EQUINE SUPERFICIAL DIGITAL FLEXOR TENDON EVALUATION USING LOW FIELD MAGNETIC RESONANCE IMAGING AND ULTRASONOGRAPHY

BY

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THESIS
Submitted in partial fulfillment of the requirements for the degree of Master of Science in VMS-Veterinary Clinical Medicine in the Graduate College of the University of Illinois at Urbana-Champaign, 2010

Urbana, Illinois

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ABSTRACT

The objective of this study was to compare ultrasonography and low field MR imaging for evaluation of equine tendon injuries over a 16 week period. The sample population was the superficial digital flexure tendon of eight young adult horses. The percentage of experimentally-induced tendon injury was evaluated in cross-section at the maximal area of injury using both ultrasonography and MR imaging at 3, 4, 6, 8, and 16 week time points post collagenase injection. MR signal intensities (T1-weighted, T2-weighted, and STIR sequences) and tendon histology (collagen content, proteoglycan content, and collagen fiber alignment) was performed at the same time points. At four weeks following collagenase injury, the area of maximal injury assessed on cross-section was similar between ultrasonographic and MR imaging. In older lesions, ultrasonography significantly (P<0.001) underestimated the area of maximal cross-sectional injury by approximately 18 % when compared to MR imaging. Signal intensity of lesions on T1-weighted images was the most hyperintense of all sequences, with lesions on STIR images being slightly less hyperintense, and the T2-weighted images the most hypointense. Tendon lesion intensity was significantly higher than the normal deep digital flexor tendon. Histologically there was a decrease in proteoglycan content, an increase in collagen content, and minimal change in fiber alignment during the 16 weeks of the study. Ultrasonography may be underestimating the extent of tendon damage in chronic cases of tendinitis. Low field magnetic resonance provides a more sensitive way to evaluate tendon injury, and should be considered in cases of tendinitis of greater than 4 weeks duration.
ACKNOWLEDGMENTS

I would like to thank my advisors. Firstly, to acknowledge Dr. Chris Byron for always providing excellent guidance and support, no matter how busy he was. Secondly, Dr. Allison Stewart who was so helpful in pushing me to complete my project and providing both the time and resources to finish this project. Finally, I would like to thank Drs. Matt Stewart and Santiago Gutierrez Nibeyro for their assistance in writing and always being available to answer questions or lending insight into my paper or project.

To my fiancée, who always gave me the support I needed when I was working and was always understanding of my schedule, and to my father, who always gave me support and guidance even when I wasn’t sure I could do it. To my mother, for always listening and being interested in what I did. Finally, to my sister for always believing in me.
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CHAPTER 1
INTRODUCTION

Lameness in the athletic horse is the most common cause of loss of use [Dyson, P.K. 2008; Jeffcott, L.B. 1982; Olivier, A. 1997; Perkins, N.R. 2005; Rossdale, P.D. 1985]. Superficial flexor tendonitis is a common career injury in sport horses. [Dyson, S.J. 2004]. When severe or with recurrence it can result in early retirement or destruction of the affected horse [Rossdale, P.D. 1985]

By definition, tendons passively transfer forces generated by muscles to the opposite side of a joint. The flexor tendons of the horse have developed to support the distal aspect of the limb during normal weight-bearing [Ross, W.S. 2003]. The flexor tendons also act elastically to help store energy for more efficient locomotion. The superficial digital flexor tendon (SDFT) has muscle fibers obliquely oriented, so maximal power can be obtained while minimizing contraction distance. The short muscle fiber and small contractile movement of the SDFT supports its capacity for shock absorption, through its innate elasticity [Dyson, S.J. 2004]. The elastic nature of tendon allows the horse to maintain high speeds while minimizing energy expenditure [Alexander, R.M. 2002].

Injury to the flexor tendons are of concern because of the limitations of tendon healing. When a tendon is injured, the resultant scar tissue is inferior to normal tendon and prone to reinjury, at a rate of anywhere from 56-66% [Dyson, S.J. 2004]. The body’s goal is for the repair process to return the tendon to as close to biomechanically normal as possible. There are two ways this can be achieved. Weaker tendons can compensate by increasing cross-sectional diameter to achieve strength closer to normal. The healing tissue can also mimic normal tendon by developing a well organized parallel fiber alignment.
Current therapies for tendinitis include conservative and surgical treatments. Currently, a paradigm shift in treatment is occurring with the introduction of tissue regenerative therapy. Along with the introduction of new treatment options, there has been advancements in diagnostic tools available for the diagnosis and monitoring of injured tendons. The increasing availability of magnetic resonance (MR) imaging and its increasing use in human medicine, due to its reproducibility and minimal operator error, have made it one of the diagnostic modalities of choice[Parizel, P.M. 1995; Shalabi, A. 2001]. Traditionally, tendon and ligament injury diagnosis utilized ultrasonography, but with the increase in MR facilities and the diagnostic benefitis associated with MRI, there has been a shift toward MR imaging. The purpose of this study was to compare ultrasound (US) and MR as imaging modalities to evaluate repair of superficial digital flexor tendinitis induced by collagenase.
CHAPTER 2
LITERATURE REVIEW

Superficial digital flexor tendinitis accounts for up to 52% of limb injuries in National Hunt horses [Williams, R.B. 2001]. In Thoroughbred racehorses, 46% of limb injuries are due to strain of the superficial digital flexure tendon [Williams, R.B. 2001]. Tendinitis is defined by tendon tenonopathy which results in disruption of the normal collagen fiber pattern. A complete understanding of the healing and treatment of tendinitis is not known at this time [Ross, W.S. 2003].

Structure and Function of Tendons

Anatomy

Tendons are divided into two categories: weight-bearing tendons and positional tendons. Weight-bearing tendons are more elastic, helping them store energy during the process of locomotion. Positional tendons require more stiffness for accurate placement of the limb or digit [Ross, W.S. 2003].

The superficial digital flexor (SDF) muscle of the forelimb is located in the middle of the flexor groups of muscles between the flexor carpi ulnaris and the deep digital flexor. The SDF muscle originates from the medial epicondyle of the humerus and a ridge on the caudal radial surface, just distal to the mid diaphysis. The SDF tendon (SDFT) inserts on the proximal eminencies of the middle phalanx, palmar to the collateral ligaments, and the distal aspect of the proximal phalanx [Ross, W.S. 2003] (Figure 1).
Structure

Tendons are complex organs composed of increasing sized fiber subunits arranged to form a larger structure (Figure 2). They are composed of fascicles, which are then composed of increasingly smaller subunits of fibers and fibrils. Collagen fibrils are synthesized as a triple helical pro-collagen molecule. As the animal ages, adjacent fibers fuse together, increasing the size of the collagen fibril. The fibers are connected and surrounded by endotendon, a continuation of the epitendon, the outer connective tissue of the tendon. Within the endotendon lies the majority of the nerve and blood supply to the tendon. The elastic nature of the equine flexor tendons allows loading and unloading of the tendon with a very small loss of energy (5%) in the form of heat. Almost 95% of applied energy is stored and released as kinetic energy [Ross, W.S. 2003].

Tendons are composed of both tenocytes and extracellular matrix. The overwhelming majority of their composition is extracellular matrix. The extracellular matrix is predominantly water (65% wet weight), collagen (35% wet weight), and non-collagenous glycoproteins (5% wet weight). Of the dry weight, collagen makes up approximately 80%, the predominant type being collagen I (>95%). The strength of tendons derives from the bonds between fibrils, not the structure of the actual fibril [Goodship, A.E. 1994]. The fibrils are strong; the strength of tendon comes from the covalent cross-linking of type I collagen, between hydrolysine and lysine residues. Non-covalent cross-links are created by proteoglycans and glycoproteins, which coat the collagen fibrils. These bonds play a significant role in the mechanical properties of tendons [Ross, W.S. 2003].

Cartilage oligometric matrix protein (COMP) consists of five subunits bound by disulfide bonds at their N-termini to form a five-armed protein which interacts with other
matrix components. [Hedbom,E. 1992]. COMP is distributed in tissues that resist load and is therefore abundant in tendons and ligaments as well as cartilage. Levels within tendons increase with growth until skeletal maturity and then slowly decline over time. The exact role of COMP is unknown at this time, however it has been shown to be important for tendon and ligament development. With mutation of the human COMP gene, people develop pseudochondroplasia characterized by laxity in tendons and ligaments, a short stature, and an early onset of osteoarthritis [Hecht,J.T. 1995]. In horses, there has been significant correlation between a tendon’s ultimate tensile strength and the COMP levels within the tendon at skeletal maturity (figure 3). COMP binds fibrillar collagens and also play a role in the organization of the collagen network of tendons and ligaments [Smith,R.K. 2000].

Proteoglycans possess a protein core and one of more side chain of sugars, called glycosaminoglycans (GAGs). There is a great diversity in the sugar side chains that attach to the protein core, resulting in a high degree of variability in the type and length of the proteoglycan. Proteoglycans can be classified as small or large chain proteoglycans. Large proteoglycans contain large numbers of GAG molecules, taking on a bottle-brush appearance due to the negative charge associated with them and the repulsive nature of multiple negative charges in close proximity. This assists in trapping water molecules, which helps to resist the compressive forces placed upon them, particularly in the metacarpophalangeal region [Rees,S.G. 2009]. The smaller proteoglycans are likely responsible for providing the electrostatic cross linking that occurs between fibrils [Danielson,K.G. 1997].
Degeneration

There are two stimuli for tendon degeneration; mechanical and physical influences. Mechanical influences are a sudden over-extension or low-grade forces that result in cumulative fatigue, resulting in microdamage of the tendon matrix. Cumulative microdamage of the tendon can eventually lead to more serious injury [Patterson-Kane, J.C. 2009].

Physical influences are also thought to play a role and the hysteresis generated during exercise has been shown to generate temperatures up to 45 degrees Celsius in the center of the tendon [Wilson, A.M. 1994]. These temperatures have been proven to be detrimental to other types of cells. While tenocytes seem to be more resilient in vitro, there is still the potential for damage to the extra cellular matrix and surrounding structures from the increase in temperature [Wilson, A.M. 1994; Hosaka, Y. 2005].

