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Equine flexor tendon imaging part 1 - recent developments in ultrasonography, with focus on the superficial digital flexor tendon

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Review

Equine flexor tendon imaging part 1 – recent developments in ultrasonography, with focus on the superficial digital flexor tendon

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Abstract

Flexor tendon injuries are a major cause of lameness in performance horses and have considerable impact on equine welfare and the wider horse industry. Ageing and repetitive strain frequently cause varying degrees of tendon micro-damage prior to the recognition of clinical tendinopathy. Whilst B-mode ultrasonography is most commonly utilized for detection and monitoring of tendon lesions at the metacarpal/metatarsal level, the emphasis of recent research has focused on the identification of subclinical tendon damage in order to prevent further tendon injury and improve outcomes. The introduction of elastography, acoustoelastography and ultrasound tissue characterisation in the field of equine orthopaedics shows promising results and might find wider use in equine practice as clinical development continues. Based on the substantial number of research studies on tendon imaging published over the past decade this literature review aims to examine the currently used ultrasonographic imaging techniques and their limitations, and to introduce and critically appraise new modalities that could potentially change the clinical approach to equine flexor tendon imaging.

Keywords: Elastography; Horse; Image; Tendinopathy; Ultrasound

Introduction

Tendon and ligament injuries are amongst the most common causes of early retirement and wastage in performance horses and account for approximately 43-54% of all musculoskeletal injuries in the equine athlete (Pinchbeck et al., 2004; Singer et al., 2008; Mitchell et al., 2020).

The majority of tendon injuries (75-93%) occurring in the equine athlete involve the superficial digital flexor tendon (SDFT), with the mid-metacarpal region of the forelimb being affected in 97-99% of cases (Ely et al., 2004; Kasashima et al., 2004; Lam et al., 2007). The prevalence of SDF tendinopathy is particularly high in racing Thoroughbreds (11-30%) and the recovery often includes a prolonged period of rehabilitation and is associated with a poor prognosis for return to high-level exercise (Lam et al., 2007; Ely et al., 2009; O'Meara et al., 2010; Kalisiak, 2012; Witte et al., 2016).

A growing body of evidence suggests that not only acute overstrain can lead to tendon injury but that the accumulation of tendon micro-damage as an effect of ageing and repetitive strain commonly precedes the development of clinical tendinopathy (Thorpe et al., 2013; Birch et al., 2016; Godinho et al., 2017; Thorpe et al., 2017; O'Brien et al., 2020). In the rapidly advancing field of diagnostic imaging, a specific focus is set on the non-invasive analysis of tendon viscoelastic properties in order to identify subclinical tendon damage and prevent injury (Docking et al., 2012; Lustgarten et al., 2015; Tamura et al., 2017a; Plevin et al., 2019).

The aim of this literature review is to provide evidence detailing the commonly used and recently developed ultrasonographic imaging techniques for the assessment of equine tendons. The current state of knowledge including implications, pitfalls and future directions of ultrasonography, elastography and ultrasound tissue characterisation for equine flexor tendon imaging will be discussed. A systematic PubMed, Medline and Google Scholar search with the following search terms was performed without restrictions: ‘tendon’ AND ‘ultrasonography’ OR ‘elastography’ OR ‘ultrasound tissue characterisation’ AND ‘equine’ OR ‘horse’. Additionally, studies were identified by searching the reference list of eligible articles (Fig. 1). Articles were excluded if they were not available in English language or if they were not published in peer reviewed journals. Additionally, references were not discussed in the manuscript where the authors did not regard them as recent or their content had already been discussed in detail in previous reviews.

Ultrasonography

Brightness-mode (B-mode) sonography displays the difference in acoustic impedance within a two-dimensional tissue cross-section and remains the modality most widely used for the assessment of tendon and ligament injuries in equine practice (Rantanen, 1982; Gaschen and Burba, 2012; Palgrave and Kidd, 2014; Berner, 2017). Tendon lesions are generally classified as core lesions, border lesion, tendon splits or diffuse decrease in echogenicity with loss of long linear parallel echoes with transverse as well as longitudinal plane ultrasonography required to determine the extent of an injury (Smith, 2008; Alzola et al., 2018).

Tendon cross-sectional area

The tendon cross-sectional area (CSA) as well as the CSA and length of a tendon lesion can be calculated with reasonable accuracy using ultrasonography (Genovese et al., 1990; Smith et al., 1994; Gillis et al., 1995a; Alzola Domingo et al., 2017; Kojah et al., 2017). Other measurements including latero-medial or dorso-palmar/plantar dimensions of tendon lesion are considered less sensitive (Reef, 2001; Smith and Cauvin, 2014).

The flexor tendon CSA may be influenced by acute changes in hoof angulation. With marked elevation of the toe, flexor tendons can appear smaller whereas significant heel elevation might lead to an increase in tendon CSA. However, a consistent impact on CSA measurements could only be demonstrated with heel or toe elevations in excess of 10° in one study (Hagen et al., 2018). Despite individual variations, conventional remedial farriery rarely alters hoof angulation greater than 5° and should therefore have limited impact on the calculation of the flexor tendon CSA (Riemersma et al., 1996; Lawson et al., 2007; Hagen et al., 2017; Hagen et al., 2018).

The age, breed and type of horse further influence the flexor tendon CSA. Reference values for the tendon CSA and echogenicity have been described for foals, young horses and adults as well as for several specific horse breeds (Table 1). Additionally, reference values are available for the proximal flexor tendons in the tarsal region (Vilar et al., 2011). It is interesting to note that besides the differences in CSA and echogenicity, breed specific differences in tendon biochemical and mechanical properties have been demonstrated recently (Ploeg et al., 2017; Verkade et al., 2019). Biomechanical testing identified a significantly higher stiffness of the SDFT in Thoroughbreds when compared to Warmblood horses which might in part explain the predisposition to SDFT injury in certain breeds like Thoroughbreds (Verkade et al., 2019).

Based on variations in functional adaption, the flexor tendon CSA does not increase in a proportional rate during maturation (Birch et al., 1999; Korosue et al., 2015). A small number of studies describe a tendon hypertrophy and reduction in echogenicity with training, but results are inconsistent and tend not to demonstrate statistically significant alterations unless clinical tendinopathy is present (Gillis et al., 1993; Birch et al., 1999; Kasashima et al., 2002; Perkins et al., 2004; Moffat et al., 2008; Avella et al., 2009; Matos Santiago Reis and Arantes Baccarin, 2010). Despite some conflicting reports, overall age, sex, height at the withers or body mass index do not appear to have a strong influence on the equine flexor tendon CSA in the adult horse (Smith et al., 1994; Gillis et al., 1995b; Gillis et al., 1995c; Moffat et al., 2008; Agut et al., 2009; Avella et al., 2009; Boehart et al., 2010a; Köster et al., 2014). Additionally, most studies have not identified a difference between limbs (left vs right forelimb), which implies that ultrasonographic examination of the contralateral limb can serve as a valid comparison provided bilateral tendinopathy can be excluded (Smith et al., 1994; Gillis et al., 1995b; Perkins et al., 2004; Moffat et al., 2008; Agut et al., 2009; Avella et al., 2009; Köster et al., 2014). A 20% increase in tendon CSA when compared to the contralateral side is regarded as a sign for tendinopathy by several authors (Smith et al., 1994; Reef, 2001; Smith, 2008; Smith and Cauvin, 2014). In case of bilateral tendinopathy, it has been suggested to analyse the SDFT:DDFT ratio to assess for tendon enlargement (Berner, 2017).

Tendon lesions with a maximal injury zone of more than 25% of the total transverse tendon CSA are usually classed as severe lesions (Reef, 2001; Smith, 2008). A recent study showed a probability of 29-35% for a successful return to racing after SDFT injury in core lesions that involved less than 50% of the tendon CSA, with a decrease to 11-16% in lesions ≥50% CSA. The study additionally found that the prognosis in cases of tendinopathy without core lesion reduced from 49-99% to just 14% if at the maximal injury zone 75% or more of the longitudinal fibre pattern was disrupted (Alzola et al., 2018).

Image acquisition during consecutive ultrasonographic examinations can be performed by different operators but CSA measurements should ideally be obtained by the same person in order to avoid inaccuracy (Pickersgill et al., 2001). An increase of a lesion CSA in excess of 10% during re-assessment is most likely indicative for a degree of re-injury and requires adjustment of an existing rehabilitation program (Reef, 2001; Smith, 2008; Smith and Cauvin, 2014; Kümmerle et al. 2019).

Tendon echogenicity

The ultrasonographic echogenicity will provide some information about the collagen density of the tendon tissue and multiple scoring systems estimating the tendon intensity (brightness), homogeneity and the degree of axial alignment of tendon fibres (seen as long linear parallel echoes) have been proposed for the grading and documentation of tendon lesions (Reef, 2001; Rantanen et al., 2011; Smith and Cauvin, 2014; Alzola Domingo et al., 2017). It has been well established that the qualitative or semiquantitative assessment of the tendon echogenicity is highly subjective and operator dependent (Crass et al., 1988; Genovese et al., 1990; van Schie et al., 1999). Good intra- and inter-rater agreement has been described for parameters including type of injury, maximal injury zone, location, tendon cross-sectional area and longitudinal fibre pattern (echo alignment), but not for echogenicity (Alzola Domingo et al., 2017).

In order to increase objectivity, it has been suggested to perform first-order gray level statistics where different shades of gray are quantified within a defined region of interest (Wood et al., 1993; Tsukiyama et al., 1996; Micklethwaite et al., 2001; Crevier-Denoix et al., 2005). It has since been demonstrated that the use of such first-order gray level analysis, where the distribution of the shades of gray is quantified without determining the relative position of the various gray levels within the image (second-order statistics), has a low sensitivity for the accurate quantification of tendon tissue (van Schie et al.,1999; van Schie et al., 2000). The tendon echogenicity is additionally influenced by the amplifier gain output and the position of the transducer. Tilting the transducer by only 3° can change the mean gray level of the tissue by as much as 40%, as the returning echo is directed away from the transducer (anisotropy phenomenon). Displacement of the transducer (2 mm) may account for a 20% difference in the gray level histogram of an acute tendon lesion (van Schie et al., 1999). As research in this area progresses, the use of ultrasound tissue characterisation (UTC) was developed to quantify the architecture of the tendon matrix more accurately (see below) (van Schie et al., 2001; 2003).

Besides the described untoward effects, tilting the transducer purposely (approximately 10°) might be useful to further assess the different components of the tendon structure. The so-called angle contrast ultrasonography (ACUS) has been introduced mainly for the evaluation of the suspensory ligament but can also be used for the assessment of the flexor tendons (Werpy and Axiak, 2013; Werpy et al., 2013; Denoix and Bertoni, 2015). Scanning a tendon off-incidence can be helpful to clearly distinguish the outline of the tendon and to differentiate between tendon fibres and scar tissue (Bubeck and Aarsvold, 2018). Angle contrast ultrasonography may also aid the identification of tendon lesions, especially longitudinal tears, which can be difficult to detect on standard ultrasonographic images (Edinger et al., 2005; Smith and Wright, 2006; Arensburg et al., 2011; Bertuglia et al., 2014).

Where a lesion is suspected within the digital flexor tendon sheath, ultrasonographic examination in a flexed limb position as well as dynamic ultrasonography of the non-weight bearing limb is additionally recommended (Seignour et al., 2012). Dynamic examination in flexion and extension allows for the visualisation of the gliding motion between the deep digital flexor tendon (DDFT), the SDFT and the palmar/plantar annular ligament and may identify adhesion formation, as well as *manica flexoria* tears (DiGiovanni et al., 2016; Garcia da Fonseca et al., 2019).

Contrast-enhanced ultrasonography (CEUS) (Fig. 2) has been suggested for the identification of longitudinal DDFT tears. The injection of 10 ml of ultrasound contrast medium containing stabilised sulphur hexafluoride microbubbles (2-3 x 10^8 microbubbles/ml), into the digital flexor tendon sheath, increased the sensitivity of angle contrast ultrasonography in an experimental study (Bertuglia et al., 2014). Contrast-enhanced ultrasonography was further validated for intra-venous and intra-arterial use in sound horses. The intra-arterial application of stabilised microbubble contrast (0.5-1 ml; 5-6 x 10^{10} microbubbles/ml) in the lateral palmar digital artery, at the level of the metacarpophalangeal joint, resulted in visible contrast enhancement in the soft tissues of the distal limb, without evident adverse reactions (Seiler et al., 2016). Further research is required to show whether the technique is useful for the characterisation of tendon injuries and how it compares with Doppler ultrasonography.

Doppler ultrasonography

Doppler imaging for the assessment of musculoskeletal pathology was initially described in human patients with rotator cuff, Achilles, or patellar tendon injury (Newman et al., 1994; Hollenberg et al., 1998; Weinberg et al., 1998). The Colour Doppler signal indicates the direction and velocity of blood flow, based on the interference of the moving blood cells with the wavelength (and hence frequency) of ultrasound waves (Naredo and Monteagudo, 2014; Palgrave and Kidd, 2014).

The blood supply of equine flexor tendons consists of a fine neurovascular network within the interfascicular matrix where small vessels run parallel to the long axis of the tendon without penetrating the collagen bundles (Edwards, 1946; O’Brien, 1997). The size and velocity of this physiological tendon blood supply is usually too small to be detected with Doppler ultrasonography, but neovascularisation that develops with inflammation and injury may become visible (Stromberg, 1971; Öhberg et al., 2001; Kristoffersen et al., 2005; Boesen et al., 2007; Murata et al., 2012). However, an increase in tendon vascularity as detected with Doppler ultrasonography has also been shown in the human Achilles tendon and the equine SDFT as an effect of training, without concurrent evidence for clinical tendinopathy (Boesen et al., 2006; Malliaras et al., 2008; Hirschmuller et al., 2010; Hirschmuller et al., 2012; Hatazoe et al., 2015).

Doppler signal that occurs in relation to a tendon injury should decline after 3-6 months, as tendon healing progresses. A study evaluating the healing process of tendon lesions found that surgically induced SDFT lesions in the hindlimb showed significantly less Doppler signal when compared to forelimb lesions, 6 months post injury (Estrada et al., 2014). During sequential examination, continuous or re-occurring Doppler signal in association with a flexor tendon lesion is suggestive of a delayed healing response or re-injury (Alfredson et al., 2003; Sharma and Maffuli, 2005; Smith and Cauvin, 2014; Hatazoe et al., 2015). For optimal visualisation of blood flow, the limb should be kept in a non-weight bearing position when Doppler ultrasonography is performed (Kristoffersen et al., 2005; Boesen et al., 2007; Rabba et al., 2018).

In human tendinopathy, Doppler ultrasonography is used to facilitate sclerosing therapy for the treatment of chronic tendinopathies. The ultrasound-guided perivascular injection of an irritant agent in cases of tendinopathy with persistent Doppler signal has been described in human patients but has only been reported in a limited number of horses (Kristoffersen et al., 2005; Alfredson and Lorentzon ,2007; Boesen et al., 2007). To the authors' knowledge there are no reports detailing risks and long-term outcomes for this form of therapy in equine patients.

