauma (“sports-induced inflammation”).³⁰

Diagnosis and Imaging of Tendon Lesions

Injuries of tendons are amongst the most challenging diagnoses in equine orthopedics. The clinical evaluation of tendons has been, and in fact to a large extent still is, based mainly on the “basic skills of veterinary profession”, i.e. meticulous palpation for heat, sensitivity, and swelling, or the old hallmarks of inflammation (“Rubor et Tumor cum Calore et Dolore”) as first described by Aulus Cornelius Celsus (25BC-50AD).

The introduction of radiography as imaging modality was a revolution in equine orthopedics, but meant relatively little for the diagnosis of tendon lesions, as tendons obviously are soft tissue structures. The breakthrough came in the early 1980s with the introduction of ultrasonography as a new tool for the imaging of equine flexor tendons.³¹ For the first time it seemed possible to get an inward view of tendon tissue, not unlike X-rays provided information about the internal structure of bone. However, the ultrasonographic image is very different from the picture produced by radiography, which is caused by the essentially different physical process by which the images are generated. These differences have far-reaching consequences for the interpretation of ultrasonographic images.

Ultrasonography is a non-invasive technique that provides an inward view into tissues by means of ultrasound waves with frequencies mostly ranging from 3.5 to 12 MHz. Imaging with ultrasound waves is based on the pulse-echo principle. The transducer emits ultrasound waves, which travel through and interact with the insonated tissues. Part of the interactions result in echoes that return to the transducer. These echoes are converted to radio-frequency signals and subsequently digitally processed before being finally displayed in the ultimate ultrasonographic image.³² In the insonated tissues, the transitions between the anatomical structures act as acoustic interfaces, due to their difference in acoustic impedance.^{33,34}

At each acoustic interface a substantial part of the ultrasound waves will be subject to either reflection or scattering (structural or diffuse), while another part of the ultrasound waves continues and is gradually absorbed.³⁵ The representation of each individual structure in the ultimate ultrasonographic image depends on the size of this structure in relation to the dimension of the 3-dimensional sample volume. The axial dimension of this sample volume is determined by the pulse length, which is typically 2 to 3 times the frequency-dependent ultrasonic wavelength.^{32,36-38} A practical derivative of this dimension is the axial resolution that can be defined as the ability to discriminate separate acoustic interfaces along the axis of the ultrasound beam

(reflector separation).³⁹ When the size of the structure exceeds the dimension of the sample volume (pulse length x beam width)^{35,36,38,39}, this sample volume contains just 1 acoustic interface. In this way, there is one acoustic pulse, one acoustic interface and one acoustic hit, and thus one returning echo. This single echo can not interfere and therefore has direct structural relationship. In case the structures are smaller than the sample volume, then the sample volume will contain 2 or more acoustic interfaces. In that case, there is one acoustic pulse but there are multiple acoustic interfaces, multiple acoustic hits and multiple, interfering, echoes. Therefore, due to the interference of echoes there is no direct relationship between the structures within the sample volume, and the ultimate echo as represented in the ultrasonographic image or, in other words, there is loss of direct structural relationship. The ultrasonographic image is thus the final, unspecific, outcome of a complex of interactions between the incident ultrasound waves and the acoustic interfaces in the insonated tendon.

By “reading” an image, the clinician in fact “translates”, deliberately or not, the ultrasonographic features to a description of the insonated tissues. In order to objectify the stage of integrity of tendons, methods have been developed to quantify the ultrasonographic information. Qualitative and semi-quantitative methods are based on a rough estimation of the echogenicity (e.g. intensity and homogeneity of the echoes) in the transverse image and the axial alignment in the longitudinal image. These methods have been applied for descriptive categorizing of lesions.⁴⁰ However, because the human vision system can not distinguish and quantify the broad range of gray levels, computerized image analysis was introduced for the calculation of the gray level of each individual pixel in the transverse ultrasonographic image. Currently, quantitative ultrasonographic tissue characterization is based on the analysis of the distribution of these gray levels as represented in the histogram of transverse images.^{41,42} These first order gray level statistics provide information about the occurrence of gray levels, however independent of location or spatial relationship.