Sub-clinical pathology within tendons has been diagnosed in asymptomatic horses at post-mortem examination. In addition, there are a large number of horses which have clinical disease in one limb and sub-clinical pathology in the opposite limb [Birch, H.L. 2008]. There are numerous studies that show deterioration in tendon quality and strength with age as well as with repeated loading [Stanley, R.L. 2008].

The strain experienced by a normal tendon varies, based on the performance discipline and intensity of the exercise that is being performed. The peak maximal forces are responsible for initiating clinical detectable tendinitis. After the tendon has been affected by degenerative changes, the force required to cause damage decreases; therefore the tendon is more prone to injury or re-injury [Birch, H.L. 2007; Birch, H.L. 2008; Hosaka, Y. 2005; O'Brien, M. 1997]. Factors that increase the peak loading of the SDFT can also increase the
risk of development of clinical tendinitis. The SDFT is preferentially loaded at the early stages of the stride [Goodship, A.E. 1994; Riemersma, D.J. 1996, Platt, D.P. 1991], therefore is the time of highest risk of injury. Increased speed has also been correlated with an increase risk of SDFT injury in racehorses [Williams, R.B. 2001]. Many SDFT injuries occur towards the end of a race or event when muscle fatigue develops. This fatigue causes an increase in peak load on the SDFT, with resultant increased risk of tendinitis.

**Stages of Tendon Injury**

Tendon injuries may have a clinical or sub-clinical phase depending on the present clinical signs. In addition, the clinical phase can be divided into acute inflammatory phase, sub-acute reparative phase, and chronic remodeling phase [Ross, W.S. 2003].

The acute inflammatory phase begins at the onset of injury and last 1-2 weeks depending on the severity of the disease and the treatment instituted at the time. The characteristics of this phase are inflammation, intratendonous hemorrhage, edema, hyperemia, and infiltration of the area with inflammatory cells [Ross, W.S. 2003].

The sub-acute reparative phase starts a few days after the injury and peaks around 3 weeks [Fackelman, G.E. 1973]. There is a strong angiogenic response and filling of the damaged tissue with fibroblasts. It is these cells that are responsible for synthesizing scar tissue. The predominant collagen type synthesized during this initial phase of healing is type III. In the initial stage of repair, the tissue that replaces the damaged tendon is much weaker than normal tendon, which predisposes the area to re-injury and an recrudescence of phase 1 and 2 injury, increasing the severity of damage [Ross, W.S. 2003].
The chronic remodeling phase takes place after several months. During this phase, there is a conversion of type III fibers to type I, the major component of normal tendon. Although this phase is important to restoring the strength of the tendon, the repaired tissue is often inferior to normal tendon, even at its strongest. This is the time when controlled exercise is important to try and promote the most appropriate remodeling of the injured area [Ross, W.S. 2003].

Clinical Signs

The onset and severity of clinical signs for SDFT injury depend widely on the location, type, and severity of the injury. When evaluating a tendon for injury, clinicians need to evaluate the lameness (if present), swelling and thickening, heat, response to digital palpation, and appearance of the tendon from multiple angles. The degree of lameness often depends on the area of the tendon that is injured and the severity of injury. Swelling will not always be present with a tendon injury, especially if it’s not in the acute stages of the injury. The degree of sensitivity to digital palpation of the area will vary with the individual patient was well as the significance of the injury. Visual assessment of the injured limb is also important to help assess the limb at multiple angles to determine if there is asymmetry [Ross, W.S. 2003].

Diagnosis

The diagnostic approach to flexor tendon injuries of the equine distal limb is extremely complex. An initial thorough physical examination along with palpation, observation of gait/lameness, and analgesic confirmation of the affected area are critical.
After locating the area that is affected, further diagnostics can be implemented to determine the cause and severity of the disease process. These include ultrasonography, thermography, radiography, nuclear scintigraphy, computed tomography, and magnetic resonance imaging. All of these imaging modalities are useful, though they are each unique in their ability to assist in diagnosis and differentiating of disease.

**Treatment of Tendon Injuries**

**Medical Treatment**

The method of treatment varies depending on the phase of injury the tendon. In the acute phase of tendon injury, there is a wide range of possible treatments. Most involve some form of rest, anti-inflammatory therapy, typically a non-steroidal anti-inflammatory agent, some form of cold therapy, and supportive bandaging. At this time, it is also important to assess the area and determine the extent of the injury, which is commonly done thorough physical exam and ultrasonographic evaluation. [Ross, W.S. 2003; Reef, A.B. 1998; Rantanen, N.W. 1998].

The damage that follows acute trauma or repetitive loading of the tendon results in a prolonged period of disability. It is uncommon for the repair process to restore a damaged tendon to its normal morphologic and functional characteristic [Ross, W.S. 2003; Thorpe, C.T. 2010]. In Thoroughbred horses, there is a high morbidity associated with injury to the flexor tendons as well as a prolonged period off work. The incidence of flexor tendinitis among racehorses has been reported to range from 46% [Williams, R.B. 2001] and 66% in sport horses, with re-injury rates as high as 56%[Fortier, L.A. 2008].
Treatment protocols and rehabilitation plans during the sub-acute phase differ significantly between individuals. Individual clinician preference and experience play a significant role in the final treatment decision/protocol. Occasionally, the decision is made to provide symptomatic treatment while still maintaining the horse in work. With this option, it is important, as it is in the acute phase, to minimize the chance of re-injury. The one important similarity in all treatment protocols is the necessity for routine evaluations, particularly ultrasonographic evaluation, to assess the progression of healing and to insure there is not a recurrence or progression of the original injury [Goodship, A.E. 1994; Ross, W.S. 2003].

Long term treatment plans vary among individuals. They can be as simple as a controlled exercise program and gradual return to work, depending on the severity of the injury [Gillis, C. 1997]. Occasionally, the addition of an irritant is also added to the treatment protocol. Internal and external methods of “blistering” have been used for decades; however there is little scientific data to support its use, other than a small amount of data to suggest that it increases angiogenesis [Silver, I.A. 1983].

There have been many attempts at scientific advancement in the area of medical treatment for tendon and ligament injury. One of these areas is that termed “regenerative medicine”. By definition, the term regenerative medicine refers to treatment with the goal to restore the normal architecture as well as the biomechanical function of the affected tissue. Since the focus of tendon treatment is to restore the tendon to as close to normal as possible, many therapies have focused on regenerative repair [Fortier, L.A. 2008; Lacitignola, L. 2008].

Intra-lesional therapies including polysulfated glycosaminoglycans (PSGAGs) [Yoon, J.H. 2005], hyaluronic acids (HA) [Gaughan, E.M. 1991; Wiig, M. 1996], and beta-
aminopropionitrile fumarate (BAPN) [Dahlgren,L.A. 2001] have been evaluated in the hope that they would improve tendon healing after injury. Some other areas that have been investigated include acellular bio-scaffolds, various growth factors and the use of mesenchymal stem cells [Fortier,L.A. 2008]. The majority of the therapies previously mentioned have advantages in tendon regeneration but also have some important limitations.

**PSGAGs.** PSGAGs have been shown to inhibit the activity of metalloproteases (MMP) and minimize the activation of macrophages [Dowling,B.A. 2000]. PSGAG injection also results in histological improvement in collagen fiber organization in a collagenase-induced SDFT injury, at 5 months post-injury [Moraes,J.R. 2009]. However, they do not have any effect on proteoglycan production by fibroblasts. Success rates vary from 46% to 76% and another study showed no difference between treatment and control groups for horses returning to function, showing that there are inconsistencies with outcomes between PSGAG-focused studies [Marr,C.M. 1993; Dyson,S.J. 2004].

**Hyaluronic Acid.** Intralesional injection of HA has shown minimal to no improvement when compared to conservative management. However, intrathecal HA has demonstrated a decrease in inflammatory cell infiltration, a reduction in intralesional hemorrhage, and a decrease in adhesion formation within the tendon sheath, compared to controls [Dyson,S.J. 2004].

**Beta-Aminopropionitrile Fumarate.** BAPN binds to the enzyme lysyl oxidase, temporarily blocking the cross linking of collagen fibers. This is thought to improve the
quality of the repair, in combination with controlled exercise, by allowing the cross-linking to develop in a more parallel alignment. By delaying cross-linking, BAPN results in a more physiological repair. With the use of BAPN, a normal ultrasonographic appearance of clinically injured tendons was seen at 20 weeks post treatment [Fortier,L.A. 2008; Genovese, R.L. 1986]. However, BAPN has also been shown to reduce collagen synthesis in equine explants in vitro, which could result in less overall collagen synthesis in a healing tendon [Dahlgren,L.A. 2001].

**Urinary Bladder Matrix.** Urinary bladder matrix (ACell), derived from the basement membrane of porcine bladders, has been investigated as an inductive scaffold following tendon injury. The use of ACell showed no significant effect compared to control groups in an experimentally induced model of tendonitis using collagenase [Fortier,L.A. 2008]. However, it was demonstrated that ACell delivers extracellular matrix components and growth factors to the damaged region of tendons, and may attract mesenchymal stem cells through the breakdown of bioactive products [Beattie,A.J. 2009; ].

**Growth Factors.** The goal of growth factors is to stimulate cell proliferation, increase extracellular matrix formation and promote vascular ingrowth of the affected area(s). They also help to down regulate catabolic matrix-degrading agents such as interleukins and matrix metalloproteinases [Molloy,T. 2003; Tang,J.B. 2003; Zhang,F. 2003; Dahlgren,L.A. 2002]. Growth factors evaluated for tendon repair include insulin-like growth factor-I (IGF-I), platelet-derived growth factor (PDGF), bone morphogenetic protein-12 (BMP-12), transforming growth factor-β (TGFβ), vascular endothelial growth factor (VEGF),
growth/differentiation factor 5 (GDF-5), and basic fibroblast growth factor (bFGF) [Molloy,T. 2003; Tang,J.B. 2003; Zhang,F. 2003; Dahlgren,L.A. 2002].

IGF-1 has been investigated extensively for tendon repair. It has been shown to return damaged fiber pattern back to a near normal ultrasonographic pattern and also improved the mechanical properties of the repaired tissue in a collagenase-induced SDFT injury model [Dahlgren,L.A. 2002,]. These outcomes were similar to a previous study that compared conservative therapy and intralesional injection with BAPN, PSGAGs, and HA [Dyson,S.J. 2004].