Areas of focal mineralization are occasionally found in equine flexor tendons, especially in the DDFT. Tendon mineralization can be an incidental finding or may potentially be associated with previous corticosteroid injection as well as clinically apparent or subclinical tendon injury (Ross et al., 2011; O'Brien et al., 2012; Zhang et al., 2013; O'Brien and Smith, 2018; Ali et al., 2021). Doppler ultrasonography is regarded as a useful indicator for the detection of clinically relevant and painful tendon mineralization in human patients (Chiou et al., 2002; Le Goff et al., 2010). Similar observations have been described in a small number of horses in a recent report (Fig. 3A) (O'Brien and Smith, 2018).

In contrast to the commonly utilized Colour Doppler, Power Doppler ultrasonography analyses the total intensity of returning signal without characterising the direction of flow (Fig. 3B). The technique is independent of the ultrasound probe being aligned to the direction of blood flow and is particularly sensitive for imaging of low-velocity flow from small vessels (Martinoli et al., 1998; Anderson and McDicken, 2002; Naredo and Monteagudo, 2014; Palgrave and Kidd, 2014). Power Doppler ultrasonography has been shown to be useful for the detection of microvascularity in both Achilles and patellar tendons in man

(Newman et al., 1994; Terslev et al., 2001; Peers et al., 2003; Richards et al., 2005). Initial reports describing the application of Power Doppler ultrasonography for the assessment of the equine suspensory ligament branches and SDF tendinopathy show promising results; however, a study directly comparing Colour- and Power Doppler ultrasonography in horses is not currently available (Rabba et al., 2018; Lacitignola et al., 2020; Rabba et al., 2020).

Elastography

Based on the principle that pathological changes often result in altered tissue stiffness, elastography was first introduced for the staging of liver fibrosis and neoplastic lesions in human medicine (Ophir et al., 1991; Sigrist et al., 2017; Kennedy et al., 2018). Later the application was adopted for musculoskeletal imaging and elastography is now available for the assessment of muscle and tendon viscoelastic properties in human and equine athletes (Ellison et al., 2014; Klauser et al., 2014; Lustgarten et al., 2014; Ooi et al., 2014; Tamura et al., 2017a; Berger et al., 2018; Prado-Costa et al., 2018).

Depending on the stress applied to the tissue and the measured physical quantity, elastography can be divided into quasi-static and dynamic imaging (Bamber et al., 2013; Sigrist et al., 2017; Washburn et al., 2018). During quasi-static strain elastography (compression elastography), the operator applies stress to the tissue manually, by applying pressure using the ultrasound transducer. The resulting tissue deformation (relative stiffness of the tissue) is measured by radiofrequency echo correlation-based tracking and subsequently transformed into a colour-coded elastogram (Fig. 4) (Klauser et al., 2014; Winn et al., 2016; Prado-Costa et al., 2018). Dynamic elastography relies on the detection of shear-wave propagation. Dynamic stress is applied to the tissue with mechanical vibrating devices or acoustic radiation force and the shear-waves created by the excitation are recorded (Ooi et al., 2014; Sigrist et al., 2017; Taljanovic et al., 2017).

An initial study investigating the use of strain elastography for the assessment of equine flexor tendons demonstrated a moderate reproducibility (68%, Kappa agreement (κ) = 0.46) and good repeatability (83%, κ = 0.78) when different operators performed elastography in horses without evidence of tendinopathy (Lustgarten et al., 2014). There was no significant difference in elastography readings of tissue stiffness when tendons were imaged in various leg positions, but the flexor tendons appeared softer in the longitudinal plane compared to the transverse plane. The same technique was subsequently applied in clinical cases of tendon and ligament injury and compared with gray-scale ultrasonography (SDFT, DDFT, accessory ligament of the DDFT, suspensory ligament) and high-field MRI (1.5T) (DDFT, suspensory ligament) (Lustgarten et al., 2015). Elastograms were assessed subjectively using a qualitative colour-grading system. Additionally, an algorithm was developed for the quantitative analysis of the percentage of each colour within a specific region of interest. A significant correlation was found between lesions detected during strain elastography, gray-scale ultrasonography, short tau inversion recovery-fast spin echo (STIR-FSE) and proton density (PD) weighted MRI sequences. Chronic / subacute tendon lesions (> 2 weeks post trauma) appeared stiffer than acute lesions when quantitative and qualitative elastographic evaluation was performed.

Further research focused on the use of strain elastography as a monitoring tool for the recovery period of Thoroughbred racehorses that had sustained an SDFT injury (Tamura et al., 2017a; Tamura et al., 2017b). A prospective longitudinal study that followed a group of horses (n = 7) with SDFT tendinopathy showed that the lesion echogenicity changed within the first two months post injury using gray-scale ultrasonography. However, evidence for a significant increase in tendon stiffness was detected 4 to 7 months later on elastograms of the affected SDFTs (Tamura et al., 2017b). A second study by the same group confirmed that a significant difference in tendon stiffness can be detected using sonoelastography during the later stages of tendon healing (5 vs 9 months post injury), where gray-scale ultrasonographic findings remain largely unchanged (Tamura et al., 2017a).

In human athletes, the use of elastography as a monitoring tool for the identification of Achilles tendons with an increased injury risk showed promising results (Ooi et al., 2015; Balaban et al., 2016; Ooi et al., 2016). Additionally, age-related changes in Achilles tendon stiffness have been characterised with elastography in man (Klauser et al., 2014; Turan et al., 2015). Since the equine SDFT is considered functionally equivalent to the human Achilles tendon, monitoring protocols for the prevention of flexor tendon injury may be adaptable for equine athletes (Patterson-Kane and Rich, 2014; Washburn et al., 2018). Whilst current reports describe a good repeatability and interobserver agreement for strain elastography in horses, the variability of the manually applied tissue compression is a limitation that is yet to overcome (Klauser et al., 2010; Lustgarten et al., 2014; Tamura et al., 2017a; Tamura et al., 2017b). Due to an uneven pressure distribution, colour artefacts can additionally occur, particularly on transverse images of the SDFT. A standoff pad reduces artefacts at the tendon margins and can additionally be used as an external standard reference for quantitative sonoelastographic evaluation but may occasionally result in reverberation artefacts (encountered as red lines) (Lustgarten et al., 2014; Tamura et al., 2017a; Tamura et al., 2017b).

When interpreting elastography it is important to consider that this technique generally measures lateral compression rather than tensile load occurring during natural locomotion (Lustgarten et al., 2014; Sigrist et al., 2017). Further research including prospective longitudinal studies with a larger number of horses as well as studies including histological and biomechanical evaluation, and potentially the development of shear-wave elastography for the use in horses are desirable before elastography may be introduced as a standard tool for monitoring of equine flexor tendons. Elastography provides additional information about the structural integrity of tendon tissue but is not intended to replace other diagnostic imaging modalities for the characterisation of tendon lesions currently (Lustgarten et al., 2015).

Acoustoelastography

Acoustoelastography is an ultrasound-based method using mathematical postprocessing for the non-invasive assessment of tendon stiffness. Whilst elastography compares between echoes obtained before and after tissue compression, acoustoelastography evaluates changes in echogenicity during deformation of the tendon from an unloaded to a loaded state. To load the SDFT, the contralateral limb is lifted off the ground in a controlled manner. The increasing stress and strain caused by tendon loading results in changes in the B-mode ultrasound echo intensity and provides the basis for the calculation of a stiffness gradient (Kobayashi and Vanderby, 2005; Duenwald et al., 2011).

The method was initially described in porcine and canine tendons and has since been used for the evaluation of the equine SDFT and DDFT in groups of clinically normal horses (Duenwald-Kuehl et al., 2012a; Ellison et al., 2013; Ellison et al., 2014; Berger et al., 2018). Good intra- and inter-observer repeatability with no significant effect of age, sex or limb was demonstrated (Ellison et al., 2013; Ellison et al., 2014). It is, however, important to note that the stiffness may not be homogeneous along the length of the tendon and can vary amongst individual horses (De Gasperi et al., 2017; Berger et al., 2018). Additionally, sedation (detomidine-butorphanol combination) appears to significantly decrease the stiffness gradient of the equine SDFT (De Gasperi et al., 2017).

In contrast to strain elastography, acoustoelastography is thought to more accurately mimic the natural situation, where tendons are exposed to longitudinal strain rather than compression (Ellison et al., 2014). Initial reports of the use of acoustoelastography for the detection of strain-induced damage or tendon tears, as well as for monitoring purposes after tendon injury are available for other species (Duenwald-Kuehl et al., 2012b; Frisch et al., 2014; Hans et al., 2014). Further research assessing the feasibility of acoustoelastography for the detection of tendon injury and monitoring of tendon healing in equine patients is warranted before this technique might be more widely used in the field of equine orthopaedics.

Ultrasound Tissue Characterisation

Computerised ultrasound tissue characterisation (UTC) uses a high-resolution ultrasound probe mounted in a tracking device with a built-in standoff pad. With standardised settings including transducer tilt angle, gain, focus and depth, approximately 600 consecutive transverse images of the tendon are acquired at a set distance (every 0.2 mm over 12 cm) and concatenated to create a three-dimensional ultrasound data-block. By correlating the pixel stability across a number of contiguous transverse images, specific algorithms can distinguish between four different structure- and non-structure-related echo-types, which reflect the integrity of the tendon matrix (Table 2). Based on this data a colour code is generated, where green displays a good pixel correlation (uniform tissue), blue ≤ 10% difference in pixels (irregular tissue), red ≥ 10% difference in pixels (fibrillar tissue) and black = no correlation (amorphous tissue). Additionally, longitudinal information (sagittal and frontal planes) can be reconstructed, and the percentage of each echo-type can be quantified for a specific tendon region (Fig. 5) (van Schie and Bakker, 2000; van Schie et al., 2001; 2003; van Schie et al., 2010; van Schie et al., 2013; Cook and Purdam, 2014).

The technique was first validated in the equine SDFT where images were compared with the histopathological characteristics of normal and diseased specimens (van Schie and Bakker, 2000; van Schie et al., 2001; 2003). A good inter-observer reliability was subsequently demonstrated for UTC of the equine SDFT as well as the human Achilles and patellar tendons (van Schie et al., 2010; Docking et al., 2015; Geburek et al., 2017; Plevin et al., 2019; Rabello et al., 2019a; van Ark et al., 2019).

Ultrasound tissue characterisation was further used as a monitoring tool for tendon healing in several experimental studies with surgically created tendon lesions in horses. Based on this research, UTC is currently considered the most objective tool for longitudinal *in vivo* assessment of tendon healing (van Schie et al., 2009; Bosch et al., 2011; David et al., 2012; Cadby et al., 2013; Geburek et al., 2017). Treatment options for equine tendinopathy including cast immobilisation, platelet-rich plasma and adipose-derived mesenchymal stromal cells have been investigated with the aid of this technique (Bosch et al., 2011; David et al., 2012; Geburek et al., 2016; Geburek et al., 2017).

Besides the application for lesion monitoring, it has been shown that UTC can be employed to illustrate the tendon’s response to exercise. When UTC examination was performed in a group of racing Thoroughbreds, it was demonstrated that the SDFT shows a significant reduction of aligned tendon bundles (echo-type I) with an increase in discontinuous secondary fascicle bundles (echo-type II) and fibrillar components (echo-type III), for two days post racing (Docking et al., 2012). The described decrease in the tendon's structural integrity appeared to be reversible and returned to baseline within approximately 72 hours. Due to this observation, it was recommended that repeated maximal exercise should be avoided for three days after competitive racing (Docking et al., 2012).

Ultrasound tissue characterisation was subsequently used in a longitudinal field study, where the SDFT of yearling Thoroughbreds was evaluated as they started race training (Plevin et al., 2019). The study showed that the ultrasonographic characteristics of the SDFT changed significantly within the first 6 months of training, which is thought to reflect the tendon's response to altered loading patterns as race training commences. The UTC equipment was considered easy to use with young horses under field conditions. However, the technique was susceptible to movement artefacts, which usually present as blue lines and necessitated repeated examination. The reference values identified within this young group of horses were ≥ 85% echo-type I and ≤ 15% echo-type II with a negligible proportion of echo-type III and IV for all tendons at all time points. In the racing group of Thoroughbreds (mean ± SD age 3.8 ± 0.6 years) described in the previous paragraph, values were somewhat different with > 90% echo-type I, < 5% echo-type II and up to 5% of echo-type IV for mature horses in training (Docking et al., 2012). The increased percentage of echo-type IV might be explained by transient intra-fascicular fluid accumulation as well as subclinical tendon matrix degradation (Docking et al., 2012; Plevin et al. 2019).

A growing body of evidence advocates the benefits of serial tendon scans in human athletes in training in order to recognise subclinical changes of the tendon architecture (Rosengarten et al., 2015; Docking et al., 2016; van Ark et al., 2016; Stanley et al., 2018; Waugh et al., 2018; Rabello et al., 2019a; Rabello et al., 2019b). Further long-term studies should show whether an increase in echo-type II may represent an early indicator for degenerative changes, and whether UTC will prove to be reliable in predicting clinical tendinopathy in equine or human athletes. A systematic review of the human literature regarding UTC emphasizes that standardised protocols should be adopted for UTC measurement and analysis (Rabello et al., 2019b). Another recent study points out that clinical symptoms remain more predictive than UTC for the presence or severity of clinical tendinopathy in man (Docking et al., 2019). This observation is most likely explained by the fact that a high proportion (approximately 80%) of tendons with imaging findings remain asymptomatic in human athletes and the question when and why lesions become painful is yet to be determined (Rio et al., 2014; McAuliffe et al., 2016).

In summary, the authors regard ultrasonographic imaging as a vital tool for the assessment of equine flexor tendons and routinely use Doppler ultrasonography for lesion monitoring. In the authors' experience the reproducibility of elastography is currently still hampered by difficulties in application of uniform pressure distribution using the ultrasound transducer. The use of dynamic shear-wave elastography might overcome some of the limitations of strain elastography in the future, as the technique additionally offers quantitative values for the characterisation of tendon tissues. However, dynamic elastography has not been developed specifically for the use in equine patients so far. The clinical application of UTC is gaining popularity in equine practice. Future longitudinal studies with larger case numbers should show whether UTC is a reliable tool not only for early detection, monitoring and prognostication but also for the prediction of tendon lesions.

Conclusion

Ultrasonography remains the technique most commonly used for the assessment of the flexor tendons in equine practice. Awareness of its potential including Doppler and contrast-enhanced ultrasonography, as well as its limitations, should prevent misinterpretation and guide the selection of cases where alternative imaging techniques might be beneficial. Based on the poor prognosis of horses with flexor tendinopathy a main focus of future diagnostic imaging lies on the detection of subclinical tendon damage before clinical tendinopathy becomes apparent. Additionally, careful monitoring of the tendon structure and viscoelastic properties supports individual rehabilitation and may decrease the risk of re-injury. The modalities developed for this purpose including elastography, acoustoelastography and ultrasound tissue characterisation should be of particular interest for equine practitioners. The majority of the imaging techniques developed for the assessment of the tendon biomechanical properties do not require a hospital setup or and may be used under field conditions.

