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

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Abstract:	<p>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.</p>

1 **Review**

2

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

5

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20

21 **Abstract**

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23 considerable impact on equine welfare and the wider horse industry. Ageing and repetitive
24 strain frequently cause varying degrees of tendon micro-damage prior to the recognition of
25 clinical tendinopathy. Whilst B-mode ultrasonography is most commonly utilized for
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33 ultrasonographic imaging techniques and their limitations, and to introduce and critically
34 appraise new modalities that could potentially change the clinical approach to equine flexor
35 tendon imaging.

36

37 *Keywords:* Elastography; Horse; Image; Tendinopathy; Ultrasound

38

39 **Introduction**

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

44

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

52

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

61

62 The aim of this literature review is to provide evidence detailing the commonly used
63 and recently developed ultrasonographic imaging techniques for the assessment of equine

64 tendons. The current state of knowledge including implications, pitfalls and future directions
65 of ultrasonography, elastography and ultrasound tissue characterisation for equine flexor
66 tendon imaging will be discussed. A systematic PubMed, Medline and Google Scholar search
67 with the following search terms was performed without restrictions: ‘tendon’ AND
68 ‘ultrasonography’ OR ‘elastography’ OR ‘ultrasound tissue characterisation’ AND ‘equine’
69 OR ‘horse’. Additionally, studies were identified by searching the reference list of eligible
70 articles (Fig. 1). Articles were excluded if they were not available in English language or if
71 they were not published in peer reviewed journals. Additionally, references were not
72 discussed in the manuscript where the authors did not regard them as recent or their content
73 had already been discussed in detail in previous reviews.

74

75 **Ultrasonography**

76 Brightness-mode (B-mode) sonography displays the difference in acoustic impedance
77 within a two-dimensional tissue cross-section and remains the modality most widely used for
78 the assessment of tendon and ligament injuries in equine practice (Rantanen, 1982; Gaschen
79 and Burba, 2012; Palgrave and Kidd, 2014; Berner, 2017). Tendon lesions are generally
80 classified as core lesions, border lesion, tendon splits or diffuse decrease in echogenicity with
81 loss of long linear parallel echoes with transverse as well as longitudinal plane
82 ultrasonography required to determine the extent of an injury (Smith, 2008; Alzola et al.,
83 2018).

84

85 *Tendon cross-sectional area*

86 The tendon cross-sectional area (CSA) as well as the CSA and length of a tendon
87 lesion can be calculated with reasonable accuracy using ultrasonography (Genovese et al.,
88 1990; Smith et al., 1994; Gillis et al., 1995a; Alzola Domingo et al., 2017; Kojah et al.,

89 2017). Other measurements including latero-medial or dorso-palmar/plantar dimensions of
90 tendon lesion are considered less sensitive (Reef, 2001; Smith and Cauvin, 2014).

91

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

100

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

111

112 Based on variations in functional adaption, the flexor tendon CSA does not increase
113 in a proportional rate during maturation (Birch et al., 1999; Korosue et al., 2015). A small

114 number of studies describe a tendon hypertrophy and reduction in echogenicity with training,
115 but results are inconsistent and tend not to demonstrate statistically significant alterations
116 unless clinical tendinopathy is present (Gillis et al., 1993; Birch et al., 1999; Kasashima et al.,
117 2002; Perkins et al., 2004; Moffat et al., 2008; Avella et al., 2009; Matos Santiago Reis and
118 Arantes Baccarin, 2010). Despite some conflicting reports, overall age, sex, height at the
119 withers or body mass index do not appear to have a strong influence on the equine flexor
120 tendon CSA in the adult horse (Smith et al., 1994; Gillis et al., 1995b; Gillis et al., 1995c;
121 Moffat et al., 2008; Agut et al., 2009; Avella et al., 2009; Boehart et al., 2010a; Köster et al.,
122 2014). Additionally, most studies have not identified a difference between limbs (left vs right
123 forelimb), which implies that ultrasonographic examination of the contralateral limb can
124 serve as a valid comparison provided bilateral tendinopathy can be excluded (Smith et al.,
125 1994; Gillis et al., 1995b; Perkins et al., 2004; Moffat et al., 2008; Agut et al., 2009; Avella et
126 al., 2009; Köster et al., 2014). A 20% increase in tendon CSA when compared to the
127 contralateral side is regarded as a sign for tendinopathy by several authors (Smith et al., 1994;
128 Reef, 2001; Smith, 2008; Smith and Cauvin, 2014). In case of bilateral tendinopathy, it has
129 been suggested to analyse the SDFT:DDFT ratio to assess for tendon enlargement (Berner,
130 2017).