**Platelet-Rich Plasma.** Platelet-rich plasma (PRP) is obtained through centrifugation or filtration of venous blood to obtain a platelet concentrate fraction for injection. Due to the concentration of platelets, PRP is an excellent natural reservoir for numerous growth factors such as PDGF, TGF-β, and VEGF. All these factors have been shown in vivo and in vitro to enhance tendon regeneration and healing [Molloy,T. 2003; Tang,J.B. 2003; Zhang,F. 2003; Dahlgren,L.A. 2002]. The growth factors are thought to be released through the degranulation of platelets, as along with other products that promote tissue repair and influence the natural response to inflammation and angiogenesis. The fibrin scaffold deposited after injection of the PRP allows cell migration and retains the growth factors in the area of injury. One of the disadvantages of PRP is its relatively acellular content [Fortier,L.A. 2008]. Tendocytes cultured in pure PRP showed an increase in matrix molecule gene expression characteristic of tendon, an increase in the collagen type I/type III ratio without an increase in catabolic MMP expression [Schnabel,L.V. 2007].
**Progenitor Cells.** The use of progenitor or mesenchymal stem cells for tendon injury has become increasingly popular. It has been demonstrated that multiple tissue types: adipose, bone marrow and tendon, contain progenitor cells [Stewart, A.A. 2009]. When looking at the treatment of ligament and tendon injuries, tendon-derived, bone marrow derived and adipose-derived cells are the most commonly researched populations [Fortier, L.A. 2008].

**Autogenous Bone Marrow-Derived Stem Cells.** The use of bone marrow and bone marrow-derived cells has increased over the last few years. Their use is supported by the results of experimental studies that have implanted them into surgically created lesions and shown an improved outcome in tissue organization, composition, and mechanics when compared to controls [Young, R.G. 1998; Awad, H.A. 1999]. Four methods are currently used clinically; the direct intralesional injection of bone marrow, injection of the concentrate generated from centrifugation of bone marrow, the injection of the PRP-like material, and administration of a cultured cell population derived from an autogenous bone marrow aspirate [Fortier, L.A. 2008]. All of these techniques have advantages and disadvantages. The majority of the scientific data address the efficacy of expanded cell populations. These studies have shown a re-injury rate of 13-18% for horses that went back to National Hunt racehorses and 13% for sport horses. This is an improvement over previous analysis of the same categories of horses [Dyson, S.J. 2004].

**Adipose-Derived Stem Cells.** Adipose-derived stem cells have also demonstrated the ability to differentiate, though to a slightly lesser degree than bone marrow-derived cells [Im, G.I. 2005; Sakaguchi, Y. 2005]. There are no large studies looking at the responses of horse
treatment using adipose-derived stem cells. There has been one small study that suggests there may be benefits to their use, based on results from a collagenase-induced model of tendon injury [Nixon, A.J. 2008].

There have been relatively few studies addressing treatment of tendon injuries with tendon-derived stem cells. Progenitor cells which can differentiate into tendon, muscle, fat and bone are present in tendon [Bi, Y. 2007; Stewart, A.A. 2009]. These cells, derived from the tissue requiring repair, have a potential advantage over those isolated from other tissue sources, though this has not been experimentally proven.

While a majority of the treatments listed above have been used successfully to help treat tendon injury in horses, none of them are perfect. All treatments have advantages and disadvantages that need to be weighed when determining the treatment protocol of choice for specific tendon injuries. Ideally a treatment will involve all three aspects of regenerative repair, providing a scaffold, growth factors, and cells.

**Surgical Treatment**

There are limited surgical treatments available for SDFT injury. Transection of the accessory ligament of the SDFT helped to return TB horses back to racing [Hogan, P.M. 1995]. There have been more recent reports with less favorable results, showing a 51% return to racing for 5 or more starts, but average earnings were decreased in comparison to age-matched controls. Transection of the accessory ligament of the SDFT has been shown to be beneficial in Standardbred (STB) racehorses for the treatment of SDF tendinitis; 88% of treated STBs went on to race more than 5 times [Hawkins, J.F. 1995]. This treatment works well in STB racehorses, but has show minimal success in other breeds or disciplines.
Tendon splitting has been suggested as an adjunctive therapy for the treatment of SDFT injury. Initially, the procedure was used to promote neovascularization of chronic tendinitis lesions [Asheim,A. 1964]. More recently, it has been used in combination with transection of the accessory ligament of the SDFT to treat acute collagenase-induced tendinitis, with favorable results [Hawkins,J.F. 1995]. There are few clinical studies that use tendon splitting alone.

**Diagnostic Imaging of Tendon Injuries**

**Thermography.** Thermography is a pictorial representation of surface temperatures of an object and can help to indicate a site of early inflammation [Purohit,R.C. 1980]. To obtain an accurate thermographic assessment, the images must be acquired in a draft-free room and it is best to have a uniform hair coat over the area that is of interest. Bandaging or topical applications of products can generate inaccurate results [Ross, W.S. 2003].

The principle behind thermography is that dissipation of heat through radiation, convection, conduction or evaporation will show up in the superficial tissue. Given that the normal equine limb is approximately 5 degrees Celsius cooler than the body, the limb should still have a relatively uniform temperature [Ross, W.S. 2003; Stein,L.E. 1988]. Anything that cause inflammation or changes in local tissue perfusion can cause alterations in the thermographic reading [Love,T.J. 1980].

The normal flexor tendons will have elliptical, bilaterally symmetric isothermic zones [Stein,L.E. 1988; Stromberg,B. 1973]. Acute tendonitis can cause an increase in surface temperature at the area of injury,. This can occur up to two weeks prior to physical evidence of pain, lameness, or swelling [Stromberg,B. 1973]. As the damaged tendon heals,
the thermal pattern will gradually become more uniform; however it will still remain slightly elevated compared to normal tendon. With deposition of scar tissue the damaged area may begin to have a decreased surface temperature, relative to the surrounding normal tendon [Stromberg, B. 1973]. During the healing phase, the thermographic and ultrasonographic findings of the tendon do not correlate well. As healing progresses, the thermal profile of the injured area becomes more diffuse and may only generate a small overall increase in the thermal emission [Stromberg, B. 1971].

**Nuclear Scintigraphy.** Nuclear scintigraphy is commonly used to assess bone abnormalities, to identify obscure lameness, and to evaluate horses with poor performance [Ross, W.S. 2003]. Nuclear scintigraphy uses a radiopharmaceutical to obtain an image which is acquired through a gamma camera, recording the gamma rays that are released from the decaying radioactive material. The time between injecting and imaging is dependent on what tissue is of most interest. There are three imaging phases: flow, pool, and delayed-phase. Flow-phase is taken within 1-3 minutes after the injection of the radiopharmaceutical and is typically used to look at the heart and first pass of the major vasculature. The pool-phase is obtained 3-25 minutes after injection, during which time the radiopharmaceutical resides in the extracellular fluid. The pool-phase images are used for the evaluation of soft tissue such as tendon, ligaments, tendon sheaths, and bursae. The final phase is the delayed-phase which is obtained 2-4 hours after injection of the radiopharmaceutical to allow for incorporation into bone [Ross, W.S. 2003].

Nuclear scintigraphy is not a highly specific imaging modality. It does however provide information on areas of the skeleton that have increased levels of bone remodeling.
Some severe bony abnormalities can also result in increased radiopharmaceutical uptake on the pool-phase of a scan, especially if the lesion is severe enough. It is important to not misinterpret them as a primary soft tissue injury. The diagnostic accuracy of nuclear scintigraphy to detect soft tissue injuries of the equine distal limb is less sensitive and specific than an ultrasonographic examination. Nuclear scintigraphy can under-represent soft tissue tendon and ligament injury [Ross, W.S. 2003]

**Computed Tomography.** Computed tomography (CT) is made up of three components; a gantry house, the x-ray tube, and reflectors. The image that is obtained is a two-dimensional representation of a three-dimensional object, based on relative brightness. There are very few indications for using CT over MR or US when evaluating tendons, with the exception of the interface of bone and ligaments or tendons with respect to pathology. [Ross, W.S. 2003]

[Puchalski,S.M. 2009; Puchalski,S.M. 2007]

**Ultrasoundography.** Ultrasonographic evaluation for the diagnosis of soft tissue injuries in horses has been used since the early 1980s. US has been extremely beneficial in the diagnosis and reevaluation of soft tissue injuries [Rantanen, N.W. 1998]. US is currently an integral tool for the evaluation of equine tendons and ligaments, though it is not without its limitations [Rantanen, N.W. 1998]. The ability to get accurate and high quality diagnostic images is influenced by many factors; operator skill, limb preparation, and the equipment being used [Ross, W.S. 2003]. Diagnostic ultrasound uses high-frequency sound waves, typically between 2 and 13MHz. The waves are produced by the transducer and propagated into the tissue. They are then reflected back at different tissue interfaces, depending on the
density and composition of the tissue encountered. The transducer reads the reflected waves as they reach the probe and the ‘echoes’ are converted into an image by the processor on the computer [Fleisher, A.C. 1989; Rantanen, N.W. 1998]. The echogenicity (brightness) of the tissue is represented by the shades of grey evident in the picture. US waves propagate differently depending on the density of the tissue they are traveling through [Reef, A.B. 1998]. Ultrasonographic waves transmit more quickly through denser tissue such as bone, where fluid-filled structures transmit the sound waves at a much slower speed. These differences in transmission of sound waves help differentiate the different tissue structures [Fleisher, A.C. 1989]. The largest differences in US reflection are those between soft tissue and air and soft tissue and bone. The differences in tissue interface allow differentiation of structures based on their echogenicity [Rantanen, N.W. 1998; Reef, A.B. 1998].