Conflict of intertest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Table 1

Reference values for the tendon cross-sectional area (CSA) as detailed for the superficial digital flexor tendon (SDFT) and deep digital flexor tendon (DDFT) of foals, young horses and adults including several specific horse breeds. The flexor tendon CSA is smallest in the mid-metacarpal region and gradually enlarges again towards the level of the fetlock. Mean values are provided, and the reader is referred to the original literature to find reference values for the different tendon levels.

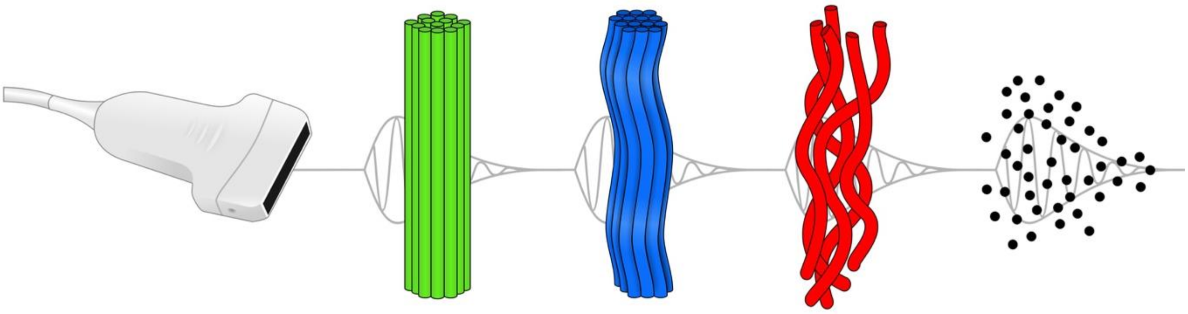
Horse - breed	CSA SDFT	CSA DDFT	Total No. horses	Literature source
Foal 1-6 months	37 – 84 mm^2	31 – 54 mm^2	7	(Korosue et al., 2015; Spinella et al., 2015; Spinella et al., 2016)
Foal 7-24 months	62 – 146 mm^2	57 – 85 mm^2	SDFT = 40 DDFT = 7	(Moffat et al., 2008; Korosue et al., 2015)
Thoroughbred	80 – 146 mm^2	72 – 213 mm^2	SDFT = 268 DDFT = 86	(Gillis et al., 1993; Smith et al., 1994; Gillis et al. 1995b; Celimli et al., 2004; Perkins et al. 2004; Avella et al., 2009; Matos Santiago Reis and Arantes

				Baccarin, 2010; Köster et al., 2014)
Standardbred	82 – 112 mm^2	90 – 165 mm^2	10	(Herslow et al., 2001; Köster et al., 2014)
Arabian horse	62 – 94 mm^2	60 – 92 mm^2	62	(Celimli et al., 2004)
Quarter Horse	78 – 97 mm^2	65 – 150 mm^2	5	(Köster et al., 2014)
Warmblood	90 – 125 mm^2	80 – 165 mm^2	10	(Köster et al., 2014)
Irish Draught cross	123 – 151 mm^2	102 – 224 mm^2	22	(Smith et al., 1994)
Purebred Spanish horse	64 – 107 mm^2	84 – 132 mm^2	20	(Agut et al., 2009)
Icelandic horse	49 – 64 mm^2	63 – 105 mm^2	50	(Boehart et al., 2010a)
Haflinger	52 – 85 mm^2	64 – 143 mm^2	30	(Boehart et al., 2010b)
Pony	71 – 83 mm^2	65 – 149 mm^2	15	(Smith et al., 1994)

Table 2

Following acquisition of three-dimensional ultrasound data, ultrasound tissue characterisation (UTC) algorithms can distinguish between four ultrasound echo-types that correlate with histo-morphological characteristics of the tendon (van Schie et al., 2013). The echo-types represent the integrity of the tendon and the level of fibrillar organisation (van Schie et al., 2010; Plevin et al., 2019; Rabello et al., 2019b).

Ultrasound tissue characterisation echo-types



Echo-type	Type I	Type II	Type III	Type IV
Pixel stability	Highly stable	Medium stable	Highly variable	Constantly low intensity and variable distribution
Tendon integrity	Reflections at intact and aligned tendon bundles	Reflections at discontinuous or wavy tendon bundles	Interfering echoes from mainly fibrillar components	Cellular components and fluid in amorphous tissue

Figure legends

Fig. 1. Flow chart detailing the systematic analysis and selection of scientific literature available on equine flexor tendon imaging. The following search terms were used for review part 1 without restrictions: ‘tendon’ AND ‘ultrasonography’ OR ‘elastography’ OR ‘ultrasound tissue characterisation’ AND ‘equine’ OR ‘horse’. Additional search terms for review part 2: ‘magnetic resonance imaging’ OR ‘computed tomography’.

Fig. 2. Longitudinal contrast-enhanced ultrasound image of the flexor tendons at the level of the digital flexor tendon sheath in a horse without evidence of orthopaedic disease. The intra-thecal injection of the digital flexor tendon sheath with 10 ml of ultrasonographic contrast medium (2 ml of room air in 8 ml of saline, shaken for 10 seconds prior to injection) aids the delineation of the superficial digital flexor tendon (SDFT) and the deep digital flexor tendon (DDFT) (black arrow) as well as the *manica flexoria* (white arrows). The limb is in a flexed position, distal is to the left (Image courtesy of Nadine Ogden, University of Liverpool 2020).

Fig. 3. (A) Colour Doppler transverse ultrasonographic image of the deep digital flexor tendon (DDFT) of an 18-year-old Pony mare with chronic DDF tendinopathy. There is positive Colour Doppler signal and focal mineralisation of the DDFT (white arrow). (B) Power Doppler longitudinal ultrasonographic image of the superficial digital flexor tendon (SDFT) of a 16-year-old Warmblood gelding six weeks after acute injury of the SDFT. Note the reduced fibre alignment and hypoechoic appearance of the SDFT with diffuse Power Doppler signal indicative for neovascularisation.

Fig. 4. Transverse elastogram (compression elastography) on the left and corresponding gray-scale ultrasonographic image (on the right) of the flexor tendons of a 14-year-old Warmblood

gelding with deep digital flexor tendinopathy. The green line (bottom left) indicates the application of adequate pressure using the ultrasound transducer. The relative stiffness of the tissue follows the colour scale (top left) where red is soft, and blue is hard. Note the diffusely heterogenous fibre pattern with small hypoechoic areas throughout the deep digital flexor tendon on the ultrasonographic image that correspond to discrete areas of decreased tissue stiffness on the elastogram.

Fig. 5. Ultrasound tissue characterisation examination showing the colour-coded pixel stability of a core lesion (cross hairs) of the superficial digital flexor tendon (SDFT) in transverse (A), longitudinal (B) and frontal plane (C) with the associated gray scale frontal plane image (D) at the same level. The intact tendon fibres of the superficial and deep digital flexor tendons appear in green colour (echo type I). The SDFT core lesion is characterised by some red (echo type III = fibrillar tissue) and mainly black colour (echo type IV = amorphous tissue) illustrating the loss of integrity of the affected tendon fibres.

Review

Equine flexor tendon imaging part 1 – recent developments in ultrasonography, with focus on the superficial digital flexor tendon

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Abstract

Flexor tendon injuries are a major cause of lameness in performance horses and have considerable impact on equine welfare and the wider horse industry. Ageing and repetitive strain frequently cause varying degrees of tendon micro-damage prior to the recognition of clinical tendinopathy. Whilst B-mode ultrasonography is most commonly utilized for detection and monitoring of tendon lesions at the metacarpal/metatarsal level, the emphasis of recent research has~~is~~ focused on the identification of subclinical tendon damage in order to prevent further tendon injury and improve outcomes. The~~ current~~ introduction of elastography, acoustoelastography and ultrasound tissue characterisation in the field of equine orthopaedics shows promising results and might find wider use in equine practice as clinical development continues. Based on the substantial number of research studies on tendon imaging published over the past decade this literature review aims to examine the currently used ultrasonographic imaging techniques and their limitations, and to introduce and critically appraise new modalities that could potentially change the clinical approach to equine flexor tendon imaging.

Keywords: Elastography; Horse; Imag~~eing~~; ~~Tendon;~~Tendinopathy; Ultrasound

Introduction

Tendon and ligament injuries are amongst the most common causes of early retirement and wastage in performance horses and account for approximately 43-54% of all musculoskeletal injuries in the equine athlete (Pinchbeck et al., 2004; Singer et al., 2008; Mitchell et al., 2020).

The majority of tendon injuries (75-93%) occurring in the equine athlete involve the superficial digital flexor tendon (SDFT), with the mid-metacarpal region of the forelimb being affected in 97-99% of cases (Ely et al., 2004; Kasashima et al., 2004; Lam et al., 2007). The prevalence of SDF tendinopathy is particularly high in racing Thoroughbreds (11-30%) and the recovery often includes a prolonged period of rehabilitation and is associated with a poor prognosis for return to high-level exercise (Lam et al., 2007; Ely et al., 2009; O'Meara et al., 2010; Kalisiak, 2012; Witte et al., 2016).

A growing body of evidence suggests that not only acute overstrain can lead to tendon injury but that the accumulation of tendon micro-damage as an effect of ageing and repetitive strain commonly precedes the development of clinical tendinopathy (Thorpe et al., 2013; Birch et al., 2016; Godinho et al., 2017; Thorpe et al., 2017; O'Brien et al., 2020). In the rapidly advancing field of diagnostic imaging, a specific focus is set on the non-invasive analysis of tendon viscoelastic properties in order to identify subclinical tendon damage and prevent injury (Docking et al., 2012; Lustgarten et al., 2015; Tamura et al., 2017a; Plevin et al., 2019).

The aim of this literature review is to provide evidence detailing the commonly used and recently developed ultrasonographic imaging techniques for the assessment of equine tendons. The current state of knowledge including implications, pitfalls and future directions of ultrasonography, elastography and ultrasound tissue characterisation for equine flexor tendon imaging will be discussed. A systematic PubMed, Medline and Google Scholar search with the following search terms was performed without restrictions: ‘tendon’ AND ‘ultrasonography’ OR ‘elastography’ OR ‘ultrasound tissue characterisation’ AND ‘equine’ OR ‘horse’. Additionally, studies were identified by searching the reference list of eligible articles (Fig. 1). Articles were excluded if they were not available in English language or if they were not published in peer reviewed journals. Additionally, references were not discussed in the manuscript where the authors did not regard them as recent or their content had already been discussed in detail in previous reviews.

Ultrasonography

Brightness-mode (B-~~brightness~~ mode) sonography displays the difference in acoustic impedance within a two-dimensional tissue cross-section and remains the modality most widely used for the assessment of tendon and ligament injuries in equine practice (Rantanen, 1982; Gaschen and Burba, 2012; Palgrave and Kidd, 2014; Berner, 2017). Tendon lesions are generally classified as core lesions, border lesion,~~ or~~ tendon splits~~,~~ or diffuse decrease in echogenicity with loss of long linear parallel echoes with transverse as well as longitudinal~~sagittal~~ plane ultrasonography required to determine the extent of an injury (Smith, 2008; Alzola et al., 2018).

Tendon cross-sectional area

The tendon cross-sectional area (CSA) as well as the CSA and length of a tendon lesion can be calculated with reasonable accuracy using ultrasonography (Genovese et al., 1990; Smith et al., 1994; Gillis et al., 1995a; Alzola Domingo et al., 2017; Kojah et al., 2017). Other measurements including latero-medial or dorso-palmar/plantar dimensions of tendon lesion are considered less sensitive (Reef, 2001; Smith and Cauvin, 2014).

The flexor tendon CSA may be influenced by acute changes in hoof angulation. With marked elevation of the toe, flexor tendons can appear smaller whereas significant heel elevation might lead to an increase in tendon CSA. However, a consistent impact on CSA measurements could only be demonstrated with heel or toe elevations in excess of 10° in one study (Hagen et al., 2018). Despite individual variations, conventional remedial farriery rarely alters hoof angulation greater than 5° and should therefore have limited impact on the calculation of the flexor tendon CSA (Riemersma et al., 1996; Lawson et al., 2007; Hagen et al., 2017; Hagen et al., 2018).

The age, breed and type of horse further influence the flexor tendon CSA. Reference values for the tendon CSA and echogenicity have been described for foals, young horses and adults as well as for several specific horse breeds (Table 1). Additionally, reference values are available for the proximal flexor tendons in the tarsal region (Vilar et al., 2011). It is interesting to note that besides the differences in CSA and echogenicity, breed specific differences in tendon biochemical and mechanical properties have been demonstrated recently (Ploeg et al., 2017; Verkade et al., 2019). Biomechanical testing identified a significantly higher stiffness of the SDFT in Thoroughbreds when compared to Warmblood horses which might in part explain the predisposition to SDFT injury in certain breeds like Thoroughbreds (Verkade et al., 2019).

Based on variations in functional adaption, the flexor tendon CSA does not increase in a proportional rate during maturation (Birch et al., 1999; Korosue et al., 2015). A small number of studies describe a tendon hypertrophy and reduction in echogenicity with training, but results are inconsistent and tend not to demonstrate statistically significant alterations unless clinical tendinopathy is present (Gillis et al., 1993; Birch et al., 1999; Kasashima et al., 2002; Perkins et al., 2004; Moffat et al., 2008; Avella et al., 2009; Matos Santiago Reis and Arantes Baccarin, 2010). Despite some conflicting reports, overall age, sex, height at the withers or body mass index do not appear to have a strong influence on the equine flexor tendon CSA in the adult horse (Smith et al., 1994; Gillis et al., 1995b; Gillis et al., 1995c; Moffat et al., 2008; Agut et al., 2009; Avella et al., 2009; Boehart et al., 2010a; Köster et al., 2014). Additionally, most studies have not identified a difference between limbs (left vs right forelimb), which implies that ultrasonographic examination of the contralateral limb can serve as a valid comparison provided bilateral tendinopathy can be excluded (Smith et al., 1994; Gillis et al., 1995b; Perkins et al., 2004; Moffat et al., 2008; Agut et al., 2009; Avella et al., 2009; Köster et al., 2014). A 20% increase in tendon CSA when compared to the contralateral side is regarded as a sign for tendinopathy by several authors (Smith et al., 1994; Reef, 2001; Smith, 2008; Smith and Cauvin, 2014). In case of bilateral tendinopathy, it has been suggested to analyse the SDFT:DDFT ratio to assess for tendon enlargement (Berner, 2017).

Tendon lesions with a maximal injury zone of more than 25% of the total transverse tendon CSA are usually classed as severe lesions (Reef, 2001; Smith, 2008). A recent study showed a probability of 29-35% for a successful return to racing after SDFT injury in core lesions that involved less than 50% of the tendon CSA, with a decrease to 11-16% in lesions ≥50% CSA. The study additionally found that the prognosis in cases of tendinopathy without core lesion reduced from 49-99% to just 14% if at the maximal injury zone 75% or more of the longitudinal fibre pattern was disrupted (Alzola et al., 2018).