131

132 Tendon lesions with a maximal injury zone of more than 25% of the total transverse
133 tendon CSA are usually classed as severe lesions (Reef, 2001; Smith, 2008). A recent study
134 showed a probability of 29-35% for a successful return to racing after SDFT injury in core
135 lesions that involved less than 50% of the tendon CSA, with a decrease to 11-16% in lesions
136 $\geq 50\%$ CSA. The study additionally found that the prognosis in cases of tendinopathy without
137 core lesion reduced from 49-99% to just 14% if at the maximal injury zone 75% or more of
138 the longitudinal fibre pattern was disrupted (Alzola et al., 2018).

139

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

146

147 *Tendon echogenicity*

148 The ultrasonographic echogenicity will provide some information about the collagen
149 density of the tendon tissue and multiple scoring systems estimating the tendon intensity
150 (brightness), homogeneity and the degree of axial alignment of tendon fibres (seen as long
151 linear parallel echoes) have been proposed for the grading and documentation of tendon
152 lesions (Reef, 2001; Rantanen et al., 2011; Smith and Cauvin, 2014; Alzola Domingo et al.,
153 2017). It has been well established that the qualitative or semiquantitative assessment of the
154 tendon echogenicity is highly subjective and operator dependent (Crass et al., 1988;
155 Genovese et al., 1990; van Schie et al., 1999). Good intra- and inter-rater agreement has been
156 described for parameters including type of injury, maximal injury zone, location, tendon
157 cross-sectional area and longitudinal fibre pattern (echo alignment), but not for echogenicity
158 (Alzola Domingo et al., 2017).

159

160 In order to increase objectivity, it has been suggested to perform first-order gray level
161 statistics where different shades of gray are quantified within a defined region of interest
162 (Wood et al., 1993; Tsukiyama et al., 1996; Micklethwaite et al., 2001; Crevier-Denoix et al.,
163 2005). It has since been demonstrated that the use of such first-order gray level analysis,

164 where the distribution of the shades of gray is quantified without determining the relative
165 position of the various gray levels within the image (second-order statistics), has a low
166 sensitivity for the accurate quantification of tendon tissue (van Schie et al., 1999; van Schie et
167 al., 2000). The tendon echogenicity is additionally influenced by the amplifier gain output
168 and the position of the transducer. Tilting the transducer by only 3° can change the mean gray
169 level of the tissue by as much as 40%, as the returning echo is directed away from the
170 transducer (anisotropy phenomenon). Displacement of the transducer (2 mm) may account
171 for a 20% difference in the gray level histogram of an acute tendon lesion (van Schie et al.,
172 1999). As research in this area progresses, the use of ultrasound tissue characterisation (UTC)
173 was developed to quantify the architecture of the tendon matrix more accurately (see below)
174 (van Schie et al., 2001; 2003).

175

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

186

187 Where a lesion is suspected within the digital flexor tendon sheath, ultrasonographic
188 examination in a flexed limb position as well as dynamic ultrasonography of the non-weight

189 bearing limb is additionally recommended (Seignour et al., 2012). Dynamic examination in
190 flexion and extension allows for the visualisation of the gliding motion between the deep
191 digital flexor tendon (DDFT), the SDFT and the palmar/plantar annular ligament and may
192 identify adhesion formation, as well as *manica flexoria* tears (DiGiovanni et al., 2016; Garcia
193 da Fonseca et al., 2019).

194

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

207

208 *Doppler ultrasonography*

209 Doppler imaging for the assessment of musculoskeletal pathology was initially
210 described in human patients with rotator cuff, Achilles, or patellar tendon injury (Newman et
211 al., 1994; Hollenberg et al., 1998; Weinberg et al., 1998). The Colour Doppler signal
212 indicates the direction and velocity of blood flow, based on the interference of the moving

213 blood cells with the wavelength (and hence frequency) of ultrasound waves (Naredo and
214 Monteagudo, 2014; Palgrave and Kidd, 2014).