When ultrasound waves encounter changes in soft tissue densities, this results in a given acoustic impedance (Z). The acoustic impedance is the product of the local tissue density (p) and the ultrasound propagation velocity (c). When the wave encounters a change in acoustic impedance, a portion of the wave is reflected back. The differences in acoustic impedance that occurs between most soft tissue and organs in the body is small, however there is a large differences in acoustic impedance between soft-tissue and bone or structures containing air [Reef, A.B. 1998; Rantanen, N.W. 1998; Fleisher, A.C. 1989]. Bone has high acoustic impedance compared to that of soft-tissue, and air has a very low acoustic impedance. This large difference in acoustic impedance results in nearly all the ultrasound waves being reflected back to the transducer, leaving few, if any, ultrasound waves to penetrate deeper into the tissues [Rantanen, N.W. 1998; Reef, A.B. 1998].
Ultrasound waves, like other waves, are reflected, refracted, scattered, attenuated and absorbed [Fleisher, A.C. 1989]. Reflection is defined as the portion of ultrasound returning from the boundary of a medium. The reflected sound waves are transmitted back to the transducer, and processed into an image. The ultrasound waves that are transmitted continue to deeper tissue to be reflected or transmitted when the next medium boundary is encountered. Refraction occurs when the ultrasound beam changes directions, which occurs when US waves pass from one tissue density to another. Optimally, most or all ultrasound waves are reflected back to the receiver and wave refraction is minimized [Rantanen, N.W. 1998; Reef, A.B. 1998].

The overall resolution of an US image depends primarily on the axial and lateral resolutions of the acquisition. Axial resolution is the ability to determine a dot along the path of the ultrasound waves. The pulse length determines this, which is determined by the probes frequency [Rantanen, N.W. 1998]. The axial resolution is directly related to the frequency of the probe. Lateral resolution is the minimum distance two dots can be from each other, at the same depth, and still be distinguished from each other. The lateral resolution usually decreases with an increase in depth of view, but is best at the focal zone(s) [Fleisher, A.C. 1989]. Ultrasound waves also attenuate, scatter, reflect, and refract, contributing to the image that is visible on the screen. [Reef, A.B. 1998; Rantanen, N.W. 1998]

**Magnetic Resonance Imaging.** MR imaging has had an important impact on the evaluation of human orthopedic disorders [Bassett, L.W. 1989]. MR imaging allows evaluation of cortical bone, bone marrow, ligaments, joint capsule, articular cartilage, muscle and tendons with a higher sensitivity than has been possible with other imaging modalities [Bassett, L.W.
Due to the high accuracy of MR imaging, it has become the diagnostic and pre-operative imagine modality of choice for human orthopedics and sports medicine physicians, which shows the huge impact that this imaging modality has had in sports medicine [Helms, C.A. 2002].

Advantages of MR imaging include visualization of the anatomy in multiple planes, with thinner image sections and superior soft tissue detail. The disadvantages of MR imaging are the non-specific nature of many of the findings, questionable correlation to the cause of the injury, the high cost of examinations, and the limited availability of MR systems [Bassett, L.W. 1989]. Due to the non-specificity of many MR imaging findings, MR evaluations should be performed and interpreted with knowledge of the results of other imaging examinations and physical examination results [Bassett, L.W. 1989; Seeger, L.L. 1989].

Magnetic resonance imaging works by observing the way protons in the body respond to an external magnetic field. Protons have a positive electrical charge; this charge spins with the proton as it spins around its axis. This moving electrical charge creates an electrical current, which induces a small magnetic force or magnetic field [Kaplan, P.A. et al. 2001]. Since they are, by definition, small magnets, they align themselves to the external magnetic field. In order to obtain a sufficient signal for imaging, a strong homogenous magnetic field is required. MRI is a two-step process: in the first stage, the proton spin orientation is manipulated by an assortment of applied magnetic fields. In the second stage, the change in orientation of the proton through its interaction with the magnetic field is detected by a coil detector. While, the signal measured from an individual proton is very small, the image is
obtained from the sum of all proton signals, resulting in a significant enough signal to be interpreted [Haacke, E.M. 1999].

Hydrogen is the atom used for magnetic resonance imaging, because of its abundance in the body and its relative stability. The magnetic, or dipole, moment of an atom is the tendency to produce motion, and is the result of the atom’s angular moment or net spin of the nucleus. In the absence of an externally applied magnetic field, the vectors of these magnetic dipole moments are randomly orientated. Once exposed to a strong magnetic field, the dipoles tend to become magnetized, resulting in them becoming aligned with the magnetic field in either a parallel or anti-parallel direction [Helms, C.A. 2002; Bassett, L.W. 1989]. Slightly more of the hydrogen protons will align themselves parallel to the magnetic field because it takes less energy than to be oriented anti-parallel. As each hydrogen nucleus is spinning on its axis, the influence of the external magnetic field produces an additional spin, or wobble called ‘precession’. The frequency (speed) of this ‘precession' is known as the resonance frequency and is proportional to the strength of the applied magnetic field. As the magnetic strength of the magnetic field increases, the precession frequency becomes greater [Bassett, L.W. 1989; Haacke, E.M. 1999].

In order to obtain an MR image, an external electromagnetic wave is sent into the patient, 90 degrees to the external magnetic field. This short burst of electromagnetic energy is termed a radio frequency (RF) pulse. This causes an exchange of energy from the RF pulse to the proton, causing a disruption in its alignment. This exchange is termed ‘resonance’ [Kaplan, P.A. et al. 2001]. The RF pulse also results in a transversal magnetization of the precessing protons. This is due to the fact that when the protons, which are precessing in random directions, exchange energy with the RF pulse, they point in the
same direction and the sum of their magnetic vectors is directed to the side that the precessing protons are pointing. This transversal magnetized vector moves with the precession protons, so that it is moving in a circle. As the proton moves towards and away from the center of the magnetic field, this constant changing position induces an electric current. This electric current moves around the magnetic field and produces a signal which is processed into the MR image [Haacke, E.M. 1999]. The signal is identified by a gradient according to its precessional frequency. There are three gradients within the magnet for image formation. They are situated with respect to the bore of the magnet, and are termed the Z (long), Y (vertical), and X (horizontal) axes. The point where all three gradients intersect is called the isocenter of the magnet [Haacke, E.M. 1999].

If the RF pulse continued, the signal obtained would not change because the magnetic field associated with the individual protons would still remain constant. Once the RF pulse is turned off, the transverse magnetization that was induced by the RF pulse begins to dissipate. This process is termed transverse relaxation. Concurrently, the longitudinal magnetization decreases. This is termed longitudinal relaxation. The energy that the protons give off as they change phases/states is released into their surroundings, termed the lattice. The process of longitudinal relation is also termed spin-lattice-relaxation due to the exchange of thermal energy to the surrounding lattice while protons return to their lower energy state. The total time it takes for the longitudinal magnetization to recover is described by the longitudinal relaxation time, and as a time constant- $T_1$. $T_1$ is defined as the time at which 63% of the original longitudinal magnetization has been reached. If the magnetic field of the MR was homogenous it would be extremely difficult to obtain a recognizable image. However, due to
the non-uniform nature of the magnetic field and the influence of protons on their neighboring protons, an image is able to be obtained [Haacke, E.M. 1999].

When the RF pulse is stopped, the transverse magnetization also dissipates. The time it takes to resolve is termed the transverse relaxation time and is described as the time constant-$T_2$. $T_2$ is defined as the time when transverse magnetization as decreased to 37% of its original value. The process of transverse relaxation is also called the spin-spin relaxation, due to the underlying interactions of spinning protons on each other [Haacke, E.M. 1999].

The specific information derived from MRI can vary according to the particular sequences employed to acquire images. Fast spin echo imaging is the most commonly used method of MR image formation and it represents a good technique for description of MR image formation. Before the MR signal of a sample of tissue within the magnet can be generated and detected, the protons must undergo three additional manipulations: (1) the net magnetic vector of the protons must be flipped 90° from the parallel position into the transverse plane; (2) the spins of the protons must be in phase; and (3) the spins must be moved into a higher energy level. All of these conditions are accomplished by the application of electromagnetic energy offset 90° from the main magnetic field which is called radiofrequency (RF) pulse. The application of an RF pulse that causes resonance to occur is called excitation [Bassett, L.W. 1989; Westbrook, C. 2005]. When the RF pulse is turned off, the protons move back to a lower energy state and thus emit RF energy which is the MR signal. This process is called relaxation. The amplitude of the MR signal is proportional to the number of spins in the sample or its proton density. When more protons are present, the intensity of the magnetization is increased and the signal detected by the RF receiver coil is greater [Westbrook, C. 2005; Haacke, E.M. 1999].
In order to obtain an image, the system must be able to locate signal spatially in three dimensions, so that each signal can be positioned at the correct point in the image. The first step involves location of the slice (slice selection). Once the slice is selected, the signal is located or encoded along both axes of the image (frequency and phase encoding). These tasks are performed by the gradients [Westbrook, C. 2005]. Gradients are alterations to the main magnetic field and are generated by certain areas of the bore of the magnet from where current is passed. The passage of current induces a gradient magnetic field, which either subtracts from or adds to the main static magnetic field B0. The magnitude of B0 is altered in a linear fashion by the gradient coils, thereby the magnetic field strength and the precessional frequency experienced by the nuclei situated along the axis of the gradient can be predicted (spatial encoding) [Westbrook, C. 2005; Haacke, E.M. 1999].

Depending on the location on the bore of the magnet, the nuclei will have an increased precessional frequency or decreased precessional frequency. Therefore, the position of the nucleus along a gradient can be identified according to its precessional frequency. There are three gradient coils situated within the bore of the magnet; Z (long), Y (vertical) and X (horizontal) [Haacke, E.M. 1999; Westbrook, C. 2005]. Another important term is the ‘magnetic isocenter’ which is the center of the axis of all three gradients and the bore of the magnet. The magnetic field strength and precessional frequency remain unaltered even when the gradients are switched on. When a gradient coil is switched on, the magnetic field strength is either subtracted from or added to B0, relative to the isocenter [Westbrook, C. 2005].
The application of all the gradients selects an individual slice and produces a frequency shift along one axis of the slice and a phase shift along the other. The system is now able to locate an individual signal within the image. When data of each signal position are collected, the information is stored as data points in the K space. K space is a spatial frequency domain where information about the frequency of a signal and where it comes from the patient is stored. In order to obtain an image, it is necessary to fill different lines of K space with data; however the K space is not the image. Each data point contains information for the whole slice as the frequencies that represent it come from the whole echo and the echo comes from the whole slice [Westbrook, C. 2005]. The final step to produce an image from the acquired data is a mathematical process called fast Fourier transform (FFT). Through FFT, data is converted into signal amplitude versus its frequency. This assigns a grayscale for each pixel in the matrix of the image [Westbrook, C. 2005; Haacke, E.M. 1999].