Image acquisition during consecutive ultrasonographic examinations can be performed by different operators but CSA measurements should ideally be obtained by the same person in order to avoid inaccuracy (Pickersgill et al., 2001). An increase of a lesion CSA in excess of 10% during re-assessment is most likely indicative for a degree of re-injury and requires adjustment of an existing rehabilitation program (Reef, 2001; Smith, 2008; Smith and Cauvin, 2014; Kümmerle et al. 2019).

Tendon echogenicity

The ultrasonographic echogenicity will provide some information about the collagen density of the tendon tissue and multiple scoring systems estimating the tendon intensity (brightness), homogeneity and the degree of axial alignment of tendon fibres (seen as long linear parallel echoes) have been proposed for the grading and documentation of tendon lesions (Reef, 2001; Rantanen et al., 2011; Smith and Cauvin, 2014; Alzola Domingo et al., 2017). It has been well established that the qualitative or semiquantitative assessment of the tendon echogenicity is highly subjective and operator dependent (Crass et al., 1988; Genovese et al., 1990; van Schie et al., 1999). Good intra- and inter-rater agreement has been described for parameters including type of injury, maximal injury zone, location, tendon cross-sectional area and longitudinal fibre pattern (echo alignment), but not for echogenicity (Alzola Domingo et al., 2017) ~~(Table 1)~~.

In order to increase objectivity, it has been suggested to perform first-order gray level statistics where different shades of gray are quantified within a defined region of interest (Wood et al., 1993; Tsukiyama et al., 1996; Micklethwaite et al., 2001; Crevier-Denoix et al., 2005). It has since been demonstrated that the use of such first-order gray level analysis, where the distribution of the shades of gray is quantified without determining the relative position of the various gray levels within the image (second-order statistics), has a low sensitivity for the accurate quantification of tendon tissue (van Schie et al.,1999; van Schie et al., 2000~~; Aggarwal and Agrawal, 2012~~). The tendon echogenicity is additionally influenced by the amplifier gain output and the position of the transducer. Tilting the transducer by only 3° can change the mean gray level of the tissue by as much as 40%, as the returning echo is directed away from the transducer (anisotropy phenomenon). Displacement of the transducer (2 mm) may account for a 20% difference in the gray level histogram of an acute tendon lesion (van Schie et al., 1999). As research in this area progresses, the use of ultrasound tissue characterisation (UTC) was developed to quantify the architecture of the tendon matrix more accurately (see below) (van Schie et al., 2001; 2003).

Besides the described untoward effects, tilting the transducer purposely (approximately 10°) might be useful to further assess the different components of the tendon structure. The so-called angle contrast ultrasonography (ACUS) has been introduced mainly for the evaluation of the suspensory ligament but can also be used for the assessment of the flexor tendons (Werpy and Axiak, 2013; Werpy et al., 2013; Denoix and Bertoni, 2015). Scanning a tendon off-incidence can be helpful to clearly distinguish the outline of the tendon and to differentiate between tendon fibres~~, fat, muscle~~ and scar tissue (Bubeck and Aarsvold, 2018). Angle contrast ultrasonography may also aid the identification of tendon lesions, especially longitudinal tears, which can be difficult to detect on standard ultrasonographic images (Edinger et al., 2005; Smith and Wright, 2006; Arensburg et al., 2011; Bertuglia et al., 2014).

Where a lesion is suspected within the digital flexor tendon sheath, ultrasonographic examination in a flexed limb position as well as dynamic ultrasonography of the non-weight bearing limb is additionally recommended (Seignour et al., 2012). Dynamic examination in flexion and extension allows for the visualisation of the gliding motion between the deep digital flexor tendon (DDFT), the SDFT and the palmar/plantar annular ligament and may identify adhesion formation, as well as *manica flexoria* tears (DiGiovanni et al., 2016; Garcia da Fonseca et al., 2019).

Contrast-enhanced ultrasonography (CEUS) (Fig. 2) has been suggested for the identification of longitudinal DDFT tears. The injection of 10 ml of ultrasound contrast medium containing stabilised sulphur hexafluoride microbubbles (2-3 x 10^8 microbubbles/ml), into the digital flexor tendon sheath, increased the sensitivity of angle contrast ultrasonography in an experimental study (Bertuglia et al., 2014). Contrast-enhanced ultrasonography was further validated for intra-venous and intra-arterial use in sound horses. The intra-arterial application of stabilised microbubble contrast (0.5-1 ml; 5-6 x 10^{10} microbubbles/ml) in the lateral palmar digital artery, at the level of the metacarpophalangeal joint, resulted in visible contrast enhancement in the soft tissues of the distal limb, without evident adverse reactions (Seiler et al., 2016). Further research is required to show whether the technique is useful for the characterisation of tendon injuries and how it compares with Doppler ultrasonography ~~(Fig. 2)~~.

Doppler ultrasonography

Doppler imaging for the assessment of musculoskeletal pathology was initially described in human patients with rotator cuff, Achilles, or patellar tendon injury (Newman et al., 1994; Hollenberg et al., 1998; Weinberg et al., 1998). The Colour Doppler signal indicates the direction and velocity of blood flow, based on the interference of the moving blood cells with the wavelength (and hence frequency) of ultrasound waves (Naredo and Monteagudo, 2014; Palgrave and Kidd, 2014).

The blood supply of equine flexor tendons consists of a fine neurovascular network within the interfascicular matrix where small vessels run parallel to the long axis of the tendon without penetrating the collagen bundles (Edwards, 1946; O’Brien, 1997). The size and velocity of this physiological tendon blood supply is usually too small to be detected with Doppler ultrasonography, but neovascularisation that develops with inflammation and injury may become visible (Stromberg, 1971; Öhberg et al., 2001; Kristoffersen et al., 2005; Boesen et al., 2007; Murata et al., 2012). However, an increase in tendon vascularity as detected with Doppler ultrasonography has also been shown in the human Achilles tendon and the equine SDFT as an effect of training, without concurrent evidence for clinical tendinopathy (Boesen et al., 2006; Malliaras et al., 2008; Hirschmuller et al., 2010; Hirschmuller et al., 2012; Hatazoe et al., 2015).

Doppler signal that occurs in relation to a tendon injury should decline after 3-6 months, as tendon healing progresses. A study evaluating the healing process of tendon lesions found that surgically induced SDFT lesions in the hindlimb showed significantly less Doppler signal when compared to forelimb lesions, 6 months post injury (Estrada et al., 2014). During sequential examination, continuous or re-occurring Doppler signal in association with a flexor tendon lesion is suggestive of a delayed healing response or re-injury (Alfredson et al., 2003; Sharma and Maffuli, 2005; Smith and Cauvin, 2014; Hatazoe et al., 2015). For optimal visualisation of blood flow, the limb should be kept in a non-weight bearing position when Doppler ultrasonography is performed (Kristoffersen et al., 2005; Boesen et al., 2007; Rabba et al., 2018).

In human tendinopathy, Doppler ultrasonography is used to facilitate sclerosing therapy for the treatment of chronic tendinopathies. The ultrasound-guided perivascular injection of an irritant agent in cases of tendinopathy~~initis~~ with persistent Doppler signal has been described in human patients but has only been reported in a limited number of horses (Kristoffersen et al., 2005; Alfredson and Lorentzon ,2007; Boesen et al., 2007). To the authors' knowledge there are no reports detailing risks and long-term outcomes for this form of therapy in equine patients.

Areas of focal mineralization are occasionally found in equine flexor tendons, especially in the DDFT. Tendon mineralization can be an incidental finding or may potentially be associated with previous corticosteroid injection as well as~~or~~ clinically apparent or subclinical tendon injury (Ross et al., 2011; O'Brien et al., 2012; Zhang et al., 2013; O'Brien and Smith, 2018; Ali et al., 2021). Doppler ultrasonography is regarded as a useful indicator for the detection of clinically relevant and painful tendon mineralization in human patients (Chiou et al., 2002; Le Goff et al., 2010). Similar observations have been described in a small number of horses in a recent report (Fig. 3A) (O'Brien and Smith, 2018).

In contrast to the commonly utilized Colour Doppler, Power Doppler ultrasonography analyses the total intensity of returning signal without characterising the direction of flow (Fig. 3B). The technique is independent of the ultrasound probe being aligned to the direction of blood flow and is particularly sensitive for imaging of low-velocity flow from small vessels (Martinoli et al., 1998; Anderson and McDicken, 2002; Naredo and Monteagudo, 2014; Palgrave and Kidd, 2014). Power Doppler ultrasonography has been shown to be useful for the detection of microvascularity in both Achilles and patellar tendons in man (Newman et al., 1994; Terslev et al., 2001; Peers et al., 2003; Richards et al., 2005). Initial reports describing the application of Power Doppler ultrasonography for the assessment of the equine suspensory ligament branches and SDF tendinopathy show promising results; however, a study directly comparing Colour- and Power Doppler ultrasonography in horses is not currently available (Rabba et al., 2018; Lacitignola et al., 2020; Rabba et al., 2020).

Elastography

Based on the principle that pathological changes often result in altered tissue stiffness, elastography was first introduced for the staging of liver fibrosis and neoplastic lesions in human medicine (Ophir et al., 1991; Sigrist et al., 2017; Kennedy et al., 2018). Later the application was adopted for musculoskeletal imaging and elastography is now available for the assessment of muscle and tendon viscoelastic properties in human and equine athletes (Ellison et al., 2014; Klauser et al., 2014; Lustgarten et al., 2014; Ooi et al., 2014; Tamura et al., 2017a; Berger et al., 2018; Prado-Costa et al., 2018).

Depending on the stress applied to the tissue and the measured physical quantity, elastography can be divided into quasi-static and dynamic imaging (Bamber et al., 2013; Sigrist et al., 2017; Washburn et al., 2018). During quasi-static strain elastography (compression elastography), the operator applies stress to the tissue manually, by applying pressure using the ultrasound transducer. The resulting tissue deformation (relative stiffness of the tissue) is measured by radiofrequency echo correlation-based tracking and subsequently transformed into a colour-coded elastogram (Fig. 4) (Klauser et al., 2014; Winn et al., 2016; Prado-Costa et al., 2018). Dynamic elastography relies on the detection of shear-wave propagation. Dynamic stress is applied to the tissue with mechanical vibrating devices or acoustic radiation force and the shear-waves created by the excitation are recorded (Ooi et al., 2014; Sigrist et al., 2017; Taljanovic et al., 2017).

An initial study investigating the use of strain elastography for the assessment of equine flexor tendons demonstrated a moderate reproducibility (68%, Kappa agreement (κ) = 0.46) and good repeatability (83%, κ = 0.78) when different operators performed elastography in horses without evidence of tendinopathy (Lustgarten et al., 2014). There was no significant difference in elastography readings of tissue stiffness when tendons were imaged in various leg positions, but the flexor tendons appeared softer in the longitudinal plane compared to the transverse plane. The same technique was subsequently applied in clinical cases of tendon and ligament injury and compared with gray-scale ultrasonography (SDFT, DDFT, accessory ligament of the DDFT, suspensory ligament) and high-field MRI (1.5T) (DDFT, suspensory ligament) (Lustgarten et al., 2015). Elastograms were assessed subjectively using a qualitative colour-grading system. Additionally, an algorithm was developed for the quantitative analysis of the percentage of each colour within a specific region of interest. A significant correlation was found between lesions detected during strain elastography, gray-scale ultrasonography, short tau inversion recovery-fast spin echo (STIR-FSE) and proton density (PD) weighted MRI sequences. Chronic / subacute tendon lesions (> 2 weeks post trauma) appeared stiffer than acute lesions when quantitative and qualitative elastographic evaluation was performed.

Further research focused on the use of strain elastography as a monitoring tool for the recovery period of Thoroughbred racehorses that had sustained an SDFT injury (Tamura et al., 2017a; Tamura et al., 2017b). A prospective longitudinal study that followed a group of horses (n = 7) with SDFT tendinopathy showed that the lesion echogenicity changed within the first two months post injury using gray-scale ultrasonography. However, evidence for a significant increase in tendon stiffness was ~~only~~ detected 4 to 7 months later on elastograms of the affected SDFTs (Tamura et al., 2017b). A second study by the same group confirmed that a significant difference in tendon stiffness can be detected using sonoelastography during the later stages of tendon healing (5 vs 9 months post injury), where gray-scale ultrasonographic findings remain largely unchanged (Tamura et al., 2017a).

In human athletes, the use of elastography as a monitoring tool for the identification of Achilles tendons with an increased injury risk showed promising results (Ooi et al., 2015; Balaban et al., 2016; Ooi et al., 2016). Additionally, age-related changes in Achilles tendon stiffness have been characterised with elastography in man (Klauser et al., 2014; Turan et al., 2015). Since the equine SDFT is considered functionally equivalent to the human Achilles tendon, monitoring protocols for the prevention of flexor tendon injury may be adaptable for equine athletes (Patterson-Kane and Rich, 2014; Washburn et al., 2018). Whilst current reports describe a good repeatability and interobserver agreement for strain elastography in horses, the variability of the manually applied tissue compression is a limitation that is yet to overcome (Klauser et al., 2010; Lustgarten et al., 2014; Tamura et al., 2017a; Tamura et al., 2017b). Due to an uneven pressure distribution, colour artefacts can additionally occur, particularly on transverse images of the SDFT. A standoff pad reduces artefacts at the tendon margins and can additionally be used as an external standard reference for quantitative sonoelastographic evaluation but may occasionally result in reverberation artefacts (encountered as red lines) (Lustgarten et al., 2014; Tamura et al., 2017a; Tamura et al., 2017b).

When interpreting elastography it is important to consider that this technique generally measures lateral compression rather than tensile load occurring during natural locomotion (Lustgarten et al., 2014; Sigrist et al., 2017). Further research including prospective longitudinal studies with a larger number of horses as well as studies including histological and biomechanical evaluation, and potentially the development of shear-wave elastography for the use in horses are desirable before elastography may be introduced as a standard tool for monitoring of equine flexor tendons. Elastography provides additional information about the structural integrity of tendon tissue but is not intended to replace other diagnostic imaging modalities for the characterisation of tendon lesions currently (Lustgarten et al., 2015).

Acoustoelastography

Acoustoelastography is an ultrasound-based method using mathematical postprocessing for the non-invasive assessment of tendon stiffness. Whilst elastography compares between echoes obtained before and after tissue compression, acoustoelastography evaluates changes in echogenicity during deformation of the tendon from an unloaded to a loaded state. To load the SDFT, the contralateral limb is lifted off the ground in a controlled manner. The increasing stress and strain caused by tendon loading results in changes in the B-mode ultrasound echo intensity and provides the basis for the calculation of a stiffness gradient (Kobayashi and Vanderby, 2005; Duenwald et al., 2011).