When the frustrated practitioner turned into the clinical researcher, he gratefully grasped the unique opportunity presented to him by the new technique and decided that this would be the ideal tool to objectively evaluate a number of treatments for tendon injuries. For ages, various treatments, ranging from the rather barbarous firing of tendons to newly developed drugs that should be injected intralesionally, had been advocated with no real means to put them to the test. Now the great moment had come. However, things turned out to be less simple than they appeared and it soon became clear that the proposed quantitative analysis techniques for ultrasonographic images based on 1st order gray level statistics would not be sufficient to do the job reliably. The only way was to go into depth. To explore at the ultrastructural level the interaction between ultrasound beam and tissue, using detailed knowledge of both the biology behind the tissue and the physics behind the ultrasound waves. An academic thesis was born.

Aim and Scope of the Study

The ultimate aim of this thesis was to use the technique of ultrasonography as a tool for the improvement of the clinical management of superficial digital flexor tendon lesions in the horse. To reach this goal some steps, that were basic but of paramount importance, had to be taken first.

The first and most important step was to answer the question what actually was to be seen in the ultrasonographic image. Very soon it turned out that this image was very susceptible to transducer handling and machine setting (**Chapter II**), thus yielding information that did not meet the basic requirement of reproducibility. As the techniques that were advocated for image analysis proved to be insufficient for the correct identification of even large differences in structural integrity of tendon sections featuring different types of lesions (**Chapter III**), a new technical approach for the interpretation and quantification of the ultrasonographic image had to be developed.

It was only through the in-depth analysis of the interaction of the ultrasound beam with the tendon tissue with its unique longitudinal fibrillar structure that a technique could be developed that enabled to make a discrimination between echoes that were directly related to biological structures and those that were the result of either artefacts or of complex interactions of ultrasound waves with structures below the limits of resolution (**Chapter IV**). This technique proved to be of great help for the determination of the degree of structural integrity of (pathologically altered) tendon tissue (**Chapter V**), but was as yet insufficient for the complete “translation” of the ultrasonographic image because the echoes that were not structure-related were not taken into account. The mathematical analysis of these echoes, generated by “speckle” and (diffractive) scatter, finally permitted the correct classification of each pixel in a given ultrasonographic image. By then, the way was free for the

ultrasonographic discrimination of the various tissue types that can be found in normal or pathologically altered tendon tissue (**Chapter VI**).

The second step was to use this new, reliable and reproducible technique for “tissue typing by computerized ultrasonography” to establish a standard for the monitoring of tendon lesions. Such a standard would serve as a reference for intervention studies. This was achieved by the long-term ultrasonographic monitoring of standardized, artificially created tendon lesions (**Chapter VII**). That same study permitted the detailed description, in terms of histomorphology, collagen fibril development and biochemical characteristics of extracellular matrix components, of the long-term outcome of the healing process of tendon lesions (**Chapter VIII**) and the validation of the *in vitro* developed technique for quantitative computerized ultrasonography under *in vivo* conditions (**Chapter IX**).

After the development of computerized ultrasonography as a reliable and quantitative tool for the assessment of tendon lesions and the detailed study of the development and characteristics of these lesions, now the first steps could be taken in the new world that had been opened up: the answering on a sound scientific basis of questions arising from clinical practice. Questions such as: is it right that there are large individual differences in the efficacy of tendon healing (**Chapter IX**)? Or the important question regarding the relationship between the ultrasonographic status of tendons with different types of lesions and their biomechanical loading capacity (**Chapter X**).

“Absque labore nihil” (no success without effort), sighed the scientist who had evolved from the clinical researcher who himself had developed out of the equine practitioner, when he wiped the sweat off his forehead. Overseeing and reflecting what had been accomplished during the past years he was not unhappy for a moment or two. Then, his mind drifted off, thinking about what more could be done to improve treatment, or even better prevention, of equine tendon injuries... (Chapter XI).

*Some men see things as they are and ask, “Why?”
I dream things that never were and ask, “Why not?”*

Robert F. Kennedy (1925 – 1968)

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