\(T_1\) depends heavily on tissue composition, structure, and surroundings. \(T_1\) is dependent on the ability of the protons to exchange thermal energy into the surrounding area, the lattice. If tissue can easily exchange energy, then \(T_1\) is shorter. However, it is difficult for water/liquids to exchange energy because it moves too rapidly through the medium. Thus, liquids have a long \(T_1\). When the lattice is composed of medium-size molecules, where energy is more quickly transferred, \(T_1\) time is much shorter. In a stronger magnetic field, the \(T_1\) time will also be increased. This is represented by Larmor’s equation, which states that the precession frequency becomes higher when the magnetic field strength increases [Haacke, E.M. 1999].
$T_2$ relaxation occurs when protons are out of phase, which occurs from either inhomogeneities of the external magnetic field, and/or inhomogeneities of the local magnetic fields within the given tissue. With water, the local magnetic fields fluctuate very quickly, and this fast movement results in an averaging out, so there is no large net difference in the internal magnetic field. Thus, with little change in the internal magnetic field, the protons stay in step for a longer period resulting in a longer $T_2$. With impure fluids, like those containing other molecules, there is a larger variation in the internal magnetic field. The larger molecules move more slowly so the local magnetic fields do not cancel each other out as quickly. Thus, with change in the internal magnetic field, the protons become out of step more quickly resulting in a shorter $T_2$ time [Haacke, E.M. 1999].

The difference in $T_1$ between tissues can be used to differentiate them. This can be accomplished by applying multiple RF pulses at different time intervals. After the initial RF pulse is applied, there is a short period (in milliseconds) termed the time to repeat (TR), before the application of another RF pulse. This should be short so that the signal that is obtained from the tissue is influenced by the difference in longitudinal magnetization ($T_1$) of the two tissues. After a $TR_{short}$, several additional RF pulses are applied to achieve a greater difference in transverse magnetic vector. This determines the signal intensity; a large vector gives a larger signal intensity. The resulting picture is termed a $T_1$-weighted image, because it is the difference in $T_1$ signal intensities of the tissues [Haacke, E.M. 1999]. $T_1$ weighted images are the result of a short TR and TE. TE is the time to echo, which means the time between the RF pulse and receiving the signal that forms the image [Kaplan, P.A. et al. 2001].
T$_2$ weighted images are also called proton density (weighted) images. This is due to the fact that the proton density, also called spin density, is influenced by the number of protons present in the tissue. If there are no protons, there will be no signal. If there are many protons, then there will be a lot of signals. T$_2$-weighted images are obtained by having a longer TR and a TE [Haacke, E.M. 1999].

In general, a short TE and TR produce T$_1$-weighted images, whereas long TE and TR produce T$_2$-weighted images. A short TE and long TR produce a proton density (PD)-weighted imaged [Zubrod, C.J. 2007]. Inversion recovery sequences can also be acquired. This is where a 180-degree (inverting) RF pulse is applied, followed by various numbers of 90-degree and 180-degree pulses of spin-echo sequences. In these cases, T$_1$ is the time from the application of one pulse to the application of another. Varying the T$_1$ time affects tissue contrast. As an example, fat suppression on short-tau inversion recovery (STIR) sequences is achieved by using a short T$_1$ [Westbrook, C. 2005].

T$_1$ weighted and PD images produce great anatomic detail, but lack obvious visualization of many lesions. T$_2$ weighted images provide greater contrast and the ability to visualize lesions more clearly, compared to T$_1$ weighted or PD images. Different tissues behave differently according to their composition, and the images that are obtained from the tissues depends on how the sequences are weighted. Tissues with a large number of mobile protons produce higher signal intensities (appearing whiter), and tissues with low density and/or immobile protons will produce less signal (appearing blacker). The same tissue can have different signal intensities based on the type of image that is applied [Haacke, E.M. 1999]. Fluid will appear very intense (whiter) on T$_2$ weighted images, whereas fluid appears moderately intense (gray) on T1-weighted images [Kaplan, P.A. et al. 2001].
MRI equipment is categorized as being “Low-Field” or “High-Field”, depending on magnet strength. The strength of the magnetic field is measured in Tesla (T). One tesla is 10,000 gauss (G). The size of the magnetic field determines if a magnet is a low-field (< 0.3 T), mid-field (0.3-0.6 T), or a high-field (1 T and greater) magnet [Haacke, E.M. 1999]. In equine medicine, MR magnets that are currently used are typically between 0.2 and 3 T [Nagy, A. 2009]. There are very few studies in equine veterinary medicine that compare low and high intensity magnetic fields; however in human medicine, there is little difference in the clinical outcome of patients diagnosed with low-field vs high-field magnets [Parizel, P.M. 1995].

The major difference between the two clinically used magnets is the image quality that is obtained; high-field magnets produce an image of better quality. This quality is achieved by a higher signal-to-noise ratio (SNR), which results in a superior image resolution [Haacke, E.M. 1999; Kaplan, P.A. et al. 2001]. To obtain a picture of similar quality with a low-field magnet, scan times need to be increased by almost 3 times (comparing 0.1 T and 1 T magnets) [Nagy, A. 2009]. This longer scan time would increase the risk of patient movement, decreasing the image quality. The other obstacle in veterinary medicine is that patients often need to be anesthetized to minimize movement during the scan. Therefore, to obtain images of similar quality to that of a high-field magnet, time under general anesthesia would be prolonged, [Nagy, A. 2009; Sampson, S.N. 2009; Schramme, M. 2010; Franci, P. 2006; Rao, C.C. 1994]. In equine musculoskeletal imaging, the high resolution of images obtained with high-field magnets allows for detection of small and lower contrast lesions that are difficult to identify with low-field MR systems.
Ultrasonographic Imaging of Tendons

When looking at the normal structures, it is important to obtain the images with the transducer at 90 degrees to the tendon in order to view and evaluate the size, shape, texture, density, position and fiber pattern of the tendon. It is important to follow a systematic approach to ultrasonography and label images so that the scan can be repeated and compared as healing occurs (figure 4). There have been several methods for determining cross-sectional area of a tendon. One method is to measure the thickness (plantar/palmar) and the width (medial to lateral) of the tendon and then multiply these measurements to obtain the cross-sectional area [Gillis, C. 1995]. The cross-sectional area does not reflect the shape of the tendon but provides a consistent number that can be compared with subsequent measurements of the same tendon over time. Another method is to use a digital pad to draw on the image, which then uses a software program to obtain recorded the date. This method was more accurate, but is also much more time-consuming than the other methods [Rantanen, N.W. 1998; Reef, A.B. 1998]. The last method is to obtain downloaded images from the US unit and import them into a computer to obtain the measurements using a special software [Reef, A.B. 1998; Rantanen, N.W. 1998].

Normal Ultrasonographic Appearance of the SDFT

The SDFT at the mid-metacarpal region has a uniform echogenicity, parallel fiber pattern, and cross-sectional area that ranges from 0.6 to 1.2 cm² depending on breed [Gillis, C. 1995; Gillis, C. 1995; Perkins, N.R. 2004; Reef, V.B. 2001]. The cross-sectional area of the forelimbs and the hindlimbs should be comparable when measured at the same level [Gillis, C. 1995; Gillis, C. 1995; Perkins, N.R. 2005; Perkins, N.R. 2004; 134 Reef, A.B.
Tendon enlargement, with a decrease in echogenicity but normal fiber alignment is an early indicator of injury [Reef, V.B. 2001]. This indicates potential areas of fiber disruption in horses with sub-clinical injury. Typically, the area of fiber damage occurs in the center of the affected tendon and appears as a hypoechoic or an anechoic area [Marr, C.M. 1993; Rantanen, N.W. 1998; Reef, A.B. 1998; Avella, C.S. 2009].

**Normal Magnetic Resonance Imaging Appearance of the SDFT**

The SDFT has low signal intensity on MR images. The low signal is due to the low density and/or low motion of the protons in the normal tendon [Werpy, N.M. 2008]. However, normal SDFT can have small areas of gray loose connective tissue that can form a lattice appearance on the transverse view, when the images are obtained with a high field magnet [Busoni, V. 2004].

**Changes on Magnetic Resonance Imaging**

Pathologic changes in tendon tissue can be reflected by changes in size, in shape and/or alterations in signal intensity. The advantage of MR is that it can detect very subtle changes that on cannot be visualized on US. MR imaging also has the advantage of being able to visualize areas that aren’t possible with ultrasound, like the distal aspect of the deep digital flexure tendon [Zubrod, C.J. 2007].

When tendons are acutely injured they can become enlarged, which is often due to inflammatory cells and fluid within the tissue [Zubrod, C.J. 2007]. When chronic injury is present, tendons often enlarge due to scar tissue deposition in and around the area of injury.
The signal intensity changes within the injured tendon can give some information about the type and chronicity of the injury [Zubrod, C.J. 2007].

Acutely injured tendons will have increased fluid content, from hemorrhage and edema, which results in increased signal intensity on all MR images. As the tendon heals the fluid content decrease and the early scar tissue formation begins [Crass, J.R. 1992]. In the intermediate stage, there is an increase in the signal intensity from the normal tendon, though it is less than that of the acute stage [Crass, J.R. 1992].

Chronically injured tendons will contain fibrous scar tissue and adhesions around the injured aspect of the tendon. The signal intensity of scar tissue is usually of low to moderate signal intensity, though if a chronic lesion is re-injured, there can be fluid accumulation and areas of higher signal intensity. With chronic scarring, the intensity of the tissue can return to almost normal, however the shape of the tissue will typically be irregular, indicating chronic disease [Zubrod, C.J. 2007].