The method was initially described in porcine and canine tendons and has since been used for the evaluation of the equine SDFT and DDFT in groups of clinically normal horses (Duenwald-Kuehl et al., 2012a; Ellison et al., 2013; Ellison et al., 2014; Berger et al., 2018). Good intra- and inter-observer repeatability with no significant effect of age, sex or limb was demonstrated (Ellison et al., 2013; Ellison et al., 2014). It is, however, important to note that the stiffness may not be homogeneous along the length of the tendon and can vary amongst individual horses (De Gasperi et al., 2017; Berger et al., 2018). Additionally, sedation (detomidine-butorphanol combination) appears to significantly decrease the stiffness gradient of the equine SDFT (De Gasperi et al., 2017).

In contrast to strain elastography, acoustoelastography is thought to more accurately mimic the natural situation, where tendons are exposed to longitudinal strain rather than compression (Ellison et al., 2014). Initial reports of the use of acoustoelastography for the detection of strain-induced damage or tendon tears, as well as for monitoring purposes after tendon injury are available for other species (Duenwald-Kuehl et al., 2012b; Frisch et al., 2014; Hans et al., 2014). Further research assessing the feasibility of acoustoelastography for the detection of tendon injury and monitoring of tendon healing in equine patients is warranted before this technique might be more widely used in the field of equine orthopaedics.

Ultrasound Tissue Characterisation

Computerised ultrasound tissue characterisation (UTC) uses a high-resolution ultrasound probe mounted in a tracking device with a built-in standoff pad. With standardised settings including transducer tilt angle, gain, focus and depth, approximately 600 consecutive transverse images of the tendon are acquired at a set distance (every 0.2 mm over 12 cm) and concatenated to create a three-dimensional ultrasound data-block. By correlating the pixel stability across a number of contiguous transverse images, specific algorithms can distinguish between four different structure- and non-structure-related echo-types, which reflect the integrity of the tendon matrix (Table 2). Based on this data a colour code is generated, where green displays a good pixel correlation (uniform tissue), blue ≤ 10% difference in pixels (irregular tissue), red ≥ 10% difference in pixels (fibrillar tissue) and black = no correlation (amorphous tissue)~~ (Table 2)~~. Additionally, longitudinal information (sagittal and frontal planes) can be reconstructed, and the percentage of each echo-type can be quantified for a specific tendon region (Fig. 5) (van Schie and Bakker, 2000; van Schie et al., 2001; 2003; van Schie et al., 2010; van Schie et al., 2013; Cook and Purdam, 2014).

The technique was first validated in the equine SDFT where images were compared with the histopathological characteristics of normal and diseased specimens (van Schie and Bakker, 2000; van Schie et al., 2001; 2003). A good inter-observer reliability was subsequently demonstrated for UTC of the equine SDFT as well as the human Achilles and patellar tendons (van Schie et al., 2010; Docking et al., 2015; Geburek et al., 2017; Plevin et al., 2019; Rabello et al., 2019a; van Ark et al., 2019).

Ultrasound tissue characterisation~~TC~~ was further used as a monitoring tool for tendon healing in several experimental studies with surgically created tendon lesions in horses. Based on this research, UTC is currently considered the most objective tool for longitudinal *in vivo* assessment of tendon healing (van Schie et al., 2009; Bosch et al., 2011; David et al., 2012; Cadby et al., 2013; Geburek et al., 2017). Treatment options for equine tendinopathy including cast immobilisation, platelet-rich plasma and adipose-derived mesenchymal stromal cells have been investigated with the aid of this technique (Bosch et al., 2011; David et al., 2012; Geburek et al., 2016; Geburek et al., 2017).

Besides the application for lesion monitoring, it has been shown that UTC can be employed to illustrate the tendon's response to exercise. When UTC examination was performed in a group of racing Thoroughbreds, it was demonstrated that the SDFT shows a significant reduction of aligned tendon bundles (echo-type I) with an increase in discontinuous secondary fascicle bundles (echo-type II) and fibrillar components (echo-type III), for two days post racing (Docking et al., 2012). The described decrease in the tendon's structural integrity appeared to be reversible and returned to baseline within approximately 72 hours. Due to this observation, it was recommended that repeated maximal exercise should be avoided~~the authors recommend avoiding repeated maximal exercise~~ for three days after competitive racing (Docking et al., 2012).

Ultrasound tissue characterisation~~TC~~ was subsequently used in a longitudinal field study, where the SDFT of yearling Thoroughbreds was evaluated as they started race training (Plevin et al., 2019). The study showed that the ultrasonographic characteristics of the SDFT changed significantly within the first 6 months of training, which is thought to reflect the tendon's response to altered loading patterns as race training commences. ~~The authors report a good feasibility for the use of~~ T~~t~~he UTC equipment was considered easy to use with~~in~~ young horses under field conditions. However, the technique was susceptible to movement artefacts, which usually present as blue lines and necessitated repeated examination. The reference values identified within this young group of horses were ≥ 85% echo-type I and ≤ 15% echo-type II with a negligible proportion of echo-type III and IV for all tendons at all time points. In the ~~previously described~~ racing group of Thoroughbreds (mean ± SD age 3.8 ± 0.6 years) described in the previous paragraph, values were somewhat different with > 90% echo-type I, < 5% echo-type II and up to 5% of echo-type IV for mature horses in training (Docking et al., 2012). The increased percentage of echo-type IV might be explained by transient intra-fascicular fluid accumulation as well as subclinical tendon matrix degradation (Docking et al., 2012; Plevin et al. 2019).

A growing body of evidence advocates the benefits of serial tendon scans in human athletes in training in order to recognise subclinical changes of the tendon architecture (Rosengarten et al., 2015; Docking et al., 2016; van Ark et al., 2016; Stanley et al., 2018; Waugh et al., 2018; Rabello et al., 2019a; Rabello et al., 2019b). Further long-term studies should show whether an increase in echo-type II may represent an early indicator for degenerative changes, and whether UTC will prove to be reliable in predicting clinical tendinopathy in equine or human athletes. A systematic review of the human literature regarding UTC emphasizes that standardised protocols should be adopted for UTC measurement and analysis (Rabello et al., 2019b). Another recent study points out that clinical symptoms remain more predictive than UTC for the presence or severity of clinical tendinopathy in man (Docking et al., 2019). This observation is most likely explained by the fact that a high proportion (approximately 80%) of tendons with imaging findings remain asymptomatic in human athletes and the question when and why lesions become painful is yet to be determined~~eluted~~ (Rio et al., 2014; McAuliffe et al., 2016).

In summary, the authors regard ultrasonographic imaging as a vital tool for the assessment of equine flexor tendons and routinely use Doppler ultrasonography for lesion monitoring. In the authors’ experience the reproducibility of elastography is currently still hampered by difficulties in application of uniform pressure distribution using the ultrasound transducer. The use of dynamic shear-wave elastography might overcome some of the limitations of strain elastography in the future, as the technique additionally offers quantitative values for the characterisation of tendon tissues. However, dynamic elastography has not been developed specifically for the use in equine patients so far. The clinical application of UTC is gaining popularity in equine practice. Future longitudinal studies with larger case numbers should show whether UTC is a reliable tool not only for early detection, monitoring and prognostication but also for the prediction of tendon lesions.

Conclusion

Ultrasonography remains the technique most commonly used for the assessment of the flexor tendons in equine practice. Awareness of its potential including Doppler and contrast-enhanced ultrasonography, as well as its limitations, should prevent misinterpretation and guide the selection of cases where alternative imaging techniques might be beneficial. Based on the poor prognosis of horses with flexor tendinopathy a main focus of future diagnostic imaging lies on the detection of subclinical tendon damage before clinical tendinopathy becomes apparent. Additionally, careful monitoring of the tendon structure and viscoelastic properties supports individual rehabilitation and may decrease the risk of re-injury. The modalities developed for this purpose including elastography, acoustoelastography and ultrasound tissue characterisation should be of particular interest for equine practitioners. The majority of the imaging techniques developed for the assessment of the tendon biomechanical properties do not require a hospital setup or ~~large funds~~ and may be used under field conditions.

Conflict of intertest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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van Schie, H.T., de Vos, R.J., de Jonge, S., Bakker, E.M., Heijboer, M.P., Verhaar, J.A., Tol, J.L., Weinans, H., 2010. Ultrasonographic tissue characterisation of human Achilles tendons: quantification of tendon structure through a novel non-invasive approach. British Journal of Sports Medicine 44, 1153-1159.

van Schie, H.T.M., Docking, S.I., Daffy, J., Praet, S.E., Rosengarten, S., Cook, J.L., 2013. Ultrasound tissue characterisation, an innovative technique for injury-prevention and monitoring of tendinopathy. British Journal of Sports Medicine 47, e2.

Verkade, M.E., Back, W., Birch, H.L., 2019. Equine digital tendons show breed-specific differences in their mechanical properties that may relate to athletic ability and predisposition to injury. Equine Veterinary Journal 52, 320-325.

Vilar, J.M., Santana, A., Espinosa, J., Spinella, G., 2011. Cross-sectional area of the tendons of the tarsal region in Standardbred trotter horses. Equine Veterinary Journal 43, 235-239.

Washburn, N., Onishi, K., Wang, J.H., 2018. Ultrasound elastography and ultrasound tissue characterisation for tendon evaluation. Journal of Orthopaedic Translation 15, 9-20.

Waugh, C.M., Alktebi, T., de Sa, A., Scott, A., 2018. Impact of rest duration on Achilles tendon structure and function following isometric training. Scandinavian Journal of Medicine & Science in Sports 28, 436-445.

Weinberg, E.P., Adams, M.J., Hollenberg, G.M., 1998. Color Doppler sonography of patellar tendinosis. American Journal of Roentgenology 171, 743-744.

Werpy, N., Axiak, L., 2013. Review of innovative ultrasound techniques for the diagnosis of musculoskeletal injury. In: Proceedings of the 59th Annual Convention of the American Association of Equine Practitioners, Nashville, Tennessee, USA, 7th-11th December 2013, pp. 209-221.

Werpy, N.M., Denoix, J.M., McIlwraith, C.W., Frisbie, D.D., 2013. Comparison between standard ultrasonography, angle contrast ultrasonography, and magnetic resonance imaging characteristics of the normal equine proximal suspensory ligament. Veterinary Radiology & Ultrasound 54, 536-547.

Winn, N., Lalam, R., Cassar-Pullicino, V., 2016. Sonoelastography in the musculoskeletal system: Current role and future directions. World Journal of Radiology 8, 868-879.

Witte, S., Dedman, C., Harriss, F., Kelly, G., Chang, Y.M., Witte, T.H., 2016. Comparison of treatment outcomes for superficial digital flexor tendonitis in National Hunt racehorses. The Veterinary Journal 216, 157-163.

Wood, A.K., Sehgal, C.M., Polansky, M., 1993. Sonographic brightness of the flexor tendons and ligaments in the metacarpal region of horses. American Journal of Veterinary Research 54, 1969-1974.

Zhang, J., Keenan, C., Wang, J.H., 2013. The effects of dexamethasone on human patellar tendon stem cells: implications for dexamethasone treatment of tendon injury. Journal of Orthopaedic Research 31, 105-110.

Table 1

Reference values for the tendon cross-sectional area (CSA) ~~and echogenicity~~ as detailed for the superficial digital flexor tendon (SDFT) and deep digital flexor tendon (DDFT) of foals, young horses and adults including several specific horse breeds. The flexor tendon CSA is smallest~~decreasing~~ in the mid-metacarpal region and gradually enlarges again towards the level of the fetlock. Mean values are provided, and the reader is referred to the original literature to find reference values for the different tendon levels.

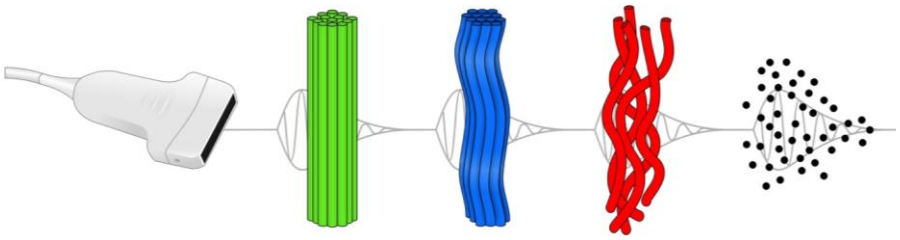
Horse - breed	CSA SDFT	CSA DDFT	Total No. horses	Literature source
Foal 1-6 months	37 – 84 mm^2	31 – 54 mm^2	7	(Korosue et al., 2015; Spinella et al., 2015; Spinella et al., 2016)
Foal 7-24 months	62 – 146 mm^2	57 – 85 mm^2	SDFT = 40 DDFT = 7	(Moffat et al., 2008; Korosue et al., 2015)
Thoroughbred	80 – 146 mm^2	72 – 213 mm^2	SDFT = 268 DDFT = 86	(Gillis et al., 1993; Smith et al., 1994; Gillis et al. 1995b; Celimli et al., 2004; Perkins et al. 2004; Avella et al., 2009; Matos Santiago Reis and Arantes

				Baccarin, 2010; Köster et al., 2014)
Standardbred	82 – 112 mm^2	90 – 165 mm^2	10	(Herslow et al., 2001; Köster et al., 2014)
Arabian horse	62 – 94 mm^2	60 – 92 mm^2	62	(Celimli et al., 2004)
Quarter Horse	78 – 97 mm^2	65 – 150 mm^2	5	(Köster et al., 2014)
Warmblood	90 – 125 mm^2	80 – 165 mm^2	10	(Köster et al., 2014)
Irish Draught cross	123 – 151 mm^2	102 – 224 mm^2	22	(Smith et al., 1994)
Purebred Spanish horse	64 – 107 mm^2	84 – 132 mm^2	20	(Agut et al., 2009)
Icelandic horse	49 – 64 mm^2	63 – 105 mm^2	50	(Boehart et al., 2010a)
Haflinger	52 – 85 mm^2	64 – 143 mm^2	30	(Boehart et al., 2010b)
Pony	71 – 83 mm^2	65 – 149 mm^2	15	(Smith et al., 1994)

Table 2

Following acquisition of three-dimensional ultrasound data, ultrasound tissue characterisation (UTC) algorithms can distinguish between four ultrasound echo-types that correlate with histo-morphological characteristics of the tendon (van Schie et al., 2013). The echo-types represent the integrity of the tendon and the level of fibrillar organisation (van Schie et al., 2010; Plevin et al., 2019; Rabello et al., 2019b).

Ultrasound tissue characterisation echo-types



Echo-type	Type I	Type II	Type III	Type IV
Pixel stability	Highly stable	Medium stable	Highly variable	Constantly low intensity and variable distribution
Tendon integrity	Reflections at intact and aligned tendon bundles	Reflections at discontinuous or wavy tendon bundles	Interfering echoes from mainly fibrillar components	Cellular components and fluid in amorphous tissue

Figure legends

Fig. 1. Flow chart detailing the systematic analysis and selection of scientific literature available on equine flexor tendon imaging. The following search terms were used for review part 1 without restrictions: ‘tendon’ AND ‘ultrasonography’ OR ‘elastography’ OR ‘ultrasound tissue characterisation’ AND ‘equine’ OR ‘horse’. Additional search terms for review part 2: ‘magnetic resonance imaging’ OR ‘computed tomography’.