215

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

227

228 Doppler signal that occurs in relation to a tendon injury should decline after 3-6
229 months, as tendon healing progresses. A study evaluating the healing process of tendon
230 lesions found that surgically induced SDFT lesions in the hindlimb showed significantly less
231 Doppler signal when compared to forelimb lesions, 6 months post injury (Estrada et al.,
232 2014). During sequential examination, continuous or re-occurring Doppler signal in
233 association with a flexor tendon lesion is suggestive of a delayed healing response or re-
234 injury (Alfredson et al., 2003; Sharma and Maffuli, 2005; Smith and Cauvin, 2014; Hatazoe
235 et al., 2015). For optimal visualisation of blood flow, the limb should be kept in a non-weight
236 bearing position when Doppler ultrasonography is performed (Kristoffersen et al., 2005;
237 Boesen et al., 2007; Rabba et al., 2018).

238

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

246

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

255

256 In contrast to the commonly utilized Colour Doppler, Power Doppler ultrasonography
257 analyses the total intensity of returning signal without characterising the direction of flow
258 (Fig. 3B). The technique is independent of the ultrasound probe being aligned to the direction
259 of blood flow and is particularly sensitive for imaging of low-velocity flow from small
260 vessels (Martinoli et al., 1998; Anderson and McDicken, 2002; Naredo and Monteagudo,
261 2014; Palgrave and Kidd, 2014). Power Doppler ultrasonography has been shown to be
262 useful for the detection of microvasculature in both Achilles and patellar tendons in man

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

268

269 **Elastography**

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

277

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

287 or acoustic radiation force and the shear-waves created by the excitation are recorded (Ooi et
288 al., 2014; Sigrist et al., 2017; Taljanovic et al., 2017).

289

290 An initial study investigating the use of strain elastography for the assessment of
291 equine flexor tendons demonstrated a moderate reproducibility (68%, Kappa agreement (κ) =
292 0.46) and good repeatability (83%, κ = 0.78) when different operators performed
293 elastography in horses without evidence of tendinopathy (Lustgarten et al., 2014). There was
294 no significant difference in elastography readings of tissue stiffness when tendons were
295 imaged in various leg positions, but the flexor tendons appeared softer in the longitudinal
296 plane compared to the transverse plane. The same technique was subsequently applied in
297 clinical cases of tendon and ligament injury and compared with gray-scale ultrasonography
298 (SDFT, DDFT, accessory ligament of the DDFT, suspensory ligament) and high-field MRI
299 (1.5T) (DDFT, suspensory ligament) (Lustgarten et al., 2015). Elastograms were assessed
300 subjectively using a qualitative colour-grading system. Additionally, an algorithm was
301 developed for the quantitative analysis of the percentage of each colour within a specific
302 region of interest. A significant correlation was found between lesions detected during strain
303 elastography, gray-scale ultrasonography, short tau inversion recovery-fast spin echo (STIR-
304 FSE) and proton density (PD) weighted MRI sequences. Chronic / subacute tendon lesions (>
305 2 weeks post trauma) appeared stiffer than acute lesions when quantitative and qualitative
306 elastographic evaluation was performed.

307

308 Further research focused on the use of strain elastography as a monitoring tool for the
309 recovery period of Thoroughbred racehorses that had sustained an SDFT injury (Tamura et
310 al., 2017a; Tamura et al., 2017b). A prospective longitudinal study that followed a group of
311 horses (n = 7) with SDFT tendinopathy showed that the lesion echogenicity changed within

312 the first two months post injury using gray-scale ultrasonography. However, evidence for a
313 significant increase in tendon stiffness was detected 4 to 7 months later on elastograms of the
314 affected SDFTs (Tamura et al., 2017b). A second study by the same group confirmed that a
315 significant difference in tendon stiffness can be detected using sonoelastography during the
316 later stages of tendon healing (5 vs 9 months post injury), where gray-scale ultrasonographic
317 findings remain largely unchanged (Tamura et al., 2017a).

318

319 In human athletes, the use of elastography as a monitoring tool for the identification
320 of Achilles tendons with an increased injury risk showed promising results (Ooi et al., 2015;
321 Balaban et al., 2016; Ooi et al., 2016). Additionally, age-related changes in Achilles tendon
322 stiffness have been characterised with elastography in man (Klauser et al., 2014; Turan et al.,
323 2015). Since the equine SDFT is considered functionally equivalent to the human Achilles
324 tendon, monitoring protocols for the prevention of flexor tendon injury may be adaptable for
325 equine athletes (Patterson-Kane and Rich, 2014; Washburn et al., 2018). Whilst current
326 reports describe a good repeatability and interobserver agreement for strain elastography in
327 horses, the variability of the manually applied tissue compression is a limitation that is yet to
328 overcome (Klauser et al., 2010; Lustgarten et al., 2014; Tamura et al., 2017a; Tamura et al.,
329 2017b). Due to an uneven pressure distribution, colour artefacts can additionally occur,
330 particularly on transverse images of the SDFT. A standoff pad reduces artefacts at the tendon
331 margins and can additionally be used as an external standard reference for quantitative
332 sonoelastographic evaluation but may occasionally result in reverberation artefacts
333 (encountered as red lines) (Lustgarten et al., 2014; Tamura et al., 2017a; Tamura et al.,
334 2017b).