**Superficial Digital Flexor Tendon on Magnetic Resonance Imaging**

**T₁ Weighted Images.** It can be difficult to determine the amount of fluid that is present in an injured tendon in T1-weighted or PD images, because increased signal intensity in the soft tissues can indicate fluid, connective tissue, or immature scar tissue. It is important to compare findings with other sequences obtained during the scan to help determine the cause of the increased signal intensity [Werpy, N.M. 2008]. If there is an increase in signal intensity on T1-weighted or PD images without concurrent changes in the STIR images, then these changes can represent permanent structural changes within the tendon.
**T₂ Weighted Image.** When soft tissues contain fluid, there will be an increase in signal intensity on the T₂ weighted and STIR images. With resolution of the fluid, the signal intensities should decrease to that approaching normal [Werpy, N.M. 2008]. These changes in the signal intensity on T₂ weighted and STIR images are dependent on the stage of the healing process. During the healing process, fibrous tissue produces an immature scar initially, which has increased signal intensity on T₁ weighted and proton density images and, to a lesser degree, increased signal intensity on STIR and T₂ weighted images. With the chronic fibrosis and scarring, there is low signal intensity on all sequences. Mature scar tissue is hard to distinguish from normal tendon, but the scarred tendon typically has an abnormal shape and irregular thickening compared to the smooth and uniformity of a normal tendon [Werpy, N.M. 2008].

**Comparing Contrast-Enhanced Computed Tomography and Magnetic Resonance Imaging**

Contrast enhancement to CT imaging allows visualization of neovascularization within a tendon lesion. There is also evidence that increased permeability of the tendon due to inflammation causes a retention of contrast material in the tendon lesion [Pollard, R.E. 2004]. When using contrast-enhancement it is possible to also obtain a cross sectional image of the tendons of the distal limb [Puchalski, S.M. 2007].

In human MR imaging, it has been shown that contrast-enhancement helps to show lesion severity. When utilized with T₁ weighting, contrast enhancement was superior at showing intratendinous lesions in people with Achilles tendinopathy [Shalabi, A. 2001; Shalabi, A. 2002; Movin, T. 1998].
Both US and MR showed good correlation with the gross findings as well as with histologic findings [Puchalski, S.M. 2009]. The presence of angiogenesis was unable to be identified in either the CT or the MR images without the addition of contrast-enhancement. When contrast was used, the MR, CT, gross and histologic examinations confirmed the presence of a tendonopathy along with increased number of small blood vessels. CT with contrast may be an alternative to MR imaging in tendons of the distal limb, since they were similar to in their ability to detect lesions [Puchalski, S.M. 2009].

**Appearance of Tendon on MR and US Images**

With both US and MR imaging, tendon injuries can be reliability observed. Both modalities showed whether a lesion was present or not and the extent of lesions [Crass, J.R. 1992]. However, MR was more effective at demonstrating other changes such as limb swelling and diffusion of edema.

Histologically, normal tendon appears as linear bundles of collagen with elongated separated, fine, connective tissue septa or endotendon. Sonographically, normal tendon is very echogenic (bright) on transverse and sagittal planes. The linear orientation of the fibers is best seen on the sagittal view. The tendon is also very sensitive to angulations of the probe and any angulations can affect the apparent echogenicity. MR of normal tendon showed little to no signal on both the T₁ and T₂ weighted images, with only faint signal from the endotendon on all views [Crass, J.R. 1992].

Histologically, acute tears are characterized by edema and hemorrhage with little cell reaction. Sonographically, there is a decrease in echogenicity and an enlargement of the tendon. On MR, there is swelling of the entire limb and, on both T₁ and T₂ weighted images,
the normally low signal intensity is increased with a similar hyperintensity to that of bone marrow [Crass, J.R. 1992; Kasashima, Y. 2002].

Histologically, at the early inflammatory phase there is hyper-cellularity with fibroblasts and mononuclear inflammatory cells separating residual tendon fibers. Sonographically, the residual fibrils are linear hyperechoic areas separated by echogenic-free zones which represent foci of cellular infiltration [Crass, J.R. 1992; Kasashima, Y. 2002]. MR images show some residual swelling with dark linear structures representing residual tendon fibrils separating by area of high signal intensity of cellular infiltration [Crass, J.R. 1992].

Histologically, the late inflammatory phase is characterized by the absence of acute inflammatory changes and the presence of hemosiderin-laden macrophages, persistent mononuclear inflammatory cell infiltration and increased ground substance, suggestive of early collagen formation. These areas of residual cellularity and early collagen formation are lucent sonographically and on MR show high signal intensity on T1 and T2 weighted sequences [Crass, J.R. 1992; Kasashima, Y. 2002].

Histologically, the early fibrotic response appears as central areas represented immature fibrous tissue which is surrounded by a more cellular area of active fibrogenesis, with the endotendon remaining hypercellular. Sonographically there is little change from the more acute phase. MR shows significantly lower signal intensity in the area of the lesion than on earlier phases [Crass, J.R. 1992].

With late fibrotic healing, histologically the tendon fibers become essentially reoriented along the tendon axis, and only mild fibrillar misalignment, cellular infiltration of the endotendon and an increased number of fibroblasts persist. Sonographically, tendons
appear normal. Both MR sequences show mild persistently increased signal from the scarred area, which was likely a manifestation of persistent cellularity [Crass, J.R. 1992]. Also, in chronic tendonitis, lesions of the same level were evident on $T_1$ weighted and $T_2$ weighted images, with the signal intensity of the $T_1$ weighted being higher than the $T_2$ weighted of the same area [Kasashima, Y. 2002]
Figure 1: Equine distal limb anatomy (Wilson, AM et al 2001)
Figure 2: Representation of hierarchical structure of equine superficial digital flexor tendon (Thorpe, Clegg & Birch 2010).
Figure 3: Effect of early exercise on tendon (Smith R.K.W. et al 2002)
Figure 4: Schematic diagram of the ultrasonographic anatomy of the palmar metacarpal soft tissues (Smith and Webbon 1994)
CHAPTER THREE
MATERIAL AND METHODS

Animals

Eight clinically normal quarter horses (seven 3 year olds and one 4 year old, mean weight of 447kg ± 10.31) were used for the study. All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee. All horses in this study were evaluated to ensure they were clinically normal without any signs of tendon injury or lameness. Lameness exams and ultrasonographic evaluation of all flexor tendons were performed prior to inclusion in this study.

Timeline

All horses had MR imaging and ultrasonography of all the superficial digital flexor tendons at the end of the study (Figure 1). Magnetic resonance imaging was only performed once for each horse. In summary, both forelimb SDF tendons were evaluated 16 weeks after injury. Two hindlimb SDF tendons were evaluated from separate horses at 3, 4, 6, and 8 weeks following injury. This study evaluated 16 forelimb SDF tendons and 8 hindlimb SDF tendons.

Induction of Tendinitis

All horses had both forelimb and one randomly selected hindlimb superficial digital flexor (SDF) tendons injected with collagenasea at the mid-metacarpal/metatarsal region under ultrasonographicb guidance, as previously described [Williams, I.F. 1984; Redding,
Horses were pre-medicated with phenylbutazone (2.2 mg/kg, IV once), procaine penicillin G (22,000 IU/kg, IM once), and received a tetanus toxoid vaccine before induction of tendonitis. The metacarpal and metatarsal regions were clipped with a #40 clipper blade, aseptically prepared, and horses were sedated with intravenous detomidine (0.01-0.015 mg/kg, IV as needed). Sixteen weeks prior to MR examination, 2,000 units of filter-sterilized bacterial collagenase were injected with a 25 gauge needle into both forelimb SDF tendons. One randomly selected hind limb SDF tendon was also collagenase-injected 15 cm distal to the point of the hock at 3, 4, 6, or 8 weeks prior to MR examination. Post-injection all horses received an addition dose of procaine penicillin G and one dose of phenylbutazone. Horses were maintained on strict stall confinement for 8 weeks post collagenase injection, after which time they were handwalked twice per day for 15 minutes until the conclusion of the study.

Ultrasonography

Twenty four hours prior to MR imaging, ultrasound imaging of the injured tendon was performed using an Aloka 7.5- 13 MHz linear transducer, set at 13 MHz for the examination. Transverse and longitudinal ultrasonographic imaging planes were acquired, using a standoff, to determine the site of maximal injury within the SDF tendon. The cross-sectional areas of the induced-tendinitis lesions were obtained at the site of maximal injury and recorded as percentage of the tendon area using the measurement tools on the ultrasonography unit (Figure 2A). All images were measured using a DICOM workstation (Figure 2B).
Magnetic Resonance

All horses were pre-medicated with xylazine at (0.02 mg/kg IV). General anesthesia was induced with diazepam at (0.08 mg/kg IV) and ketamine at (2.2 mg/kg IV), and was maintained with inhalational anesthesia using isoflurane. Low field magnetic resonance images were acquired using a 0.25 Tesla MR system and a custom-designed equine extremity coil. The MR protocol used in this study is summarized in Table 1.

MR Imaging Analysis

Quantitative measurements including cross-sectional area (Figure 3) and signal intensity values (Figure 4) were obtained at the level of maximal cross-section tendon injury in all pulse sequences using the freehand region of interest (ROI) measurement tool of the DICOM workstation. In each sequence, an average signal intensity (ASI) was calculated for the injured SDF tendon and the normal DDF at the level of maximal tendon injury by dividing the signal intensity value of the ROI (20mm²) by the signal intensity value obtained from a hypointense area (background noise) beyond the margins of the limb image using a 20mm² box and placing it on the lateral side. Measurements were repeated five times.

Histology

Tendon specimens were collected from four horses for histological evaluation of the SDF tendons for a total of 16 limbs. These horses had hindlimb SDF tendon lesions created 3, 4, 6, and 8 weeks prior to MR imaging acquisition and euthanasia. In addition, all four horses provided 16-week post-injection specimens and unaltered control SDF specimens. The tendon specimens encompassed the site where the MR and ultrasonographic imaging
was performed (the maximal injury zone). The normal unaltered hindlimb SDF tendon specimens were collected from contralateral limb at the same level as the collagenase-induced lesions. Tendon samples were fixed in 4% paraformaldehyde and paraffin-embedded using standard protocols [Rich, L. 2005].