Fig. 2. Longitudinal contrast-enhanced ultrasound image of the flexor tendons at the level of the digital flexor tendon sheath in a horse without evidence of orthopaedic disease. The intra-thecal injection of the digital flexor tendon sheath with 10 ml of ultrasonographic contrast medium (2 ml of room air in 8 ml of saline, shaken for 10 seconds prior to injection) aids the delineation of the superficial digital flexor tendon (SDFT) and the deep digital flexor tendon (DDFT) (black arrow) as well as the *manica flexoria* (white arrows). The limb is in a flexed position, distal is to the left (Image courtesy of Nadine Ogden, University of Liverpool 2020).

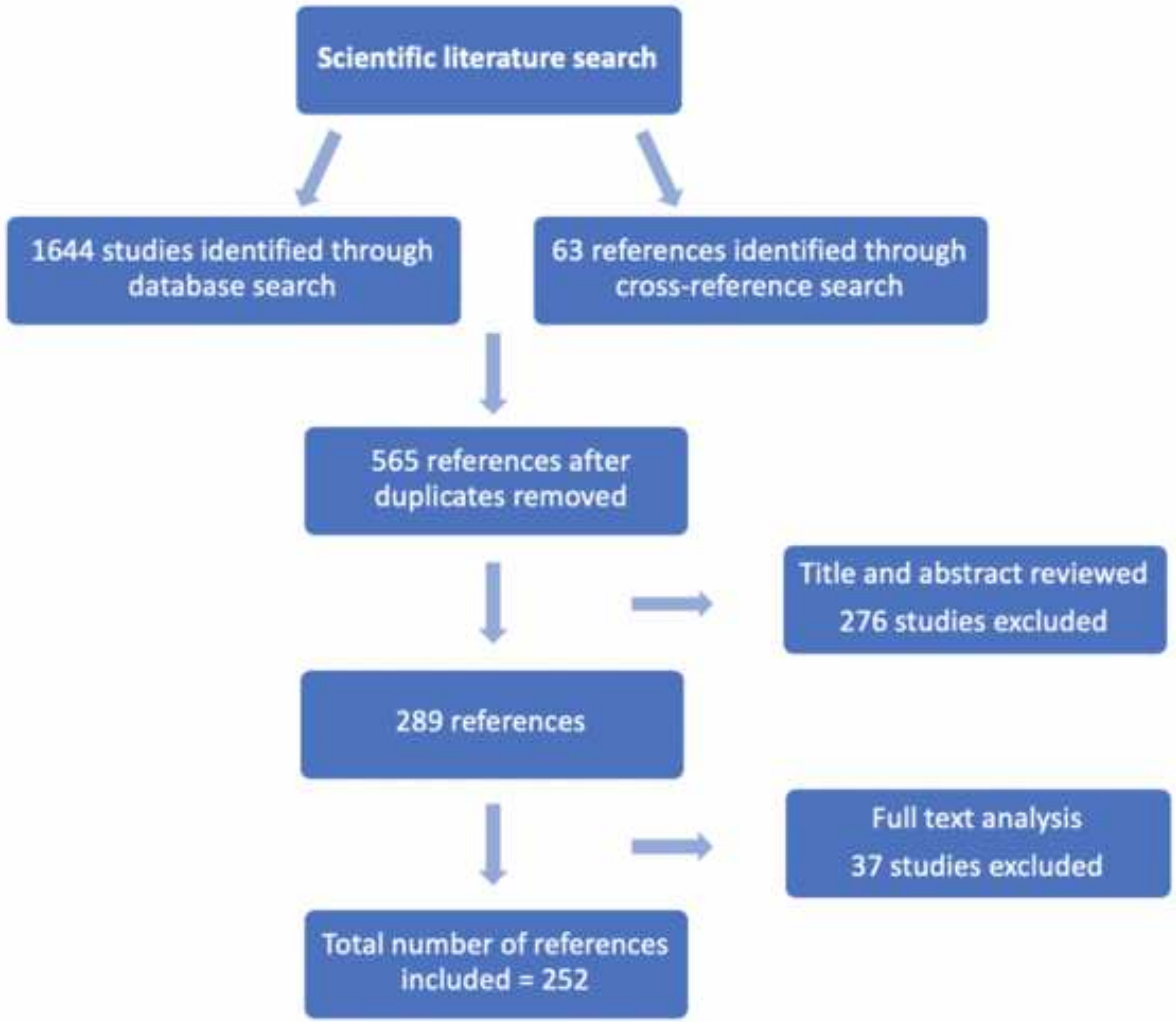
Fig. 3. (A) Colour Doppler transverse ultrasonographic image of the deep digital flexor tendon (DDFT) of an 18-year-old Pony mare with chronic DDF tendinopathy. There is positive Colour Doppler signal and focal mineralisation of the DDFT (white arrow). (B) Power Doppler longitudinal ultrasonographic image of the superficial digital flexor tendon (SDFT) of a 16-year-old Warmblood gelding six weeks after acute injury of the SDFT. Note the reduced fibre alignment and hypoechoic appearance of the SDFT with diffuse Power Doppler signal indicative for neovascularisation.

Fig. 4. Transverse elastogram (compression elastography) on the left and corresponding gray-scale ultrasonographic image (on the right) of the flexor tendons of a 14-year-old Warmblood

gelding with deep digital flexor tendinopathy. The green line (bottom left) indicates the application of adequate pressure using the ultrasound transducer. The relative stiffness of the tissue follows the colour scale (top left) where red is soft, and blue is hard. Note the diffusely heterogenous fibre pattern with small hypoechoic areas throughout the deep digital flexor tendon on the ultrasonographic image that correspond to discrete areas of decreased tissue stiffness on the elastogram.

Fig. 5. Ultrasound tissue characterisation examination showing the colour-coded pixel stability of a core lesion (cross hairs) of the superficial digital flexor tendon (SDFT) in transverse (A), longitudinal (B) and frontal plane (C) with the associated gray scale frontal plane image (D) at the same level. The intact tendon fibres of the superficial and deep digital flexor tendons appear in green colour (echo type I). The SDFT core lesion is characterised by some red (echo type III = fibrillar tissue) and mainly black colour (echo type IV = amorphous tissue) illustrating the loss of integrity of the affected tendon fibres.

Figure 1


Scientific literature search
1644 studies identified through database search
63 references identified through cross-reference search
565 references after duplicates removed
Title and abstract reviewed
276 studies excluded
289 references
Full text analysis
37 studies excluded
Total number of references included = 252


SDFT
DDFT
2

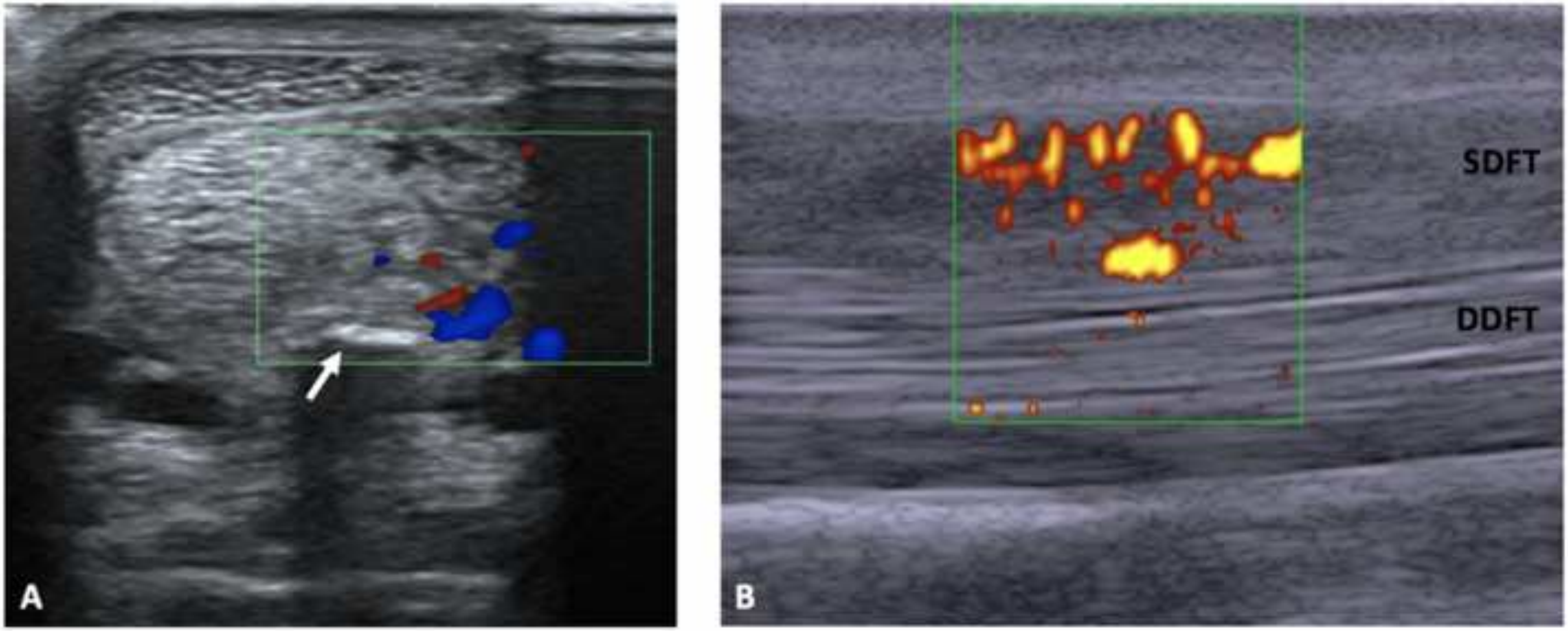
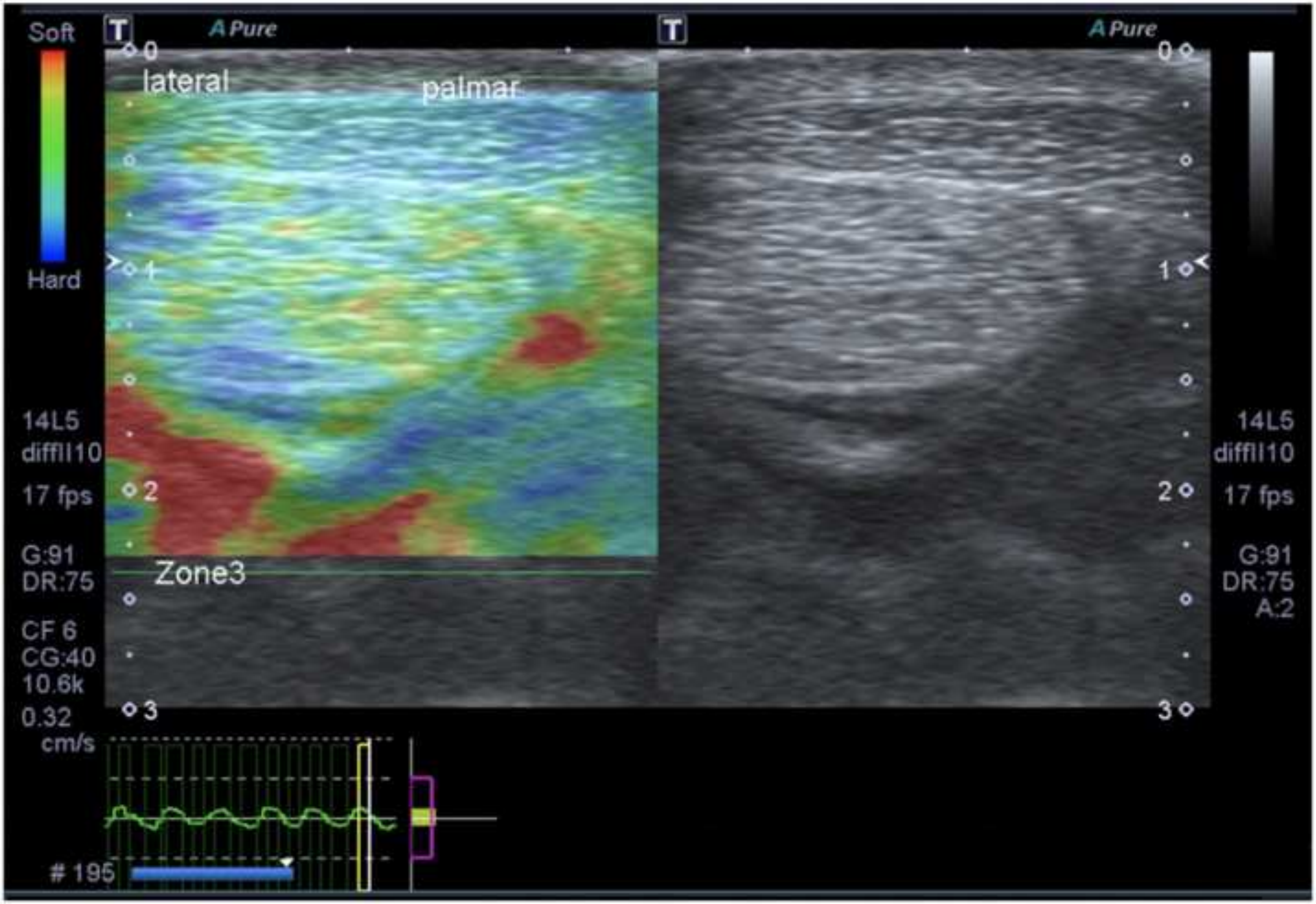