**Collagen Content, Proteoglycan Content, and Collagen Fiber Alignment**

Sections were cut to 6-micrometer thickness using a microtome and stained with Picro-Sirius Red and Toluidine blue, stains specific to collagen and proteoglycan respectively. Collagen and proteoglycan contents were evaluated using Axio Vision ® 4.7 software at 10 times magnification. Collagen fiber alignment was determined using Picro-Sirius Red stained images at 2.5 times magnification. For each specimen, ten sections, separated by at least 150 microns, were prepared. In each section, proximal, middle, and distal sites were evaluated resulting in 30 images assessed for collagen content, proteoglycan content, and collagen alignment from each tendon. Picro-Sirius Red images were analyzed using the defined analysis algorithm to assess areas for collagen as previously described (Figure 5A) [Rich, L. 2005; Whittaker, P. 1994]. Similarly, Toluidine blue-stained images were analyzed using the defined algorithm to assess areas for proteoglycan as previously described (Figure 6A) [Fung, D.T. 2009]. Three point angles were drawn on Picro-Sirius Red-stained images for the changes in collagen fiber alignment to obtain numerical values in degrees as previously described (Figure 7A and B) [Fung, D.T. 2009].
Statistical Analyses

Two-way repeated-measures ANOVA, using commercially available software\textsuperscript{9}, was used to evaluate the differences between the total cross-sectional areas determined by ultrasound and MR (T1, T2, and STIR) image analyses. In addition, MR signal intensities of the injured superficial digital flexor tendon was compared to the normal deep digital flexor tendon over time within each sequence (T1, T2, and STIR). Holm-Sidak post hoc tests were used when indicated. Values of $p \leq 0.05$ were considered significant. Descriptive data were used to report the signal intensities of each sequence (T1, T2, and STIR), as well as the histological changes in collagen content, proteoglycan content, and collagen alignment. All data were reported as mean +/- standard error.
CHAPTER FOUR

RESULTS

Percentage of Cross-Sectional Tendon Damage

The percentage of damaged tendon was not determined on four of the hindlimb SDF tendons at 6 and 8 weeks post injury due to data loss. At four weeks following collagenase injury, there was no significant (P=0.81) difference in the cross-sectional percentage of damaged tendon at the site of maximal injury when comparing ultrasonographic images to MR images (Table 2). At sixteen weeks following injury, the percentage of damaged tendon at the site of maximal injury was significantly (P<0.001) less in ultrasonographic images (approximately 18 % less) in comparison to MR images (Table 2).

Average MR Lesion Signal Intensities

At all time points, the lesion signal intensity of T1-weighted images was greatest, STIR lesion signal intensities were intermediate, and T2-weighted lesion signals were the least intense (Figure 9). On the T1-weighted images, the signal intensity of injured superficial digital flexor tendon was significantly (P=0.001) higher than the signal intensity of the normal deep digital flexor tendon at all time points following injury (Figure 10). There was no significant (P=0.264) decrease in the T1-lesion signal intensity of injured tendons over time. However, the T1-weighted images showed the largest differences in signal intensity (17.0- 12.9) between the injured and normal tendon. On the STIR images, the signal intensity of injured superficial digital flexor tendon was significantly (P=0.037) higher than the signal intensity of the deep digital flexor tendon at all time points following injury.
(Figure 11). There was no significant (P=0.66) decrease in the STIR-lesion signal intensity of injured tendons over time. On the T2-weight images, the lesion signal intensity of injured superficial digital flexor tendon was significantly (P=0.001) higher than the signal intensity of the normal deep digital flexor tendon at 16 weeks following injury (Figure 12). There was only a trend (P=0.087) for increased lesion signal intensity of the injured superficial digital flexor tendon when compared to the normal deep digital flexor tendon at three to eight weeks following injury. There was no significant (P=0.498) decrease in the T2-lesion signal intensity of injured tendons over time. However, the T2-weighted images showed the smallest differences in lesion signal intensity (2.0-3.7) between the injured and normal tendon. Individual examples of ultrasound and low field MR imaging of tendon injury at four and 16 weeks are shown in Figures 14 and 15.

**Collagen Content**

Minimal changes in collagen content were evident over time (Figure 5B). Specifically, a 10% increase in collagen was noted between three weeks and eight weeks post tendon injury. At 16 weeks post injury, 60% collagen was present when compared to the normal tendon.

**Proteoglycan Content**

In contrast, a gradual decrease in proteoglycan content was noted over time (Figure 6B). A 45% decrease in proteoglycan content was seen between three weeks and eight weeks post tendon injury. At 16 weeks post tendon injury a 2.3 fold increase in proteoglycan content was still present when compared to the normal tendon.
Collagen Fiber Alignment

Minimal changes in collagen fiber alignment were seen in the injured tendon over the 16 weeks post-injury (Figure 7C). However, fiber alignment was markedly different at all time points post-injury from those seen in the normal tendon. The collagen fiber alignment angles varied from 19 degrees to 34 degrees compared to the small 3.5 degree variations in angle derived from normal tendon.
Table 1. A summary of the MR protocol used in this study. Spin-echo sequences and T1-weighted images (SE T1), T2-weighted images (SE T2), and short tau inversion recovery (STIR) were acquired. Abbreviations: Inversion Time (TI), Echo Time (TE), Time to Repetition (TR), Spin Echo (SE), T1-weighted (T1), T2-weighted (T2), short tau Inversion Recovery (STIR), Field of View (FOV), Number of Excitations (NEX). Phase encoding was in the Y plane.

<table>
<thead>
<tr>
<th>Plane</th>
<th>Sequence</th>
<th>TR (msec)</th>
<th>TE (msec)</th>
<th>Flip Angle (°)</th>
<th>FOV (mm)</th>
<th>Matrix size</th>
<th>NEX</th>
<th>Slice width (mm)</th>
<th>Gap (mm)</th>
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</thead>
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<td>180x180</td>
<td>256x256</td>
<td>2</td>
<td>4</td>
<td>.4</td>
</tr>
<tr>
<td>Trans</td>
<td>SE T2</td>
<td>3300</td>
<td>80</td>
<td>90</td>
<td>180x180</td>
<td>256x256</td>
<td>2</td>
<td>4</td>
<td>.4</td>
</tr>
<tr>
<td>Trans</td>
<td>STIR</td>
<td>2330</td>
<td>24 (TI 85)</td>
<td>90</td>
<td>180x180</td>
<td>256x256</td>
<td>2</td>
<td>4</td>
<td>.4</td>
</tr>
</tbody>
</table>
Table 2. Percentage of tendon cross-sectional damage ± SD at ≤ 4 weeks and 16 weeks are listed according to measurements obtained from ultrasound (US) and magnetic resonance (MR). MR imaging sequences are listed as T1-weighted (T1), T2-weighted (T2), and (STIR). * Significant ($P < 0.05$) effect of imaging modality based on calculated measurements of percent cross-sectional damage for each imaging modality. Measurements based on a percentage (cross-sectional area of injury/ cross-section of the tendon)

<table>
<thead>
<tr>
<th>Time</th>
<th>US</th>
<th>T1</th>
<th>T2</th>
<th>STIR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 4 weeks</td>
<td>51.2 ± 8.9</td>
<td>54.2 ± 9.6</td>
<td>52.4 ± 7.3</td>
<td>60.8 ± 0.7</td>
<td>$P = 0.81$</td>
</tr>
<tr>
<td>16 weeks</td>
<td>41.8 ± 6.0</td>
<td>59.5 ± 13.2*</td>
<td>59.5 ± 13.5*</td>
<td>55.6 ± 14.1*</td>
<td>$P &lt; 0.001*$</td>
</tr>
</tbody>
</table>
**Timeline**