A
B
SDFT
DDFT

Figure 4


Soft
Hard
A Pure
A Pure
lateral
palmar
Zone3
0
1
2
3
14L5
diffII10
17 fps
G:91
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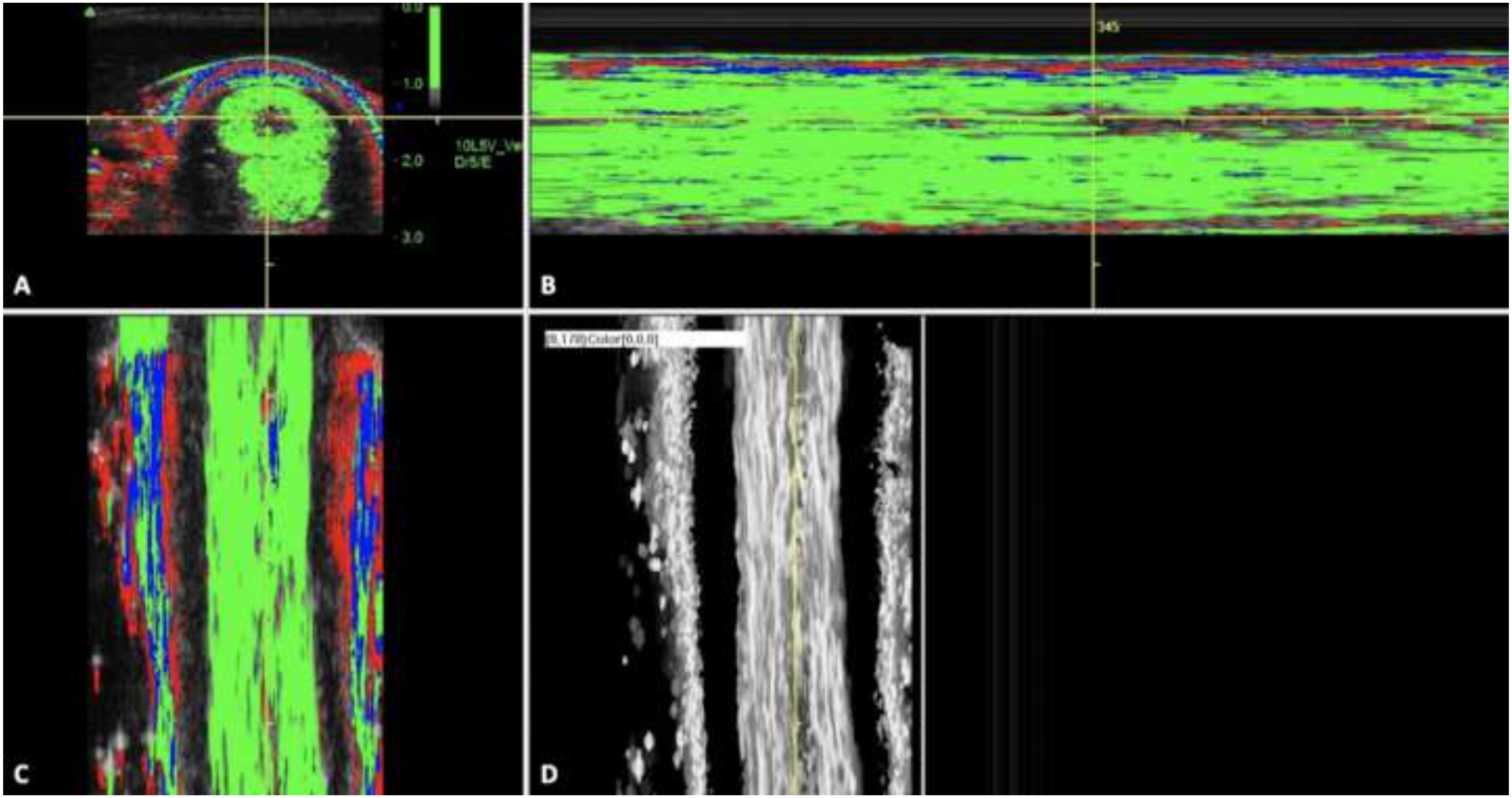
0.32
cm/s
A:2
195


0.0
1.0
2.0
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10LSV_Ve
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A
345
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Highlights review part 1 – ultrasonography

- Power Doppler and contrast add value to B-mode ultrasonography in equine flexor tendon imaging
- Elastography may be used as a monitoring tool in equine flexor tendinopathy
- Training can be adjusted based on tendon ultrasound tissue characterisation as it indicates tendon micro-damage

Revision note – tendon imaging review part 1

The authors would like to thank the Reviewers for their valuable comments and suggestions concerning the manuscript ‘Equine flexor tendon imaging part 1 – recent developments in ultrasonography’.
The manuscript has been revised accordingly and the authors’ response is detailed below. Changes to the manuscript are highlighted as track changes (reviewing mode). The authors hope that the amended manuscript will satisfy the reviewers concerns and is now considered suitable for publication.

Reviewer #1

Reviewer comment: *A very well-written, concise and informative summary of the currently available ultrasonographic technology for assessment of tendon pathology. The article is well-referenced and easy for the reader to follow. As this is an imaging paper, a few more figures would be useful to illustrate certain concepts, such as a comparative image series documenting changes in doppler flow during lesion healing, however it is appreciated that these images may be challenging to obtain in a comparative fashion in a clinical setting. Additionally, the inclusion of UTC images may be of interest to the reader.*

Authors response: The authors would like to thank reviewer 1 for this valuable suggestion, an additional figure showing UTC imaging was added to the manuscript (Figure 5).

Reviewer comment: *I feel that the manuscript overall, whilst an excellent summary of the available technology, lacks a degree of clinical relevance. It would be useful to have a feel of the authors' experiences of using this technology (eg is doppler a routine part of their clinical imaging practice?) and useful for the reader to have an impression of whether emerging technologies like UTC will address the question of subclinical tendon degeneration prior to lesions becoming clinically relevant. Another key question which would be useful for the authors to offer an opinion on would be the use of these technologies in the monitoring of tendon healing. I think the manuscript would be strengthened by a paragraph integrating the excellent summaries of each of the imaging modalities described with the clinical relevance and perhaps the authors' view of the future of ultrasonographic tendon imaging.*

Authors response: A paragraph detailing the clinical relevance and the authors’ personal view on the described imaging modalities as well as the future prospects of ultrasonographic techniques in equine flexor tendon imaging was added (lines 475-486).

Reviewer comment: *Lines 43-49 - should this be qualified as occurring in the equine athlete? The incidence of SDFT lesions in pleasure horses is much lower than this.*

Authors response: The authors agree, ‘occurring in the equine athlete’ was added to the sentence (line 50).

<u>Reviewer comment:</u> *Lines 168-199 - Has there been any work comparing the histological properties of equine tendons with high doppler signal to the ultrasonographic images and biomechanical properties of the tendons?*

<u>Authors response:</u> To the authors' knowledge studies comparing tendon biomechanical properties with Doppler ultrasonographic images are sparse in the equine literature. There is some histological and biomechanical information regarding induced SDFT lesions, for example in the study conducted by Roberto Estrada et al. (2014). This describes a difference in Colour Doppler signal between fore- and hindlimbs that does not correlate with the ultimate tensile strength, elastic modulus or histological findings of the tendons (lines 242-245). Additionally, B-mode and Power Doppler ultrasonography were compared in the work published by Rabba et al. (2018; 2020). This research found Power Doppler signal in equine suspensory branches where B-mode ultrasonography appeared normal (lines 278-282).

Estrada, R.J., van Weeren, P.R., van de Lest, C.H., Boere, J., Reyes, M., Ionita, J.C., Estrada, M., Lischer, C.J., 2014. Comparison of healing in forelimb and hindlimb surgically induced core lesions of the equine superficial digital flexor tendon. Veterinary and Comparative Orthopaedics and Traumatology 27, 358-365.

Rabba, S., Grulke, S., Verwilghen, D., Evrard, L., Busoni, V., 2018. B-mode and Power Doppler ultrasonography of the equine suspensory ligament branches: A descriptive study on 13 horses. Veterinary Radiology & Ultrasound 59, 453-460.

Rabba, S., Petrucci, V., Petrizzi, L., Giommi, D.W., Busoni, V., 2020. B-Mode ultrasonographic abnormalities and Power Doppler signal in suspensory ligament branches of nonlame working Quarter Horses. Journal of Equine Veterinary Science 94, 103254.

<u>Reviewer comment:</u> *Lines 219-261 - it is my impression from the literature and from these paragraphs, that no comparisons of histopathological and biomechanical characteristics of equine tendons and elastography findings have been published. Could the authors please clarify this?*

<u>Authors response:</u> To the authors knowledge there is no published work comparing histopathological and biomechanical characteristics with elastographic findings in equine tendons (lines 355-356). A histological study is available assessing the human Achilles tendon and found a higher sensitivity for the prediction of histopathologic degeneration using sonoelastography when compared to B-mode ultrasonography (Klauser et al. 2013).

Klauser A.S.; Miyamato, H.; Tamegger M. et al. (2013) Achilles tendon assessed with sonoelastography: histologic agreement. Radiology 267, 837-842.

Reviewer comment: Line 223 - should this read no significant differences in elastography readings of tissue stiffness rather than just tissue stiffness?

Author response: The authors agree, and the sentence was adjusted accordingly (line 309).

Reviewer #2:

Reviewer comment: This review is very well written and the authors have worked hard to do a thorough literature review. However, I have some concern regarding the title of the review and the use of the broad term 'flexor tendon imaging'. Whilst the author having performed a thorough review about ultrasonography imaging of the flexor tendons at metacarpal level, they have not included lesions of the deep digital flexor tendon, especially at their main occurrence level within the feet. The author report that the majority of tendon lesions occur in the SDFT with the metacarpal region mostly affected, but the cited references have only investigated race horses in training. The authors fail to provide a reference comparing the comparison of the occurrence of lesions of the DDFT further distally with the more proximally affected SDFT. However, to the best of my knowledge, there is no reference truly comparing the frequency of SDFT and DDFT lesions in a wider horse population. Therefore, it remains debatable, which of these lesions are truly more common. Interestingly, three of all four included ultrasound images of tendinopathies are displaying a DDFT lesion rather than a SDFT lesions.
As the authors are not including ultrasonography of the DDFT at the level of the feet, where these lesions are more commonly observed, I would suggest rephrasing the manuscript title and relevant sentences of the manuscript accordingly.

Authors response: The authors agree, even though the literature search was by no means restricted to the superficial digital flexor tendon, the majority of studies investigating new ultrasonographic techniques, focus on the SDFT of Thoroughbred racehorses. The title and relevant sentences of the manuscript were altered accordingly lines (lines 3-4; 26; 33; 50; 68).
The cited studies by Singer et al. (2008) and Mitchell et al. (2020) (lines 47-48) describe performance horses (Event/Sport horses) detailing the frequency of SDFT and DDFT lesions in this type of horse population, but the exact level of the lesion (foot vs more proximal) is not detailed.
Whilst the ultrasonographic evaluation of the DDFT at the level of the foot has been described, the emerging availability of advanced diagnostic imaging techniques including MRI and CT has largely replaced the use of ultrasonography at this level. The prevalence and imaging techniques used for the assessment of the DDFT are described in detail in review part 2.

Singer, E.R., Barnes, J., Saxby, F., Murray, J.K., 2008. Injuries in the event horse: training versus competition. The Veterinary Journal 175, 76-81.

Mitchell, R.D., DaSilva, D.D., Rosenbaum, C.F., Blikslager, A.T., Edwards, R.B., 2020. Ultrasound findings in tendons and ligaments of lame sport horses competing or training in South Florida venues during the winter seasons of 2007 through 2016. Equine Veterinary Education, (Epub ahead of print) doi: 10.1111/eve.13298.

<u>Reviewer comment:</u> *As mentioned in the title, this review is focused on the on ultrasonography, however, in the abstract and in the article the phrase 'imaging modalities' is used, despite the authors not mentioning other modalities in depth besides ultrasound based ones.*

<u>Authors response:</u> The authors appreciate the reviewers concern and have changed the manuscript accordingly (lines 33; 68). The term ‘imaging techniques’ was rephrased to ‘ultrasonographic imaging techniques’ throughout the manuscript.

<u>Reviewer comment:</u> *The inclusion criteria for this literature review are not very precise mentioned in the main text. On what was the inclusion and exclusion of the articles based? Figure 1 shows how many articles were found and which were selected as references but does not explain why articles were excluded.*

<u>Authors response:</u> The authors would like to thank reviewer 2 for this valid point. The search terms as well as the exclusion criteria are now stated in the introduction section (lines 71-78). Articles were excluded if they were not available in English language or if they were not published in peer reviewed journals. Additionally, articles were not mentioned in the manuscript where the authors felt that they were not very recent, and their content had already been discussed in previously published review articles or was regarded as common knowledge at this point in time.

<u>Reviewer comment:</u> *The phrasing of the two last bullet points of the highlights is a bit suboptimal:*
- The authors state that 'elastography provides a useful monitoring tool…', but in the main text they mention that further studies and developments 'are desirable before elastography may be introduced as a standard tool for monitoring'.
- In the last bullet point they state 'UTC indicates tendon micro-damage and may prevent injury'. Whilst I understand what the authors are referring to, UTC itself will not prevent injuries. Rather its ability to recognise early damage and consequent adaption of the training programme will help to prevent injuries.
Please revise these two bullet points.

<u>Authors response:</u> The authors agree and both bullet points were rephrased accordingly.

<u>Reviewer comment:</u> *Line 25: As mentioned above consider to be more precise of the area investigated in this review. Maybe add 'metacarpal / metatarsal level'.*

<u>Authors response:</u> The authors agree, 'metacarpal / metatarsal level' was added to the sentence (line 26).

Reviewer comment: *Line 31: Reconsider the use of the term 'imaging techniques', as this review almost exclusively focuses on ultrasound. Or phrase more precisely that this first part focuses on ultrasonography.*

Authors response: The term ‘imaging techniques’ was rephrased and ‘ultrasonographic imaging techniques’ is now used throughout the manuscript to be more precise (lines 33; 68).

Reviewer comment: *Line 43: All cited references are based on racing horses, either national hunt or flat. As this might be true for this population of horses, the authors should state this more clearly. Furthermore, as mentioned above, SDFT lesion are more common at metacarpal level, however, injuries of the DDFT are more commonly observed within the foot. The authors do not mention at this paragraph and throughout the text lesions of the DDFT, which can be commonly observed at feet level in other horse populations. Therefore, please rephrase this paragraph or provide a reference comparing the prevalence of DDFT and SDFT lesion at both levels.*

Authors response: The authors appreciate the reviewers’ concern and the sentence was changed as suggested by reviewer 1: ‘in the equine athlete’ (line 50).
More detail about the location and prevalence of DDFT lesions is given in review part 2. The second part of the review deals with advanced diagnostic imaging (MRI/CT) which is used for the assessment of the DDFT more frequently, especially when lesions are suspected at the level of the foot. Due to concerns with the word count the authors tried to keep each introduction quite short and discuss the prevalence of SDFT/DDFT lesions in the parts of the review where each of them seems more relevant.

Reviewer comment: *Line 61: As mentioned above, please phrase more clearly that this part of the review is rather based on ultrasound than all imaging techniques.*

Authors response: The term ‘imaging techniques’ was replaced by ‘ultrasonographic imaging techniques’ (lines 33; 68).

Reviewer comment: *Line 64: For ease of understanding, I would suggest to provide the keywords within the text.*

Authors response: The search terms were added to the introduction as suggested (lines 72-74).

Reviewer comment: *Line 68: Rather start the sentence with the written out term and provide abbreviation in brackets 'Brightness mode (B-mode)'.*

Authors response: The wording was re-ordered as suggested (line 81).

Reviewer comment: Line 73: Consider using 'longitudinal' instead of 'sagittal'.

Authors response: The word ‘sagittal’ was replaced with ‘longitudinal’ (line 86).

Reviewer comment: Line 95: The provided reference by Aggarwal and Agrawal, 2012 is rather about brain MRI than tendon tissue and appears not to truly fit here.

Authors response: Just to explain, this references was added as it details the difference between first- and second-order gray level statistics in a clear fashion. The authors however agree that it might be confusing in this context and the references was therefore removed (lines 176-177).

Reviewer comment: Line 128-133: The study by Seiler et al. only investigated normal tendons, however this is not truly reflected in this paragraph. Please consider rephrasing. Additionally, the provided figure 2 is presumably a sound horse.

Authors response: The authors would like to thank reviewer 2 for this valuable comment. It is now stated that both, the horses in the study published by Seiler et al. 2016 and the horse in Figure 2 were sound horses (lines 212 and 1241).

Reviewer comment: Line 170: Reconsider the use of the term 'tendinitis'. Might be replaced with 'tendon injury' or 'tendinopathy'.

Authors response: The term ‘tendinitis’ was replaced with ‘tendinopathy’ (line 254).

Reviewer comment: Line 22-227: The cited reference has mainly performed high-field MRI in horses with disease of the suspensory ligament and the ALDDFT, but did not had MRI done in other tendon structures. Therefore, the sentence appears to be misleading. I would suggest to state more precisely which structures were specifically investigated with MRI. Please clarify.

Authors response: The authors have re-reviewed the cited paper (Lustgarten et al. 2015) and the structures that were investigated with high-field MRI were stated to be the following: ‘Twenty-one horses had lesions diagnosed with MRI. Several of these horses had multiple lesions for a total of 24 lesions detected on MRI. These included four lesions of the deep digital flexor tendon within the distal digital sheath, 13 lesions of the suspensory branches, and seven lesions of the hindlimb proximal suspensory ligament.’
However, the lesions that were diagnosed with MRI and could also be detected using elastography were mainly suspensory branch lesions. The imaged structures are now stated more clearly in the manuscript (lines 313-314).

Lustgarten M.; Redding W.R.; Labens R. et al. (2015) Elastographic evaluation of naturally occuring tendon and ligament injuries of the equine distal limb. Veterinary Radiology & Ultrasound 56, 670-679.

<u>Reviewer comment:</u> *Line 231: The time frame for chronic lesions appears shorter than the routinely used. The authors of the study combined subacute and chronic lesion. Therefore, it might be more precise to state chronic / subacute lesion (> 2 weeks post trauma).*

<u>Authors response:</u> The word 'subacute' was added to the sentence (line 319).

<u>Reviewer comment:</u> *Line 239-241: This sentence appears confusing. As far as I understood it, stiffness was longer present than gray-scale ultrasonographic changes. However, the sentence states '…increase in tendon stiffness was ONLY detected 4 to 7 months…'. Please clarify.*

<u>Authors response:</u> The authors have reviewed the cited literature again and confirm that the lesion echogenicity of equine SDFT lesion using gray-scale ultrasonography changes within the first three months post injury and remains largely unchanged thereafter. It is however well known that tendon healing and a return to adequate tendon stiffness that would withstand the strains of exercise takes longer than three months to occur (Patterson-Kane and Firth 2009). The significant increase in tendon stiffness that develops at a later stage (5-9 months post injury) cannot be detected using gray-scale ultrasonography. This observation implies the risk that horses might return to exercise based on gray-scale ultrasonographic imaging that shows adequate echogenicity and texture of the tendon defect whilst the tendon stiffness is still low. The authors therefore propose that sonoelastography has the advantage that adequate tendon stiffness can be confirmed prior to returning the horse to exercise (Tamura et al. 2017a/b). It is correct that stiffness can be detected for longer but the significant increase in stiffness seems to be the key observation. The word 'only' was removed from the sentence (line 328).

Patterson-Kane J.C.; Firth E.C. (2009) The pathobiology of exercise-induced superficial digital flexor tendon injury in Thoroughbred racehorses. The Veterinary Journal 181, 79-89.

Tamura N.; Kuroda T.; Kotoyori Y. et al. (2017a) Application of sonoelastography for evaluating the stiffness of equine superficial digital flexor tendon during healing. The Veterinary Record 180, 120.

Tamura N.; Nukada T.; Kato T. et al. (2017b) The use of sonoelastography to assess the recovery of stiffness after equine superficial digital flexor tendon injuries: a preliminary prospective longitudinal study of the healing process. Equine Veterinary Journal 49, 590-595.

<u>Reviewer comment:</u> *Line 326 and 345: Abbreviations should not be used at the beginning of a sentence. Please revise.*

<u>Authors response:</u> The abbreviations were written out at the beginning of both sentences (lines 416 and 437).

Reviewer comment: Line 354: Not really clear to which the described group of Thoroughbreds is referring. I presume the racing group in the paragraph above. I would suggest rephrasing this sentence to ease understanding.

Authors response: The authors agree and the sentence was rephrased accordingly (lines 446-449).

Reviewer #3:

Reviewer comment: This review considers a variety of ultrasonographic techniques that could be used for the assessment of equine tendon injuries, considering also relevant human literature. It is generally well written and sound, although there are some notable omissions and some of the conclusions are potentially misleading. The choice of figures is curious, given that the authors have stressed in the Introduction that SDFT injuries in racing TBs are of highest concern. The figures relate to older non-racehorses and there are more images of the DDFT than the SDFT.

Authors response: The authors would like to thank reviewer 3 for the in depth and very detailed and helpful consideration of the manuscript. The authors hope that they were able to address the reviewers' concerns as outlined below. A figure of a SDFT lesion in a young horse was additionally included (Figure 5).

Reviewer comment: Line 25 has (not is)

Authors response: The authors apologize for the grammatical error, the sentence was corrected (line 27).

Reviewer comment: Line 27 'current' is redundant

Authors response: The word 'current' was removed from the sentence (line 28).

Reviewer comment: Line 35 tendon is in title; suggest substituting another word. Key words are supposed to provide additional words for a search engine that are not in the title

Authors response: The authors would like to thank reviewer 3 for this valuable suggestion, the term 'tendon' was replaced with 'tendinopathy' and 'imaging' was replaced with 'image' (line 37).

Reviewer comment: Line 64 Over what time period was the published literature reviewed?

<u>Authors response:</u> The time period was not restricted during the literature search (line 72). The authors however focused on the literature published over the past decade when compiling the manuscript.

<u>Reviewer comment:</u> Line 73 or diffuse decrease in echogenicity with loss of long linear parallel echoes in longitudinal images

<u>Authors response:</u> This very valid suggestion was added to the sentence (lines 85-86).

<u>Reviewer comment:</u> Line 80 and elsewhere Although commonly described as 'fibre pattern' this is not strictly accurate - what you see in a normal tendon is long linear parallel echoes

<u>Authors response:</u> The authors agree and the paragraph was adjusted accordingly (lines 86; 159-160).

<u>Reviewer comment:</u> Line 85 Tendon CSA measurements have been shown to be user-dependent in previous studies

<u>Authors response:</u> Originally the manuscript included a paragraph detailing the studies that are available on CSA measurements. The paragraph was removed as the manuscript exceeded the allowed word count. The paragraph is now added again for the reviewers' information (lines 90-154). Whilst the authors feel that the paragraph would add value to the paper, we will defer to the editor as to whether it should be included in the manuscript.

<u>Reviewer comment:</u> Line 86 the reference to Table 1 belongs next to CSA in line 85

<u>Authors response:</u> The references was moved to be found in the added section about tendon CSA (line 112).

<u>Reviewer comment:</u> Line 110 The authors discuss off-incidence imaging and say 'Scanning a tendon off-incidence can be helpful to clearly distinguish the outline of the tendon and to differentiate between tendon fibres, fat, muscle and scar tissue'. I think that this refers to the suspensory ligament. There should not be fat and muscle in a tendon. Focus on the SDFT and tell us exactly why and when we should use off-incidence imaging.

<u>Authors response:</u> The authors appreciate this valuable comment and the sentence was adjusted as requested (line 192).

<u>Reviewer comment:</u> *The authors at no point indicate that in the distal metacarpal region in order to image the medial and lateral borders of the SDFT the transducer will need to be rocked medially and laterally.*
Nor do they mention that if there is an acentric core lesion, longitudinal images need to be acquired in the same plane as the lesion as well as from the palmar midline.
Moreover, especially with recurrent SDFT injuries in racing TBs, the SDFT branches in the pastern region may have concurrent injury.
There is no mention/ discussion about serial monitoring the SDFT during rehabilitation - the usefulness and limitations

<u>Authors response:</u> The authors totally agree with all of the described but feel that the manuscript should not be considered a guide to ultrasonography of equine flexor tendons. Very good and detailed books as well as practical courses are available for this purpose. The manuscript aims to summarize new techniques and research findings that might be of particular interest for the educated practitioner with basic knowledge in equine flexor tendon ultrasonography.

<u>Reviewer comment:</u> *Line 121 adhesions, as well as* or adhesion formation, as well as

<u>Authors response:</u> The authors apologize for the grammatical error, the sentence was corrected (line 204).

<u>Reviewer comment:</u> *Line 133 Is there any published evidence that this invasive technique provides useful clinical information?*

<u>Authors response:</u> The authors appreciate the reviewer’s concern and the short answer to the question is ‘no’. However, the scope of this review is to detail recent developments and new techniques in ultrasonography which includes some techniques with more and some with less published evidence justifying their use. It is clearly stated in lines 216-218 that further research is required before this technique may find clinical application.

<u>Reviewer comment:</u> Line 134 Why is the reference to Fig. 2 in this position?

<u>Authors response:</u> The reference to Figure 2 was moved to the beginning of the paragraph (line 207).

<u>Reviewer comment:</u> *Line 177 Reference needed. What causes 'Tendon mineralization can be an incidental finding'? Why is there? Why is there a much higher frequency of occurrence in the DDFT in the fetlock region and rarely anywhere in the SDFT?*

<u>Authors response:</u> The authors believe that besides the described injury or corticosteroid related pathogenesis, tendon mineralisation might be a result of progressing chondroid metaplasia. This would also explain why the DDFT is commonly affected at the level of the fetlock canal where

chondrogenic differentiation is usually seen in this tendon. For further detail on the dissemination of chondroid tissue in the SDFT the authors would like to refer to the research by Ali et al. 2021. The sentence was adjusted, and the reference was added (lines 262-264).

Ali O.; Ehrle A.; Comerford E.J. et al. (2021) Intrafascicular chondroid-like bodies in the ageing equine superficial digital flexor tendon comprise glycosaminoglycans and type II collagen. Journal of Orthopaedic Research, e-pub ahead of print.

Reviewer comment: Line 178 clinically apparent or subclinical injury?

Authors response: As far as it is known, both clinically apparent as well as subclinical tendon injuries can result in tendon mineralisation. The sentence was adjusted as indicated for clarity (lines 262-264).

Reviewer comment: Line 231 Abbreviations have not been defined

Authors response: Abbreviations are now defined (lines 318-319).

Reviewer comment: Line 236-244 So what do you conclude about the functional usefulness of the technique based on the evidence presented?

Authors response: A paragraph detailing the authors' personal impression on the usefulness of the described techniques was added towards the end of the manuscript, just before the conclusion as requested by reviewer 1 (lines 475-486).

Reviewer comment: Line 271 Has any study correlated imaging findings with histopathology or functional characteristics such as load to failure?

Authors response: To the authors knowledge there is no published work comparing histopathological and biomechanical characteristics with elastographic findings in equine tendons (line 355-356). A histological study is available assessing the human Achilles tendon and found a higher sensitivity for the prediction of histopathologic degeneration using sonoelastography when compared to B-mode ultrasonography (Klauser et al. 2013).

Klauser A.S.; Miyamato, H.; Tamegger M. et al. (2013) Achilles tendon assessed with sonoelastography: histologic agreement. Radiology 267, 837-842.

Reviewer comment: Line 342 Which authors? Docking et al?
Why not rephrase as '.. ... it was recommended that repeated maximal exercise should be avoided for ..... (Docking et al)'

Authors response: The authors would like to thank reviewer 3 for this very valuable suggestion, the sentence was adjusted accordingly (lines 434-435).

Reviewer comment: Line 349 reported
But I would suggest rephrasing this sentence, which does not read well.
The UTC equipment was considered easy to use with young horses under field conditions.

Authors response: The authors really appreciate the sentences suggested by reviewer 3 which reads much better indeed. The sentence was adjusted (lines 441-442).

Reviewer comment: Line 354 please make it clear that these were racing Thoroughbreds and indicate age range

Authors response: The sentence now includes the requested information (lines 446-447).

Reviewer comment: What is the significance of echo type IV? Please explain to the reader.

Authors response: A sentence detailing possible explanations for the increased percentage of echo-type IV was added to the paragraph (lines 449-451).

Reviewer comment: Line 371 determined may be a better term than eluted in this context

Authors response: The term 'eluted' was replaced by 'determined' (line 473).

Reviewer comment: Line 379 But would people alter management on the basis of the finding of subclinical change, especially when you have stated above that a high % of imaging findings remain asymptomatic in human athletes?

Authors response: This is a very good question. It probably largely depends on the owner and the purpose of the horse. A racehorse trainer will most likely not change the entire training schedule of a horse just because of some elastography/UTC readings but things might be different in privately owned general purpose/riding horses.
Further research is required in order to find out how high the percentage of asymptomatic, subclinical tendinopathy (that show up using the described ultrasonographic imaging techniques) really is in the equine athlete. Studies like the one published by Docking et al. 2012 are a first step in understanding the influence of exercise on the described imaging findings and provides practical training advice.
As there is no imaging to date that really reliably predetermines the development of clinical tendinopathy the sentence in line 493 was phrased in a way that this achievement remains a future prospect.

Docking S.I.; Daffy J.; van Schie H.T. et al. (2012) Tendon structure changes after maximal exercise in the Thoroughbred horse: use of ultrasound tissue characterisation to detect in vivo tendon response. The Veterinary Journal 194, 338-342.

Reviewer comment: Line 385 UTC is expensive and beyond the range of most equine practitioners!

Authors response: The authors agree, the ultrasonographic imaging techniques were regarded as affordable when compared to the techniques described in part 2 of the review (CT and MRI). The price of UTC (approx. 60.000 Euro) is higher than that of a modern ultrasound scanner but similar to that of a portable digital radiography system and might therefore potentially be considered by larger practices. The sentence was however adjusted accordingly (line 500).

Reviewer comment: line 1034 what do you mean by 'is decreasing' - compared with what? What value is this statement?
What real value does this table have given the huge variances reported?
Why is echogenicity included in line 1032?

Authors response: The sentence was altered for clarity, the SDFT CSA is smallest in the mid-metacarpal region which implies that the CSA is not consistent throughout the tendon (line 1219).
The authors agree, there is a huge variance between breeds and the variance between individual horses probably plays an additional role. As the scope of this review is to summarize the literature and there is a significant number of studies published on this specific topic (CSA reference values in different horse breeds) the authors felt that the information should be included. Whilst it might go too far to discuss and compare all those studies in detail, the table serves the purpose to give the reader an overview of the published work. The authors would further like to refer to the added paragraph on CSA measurements (lines 90-154).
Some of the papers contain reference values for the tendon echogenicity. However, as the echogenicity values were not included in the table the word ‘echogenicity’ was removed (line 1217).

Reviewer comment: Line 1048 It is stated here that the equine literature was searched but the authors have provided numerous references to human studies. Please clarify.

Authors response: The human studies cited in this text were not specifically included in the systematic literature search and are not reflected in the numbers given in Figure 1. Human studies were added to give background information regarding the described ultrasonographic imaging techniques (usually in the first paragraph) and where the authors felt that it might be of interest to the reader to get additional information from human studies (where the information form equine research is still sparse).

<u>Reviewer comment:</u> *Figs. 3, 4 It seems a bit odd to use images of the DDFT of older sports horses when you have said that injuries are most common in the SDFT of TB racehorses*

<u>Authors response:</u> An additional figure showing UTC imaging of a SDFT lesion in a young horse was added (Figure 5).