8 horses: collagenase injection of RF and LF SDF tendons

2/8 horses: collagenase injection of LH SDF tendon

2/8 horses: collagenase injection of LH SDF tendon

2/8 horses: collagenase injection of LH SDF tendon

2/8 horses: collagenase injection of LH SDF tendon

8 horses: US & MRI prior to tendon collection

**Figure 5** - Timeline for eight horses of collagenase injection of superficial digital flexor tendons. On day 0 all eight horses had the right front (RF) and (LF) superficial digital flexor tendon (SDF) injected. At 4, 8, 12, and 13 weeks prior to euthanasia two horses had their left hind (LH) SDF tendon injected with collagenase. This created two tendon SDF lesions at 12, 8, 4, and 3 weeks. All horses had ultrasound and MR imaging performed at the end of 16 weeks.
Figure 6- Ultrasonographic image demonstrating the percentage of cross-sectional tendon injury calculated at 16 weeks using the Aloka ultrasound. In this image, the percentage was calculated by dividing the cross-sectional area of tendon injured (0.12 cm²) by the cross-sectional area of the entire SDF tendon (1.69 cm²) in the area of maximal injury.
Figure 7- Ultrasonographic image in DICOM workstation demonstrating the percentage of cross-sectional tendon injury calculated at 16 weeks. In this image, the percentage was calculated by dividing the cross-sectional area (AR) of tendon injured (yellow outline, 27.09 mm²) by the cross-sectional area (AR) of the entire SDF tendon (red outline, 148.85 mm²) in the area of maximal injury.
Figure 8- T1-weighted image demonstrating the percentage of cross-sectional area (AR) tendon injury calculated by dividing the damaged area (yellow outline) by the total cross sectional area (AR) of the SDF tendon (red outline).
Figure 9- T1-weighted image demonstrating the signal intensity calculations where the average signal intensity (ASI) was calculated from five signal intensities derived from the regions of interest (20 mm² yellow box) at the maximal zone of injury divided by five signal intensities derived from the background depicted by the 20 mm² red box (ASI of injured tendon/ASI of surrounding air). AR = cross-sectional area of the square, AV = average signal intensity, SD = standard deviation of lesion signal intensities within the square.
Figure 10- (A) Representative histological image of a normal tendon stained with Picro-Sirius Red taken at 10 times magnification showing the percentage area for collagen staining in green. (B) Percentage of collagen staining calculated over time (3, 4, 6, 8, and 16 weeks) following tendon injury. (n= the number of tendons sampled for each time point)
**Figure 11-** (A) Representative histological image of a tendon lesion 16 weeks following collagenase injection, stained with Toluidine blue taken at 10 times magnification showing the percentage area for proteoglycan staining in green. (B) Percentage of proteoglycan staining calculated over time (3, 4, 6, 8, and 16 weeks) following tendon injury. (n= the number of tendons sampled for each time point)
Figure 12- (A) Representative histological images taken at 2.5 times magnification showing the angles for calculation of fiber alignment in a normal tendon and (B) a tendon that had been injured for 3 weeks. (C) Angle of collagen fiber alignment calculated over time (3, 4, 6, 8, and 16 weeks) following tendon injury. (n= the number of tendons sampled for each time point)
**Figure 13** - Signal intensity of lesions from T1-Weighted (T1), T2-Weighted (T2), and short tau inversion recovery (STIR) images taken at 3, 4, 6, 8, and 16 weeks following injury. Signal intensity of lesion calculations are given on the Y-axis and time is on the x-axis. * Lesion signal intensity significantly different between the SDF tendon lesion and normal DDF tendon at the same time point (P<0.05).
Figure 14- T1- weighted images of injured SDF tendon compared to the normal deep digital flexor (DDF) tendon. Signal intensity of lesion calculations are given on the Y-axis and time is on the X-axis. * Lesion signal intensity significantly different between the SDF tendon lesion and normal DDF tendon at the same time point (P<0.05).
Figure 15- STIR images of injured SDF tendon compared to the normal deep digital flexor (DDF) tendon. Signal intensity of lesion calculations are given on the Y-axis and time is on the X-axis. * Lesion signal intensity significantly different between the SDF tendon lesion and normal DDF tendon at the same time point (P<0.05).
Figure 16- T2- weighted images of injured SDF tendon compared to the normal deep digital flexor (DDF) tendon. Signal intensity of lesion calculations are given on the Y-axis and time is on the X- axis. * Lesion signal intensity significantly different between the SDF tendon lesion and normal DDF tendon at the same time point (P<0.05).
Figure 17- Image of tendon cross-sectional damage at 4 weeks is listed according to ultrasound (US) and magnetic resonance (MR). Representative images from each modality are shown below their area of cross-sectional injury (CSI) and their corresponding signal intensities (SI) for this injured tendon. T1-weighted (T1), T2-weighted (T2), short tau Inversion Recovery (STIR).
**Figure 18**- Image of tendon cross-sectional damage at 16 weeks is listed according to ultrasound (US) and magnetic resonance (MR) imaging. Representative images from each modality are shown below their area of cross-sectional injury (CSI) and their corresponding signal intensities (SI) for this injured tendon. T1-weighted (T1), T2-weighted (T2), short tau Inversion Recovery (STIR).
CHAPTER FIVE

DISCUSSION

Results of this study showed that the area of maximal injury assessed on cross-section in acute tendinopathies was similar between ultrasonographic and low field MR imaging in the four weeks following collagenase injection. However, in older lesions, ultrasonography had significantly smaller areas of maximal cross-sectional injury by approximately 18% when compared to MR images of the same site. These findings suggest that ultrasonography is similar to MR images for identifying the injured cross-sectional area during the acute phase of injury, but shows smaller areas at later times. Thus our hypothesis that ultrasound would have smaller areas of maximal cross-sectional injury when compared to MR images was only supported 16 weeks following injury. Magnetic resonance imaging appears to show a larger area of tendon damage in injuries of greater than 4 weeks duration injuries. Another study in which MR more accurately represents the severity of tendon injury found on histological examination following euthanasia [Kasahima, Y. 2002]. One of the limitations of this study was the palmar border of the tendon injury was less well defined using collagenase-induced tendon injury when compared to naturally occurring tendon injury.

While the lesion signal intensity on all three MR sequences decreased of time, this decrease was not significant. Lesion signal intensity on T1-weighted images was the highest of all the sequences performed and showed the greatest difference form the signal intensity of the normal deep digital flexor tendon. The lesion signal intensities on the STIR images were slightly less than the T1-weighted images. The lesion signal intensities on the T2-weighted
images were lowest and showed the least difference from the normal deep digital flexor tendon. A previous study has shown that equine tendon had a persistent increase in T1-weighted signal intensity for greater than six months following the onset of injury, while the T2-weighted signal intensity decreased over time [Kasashima, Y. 2002]. The present study had similar findings, although the horses were only followed for 4 months post injury. These horses with tendon injury of 6 months duration still had evidence of slight neovascularization and irregular collagen fiber arrangement by histological assessment. The persistence of increased T1-weighted signal intensity has been described in injured human tendons that have been healing for months to years [Shalabi, A. 2001; Shalabi, A. 2004].

Clinically, T2-weighted images may be more useful to assess tendon healing because the signal intensities on T2-weighted images decreases over time whereas the signal intensity on T1-weighted images remain high for years [Kasashima, Y. 2002; Shalabi, A. 2001]. The T2-weighted images are also less susceptible to artifact [Hayes, C.W. 1996]. Tissue T2 have a prolonged repetition time and is more sensitive to changes in water content and cellular infiltration [Benjamin, M. 2006]. Based on this evidence, the T2-weighted images may be more valuable for imaging lesions [Crass, J.R. 1992]. In contrast, evaluation of tendons over time using the STIR sequences has not been described. In this study the STIR tendon lesion signal intensity closely mirrored the T1 lesion signal intensity over time, with the exception of the 6 weeks post collagenase injury. The increase in STIR signal at 6 weeks was mediated by an increase in STIR lesion signal from one horse. Given that STIR sequences are designed to eliminate fat-derived signals, and adipose tissue is not a major component of normal or healing tendon, it could be expected that STIR data correspond closely with T1-weighted profiles. Similar to other studies [Kasashima, Y. 2002; Shalabi, A. 2004]...
this study supports that multiple MR sequences are necessary to provide the most information about injured tendons. In this study proton density sequences were not used because of the length of time required for low field magnet acquisition of the T1, T2, and STIR sequences on all four tendons.

In healing tendons there are increased amounts of glycosaminoglycan (GAG)-substituted proteoglycans deposited in the repair matrix [Kasashima, Y. 2002]. The negative charges associated with the GAG sulfate residues bind water molecules and contribute to the increased signal intensity of the lesion seen with MR over time. This GAG-mediated increase in tissue hydration would provide an increased lesion signal on T-2 and could be misinterpreted as being due to active inflammation, persistent edema, or neovascularization [Kasashima, Y. 2002]. Four horses from this study had histological evaluations performed at the site of maximal injury at 3, 4, 6, 8, and 16 weeks following injury. Histologically there was a decrease in proteoglycan content, minimal changes in collagen content, and minimal change in fiber alignment during the 16 weeks of the study. This decrease in proteoglycan content corresponded with the slow decline in T-2 lesion signal intensities seen on MR imaging over time. The lesion signal intensities detected on T1 could be due to changes in collagen fiber alignment. While our histological changes were quantitative, these changes were described as a trend based on low numbers (one tendon at time points 3, 4, 6, and 8 weeks and eight tendons at 16 weeks). This study was limited by the small number of horses in the study and limited histological samples.

Based on this study, both low field MR imaging and ultrasound are valuable diagnostic modalities for assessing acute tendon injury. In this study horses were managed post-collagenase injury with stall rest and hand walking and extrapolation of these results to
clinical cases of naturally occurring tendon injury with increasing exercise should be made with care. Allowing for this, ultrasonography may be underestimating the extent of tendon damage in cases of greater than 4 weeks duration. It is possible that the flexor tendon reinjury rate is high because practitioners increase the exercise level of the horse prematurely, on the basis of ultrasound findings of tendon pathology.

Perhaps a more critical evaluation of ultrasonographic findings could prevent tendon reinjury. For example, Genovese et al. has shown that the longitudinal fiber pattern seen on ultrasonographic evaluation is the most accurate predictor for successful return to work in racing Thoroughbreds [Genovese, R.L. 2008]. Werpy et al suggested the use of off-angled ultrasonographic images, where the ultrasound beam is not oriented 90 degrees to the tendon on cross-sectional evaluation [Werpy, N.M. 2008]. The off-angle image takes into consideration that the echogenicity of normal tendon becomes less echogenic when the angle of the beam is not oriented at 90 degrees to the fiber pattern. The off-angled technique provides an additional method for detecting small changes in echogenicity indicative of subtle injuries. Accepting these possibilities, low field MR provides a more sensitive determination of tendon abnormalities and a more detailed understanding of the cellular and tissue pathology of greater than 4 weeks duration.

It is unknown whether the T1- weighted the tendon lesion signal intensity will ever return to normal. In fact, several studies suggest the T1 lesion signal intensity will remain high [Kasashima, Y. 2002; Sampson, S.N. 2007]. This study was limited in duration and ideally these tendon lesions would be followed over several years. Not surprisingly the STIR images were similar to the T1-weighted images where fat suppression in tendon would have minimal effect. Similar to other studies [Crass, J.R. 1992], in this study the T2- weighted
images subjectively correlated best to the ultrasonographic progression of healing. In this
respect, MR should be considered for evaluations of chronic tendon injuries in horses where
a successful return to competition is critical.
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APPENDIX

a. Collagenase, purified, Worthingtin Biochemical Corporation, Lakewood, NJ
b. Ultrasound Model SSD-4000, Aloka, Tokyo, Japan
c. Phenylbutazone, Sparhwak Laboratories Inc., Lenexa, KS
d. Procaine Penicillin G, Phoenix Pharmaceutical, St. Joseph, MO
e. Tetanus Toxoid, Fort Dodge, Fort Dodge, IA
f. Detomidine Hydrochloride, Pfizer Animal Health, New York, New York
h. Xylazine, Akon, Decatur, IL
i. Diazepam, Hospira Inc. Lake Forest, IL
j. VetaKet, IUX Health, St. Joseph, MO
k. Isoflurane, Hospira Inc. Lake Forest, IL
l. 0.25 T low-field strength VetMR Grande unit Esaote Biomedica, Genova, Italy
m. Picro-Sirius Red, Sigma Aldrich, St. Louis, MO.

n. Toluidine blue, Sigma Aldrich, St. Louis, MO.
o. Axio Vision ® 4.7 software, Carl Zeiss International, Thornwood, NY.