335

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

346

347 *Acoustoelastography*

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

356

357 The method was initially described in porcine and canine tendons and has since been
358 used for the evaluation of the equine SDFT and DDFT in groups of clinically normal horses
359 (Duenwald-Kuehl et al., 2012a; Ellison et al., 2013; Ellison et al., 2014; Berger et al., 2018).
360 Good intra- and inter-observer repeatability with no significant effect of age, sex or limb was

361 demonstrated (Ellison et al., 2013; Ellison et al., 2014). It is, however, important to note that
362 the stiffness may not be homogeneous along the length of the tendon and can vary amongst
363 individual horses (De Gasperi et al., 2017; Berger et al., 2018). Additionally, sedation
364 (detomidine-butorphanol combination) appears to significantly decrease the stiffness gradient
365 of the equine SDFT (De Gasperi et al., 2017).

366

367 In contrast to strain elastography, acoustoelastography is thought to more accurately
368 mimic the natural situation, where tendons are exposed to longitudinal strain rather than
369 compression (Ellison et al., 2014). Initial reports of the use of acoustoelastography for the
370 detection of strain-induced damage or tendon tears, as well as for monitoring purposes after
371 tendon injury are available for other species (Duenwald-Kuehl et al., 2012b; Frisch et al.,
372 2014; Hans et al., 2014). Further research assessing the feasibility of acoustoelastography for
373 the detection of tendon injury and monitoring of tendon healing in equine patients is
374 warranted before this technique might be more widely used in the field of equine
375 orthopaedics.

376

377 **Ultrasound Tissue Characterisation**

378 Computerised ultrasound tissue characterisation (UTC) uses a high-resolution
379 ultrasound probe mounted in a tracking device with a built-in standoff pad. With standardised
380 settings including transducer tilt angle, gain, focus and depth, approximately 600 consecutive
381 transverse images of the tendon are acquired at a set distance (every 0.2 mm over 12 cm) and
382 concatenated to create a three-dimensional ultrasound data-block. By correlating the pixel
383 stability across a number of contiguous transverse images, specific algorithms can distinguish
384 between four different structure- and non-structure-related echo-types, which reflect the
385 integrity of the tendon matrix (Table 2). Based on this data a colour code is generated, where

386 green displays a good pixel correlation (uniform tissue), blue $\leq 10\%$ difference in pixels
387 (irregular tissue), red $\geq 10\%$ difference in pixels (fibrillar tissue) and black = no correlation
388 (amorphous tissue). Additionally, longitudinal information (sagittal and frontal planes) can be
389 reconstructed, and the percentage of each echo-type can be quantified for a specific tendon
390 region (Fig. 5) (van Schie and Bakker, 2000; van Schie et al., 2001; 2003; van Schie et al.,
391 2010; van Schie et al., 2013; Cook and Purdam, 2014).

392

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

399

400 Ultrasound tissue characterisation was further used as a monitoring tool for tendon
401 healing in several experimental studies with surgically created tendon lesions in horses.
402 Based on this research, UTC is currently considered the most objective tool for longitudinal
403 *in vivo* assessment of tendon healing (van Schie et al., 2009; Bosch et al., 2011; David et al.,
404 2012; Cadby et al., 2013; Geburek et al., 2017). Treatment options for equine tendinopathy
405 including cast immobilisation, platelet-rich plasma and adipose-derived mesenchymal
406 stromal cells have been investigated with the aid of this technique (Bosch et al., 2011; David
407 et al., 2012; Geburek et al., 2016; Geburek et al., 2017).

408

409 Besides the application for lesion monitoring, it has been shown that UTC can be
410 employed to illustrate the tendon's response to exercise. When UTC examination was

411 performed in a group of racing Thoroughbreds, it was demonstrated that the SDFT shows a
412 significant reduction of aligned tendon bundles (echo-type I) with an increase in
413 discontinuous secondary fascicle bundles (echo-type II) and fibrillar components (echo-type
414 III), for two days post racing (Docking et al., 2012). The described decrease in the tendon's
415 structural integrity appeared to be reversible and returned to baseline within approximately
416 72 hours. Due to this observation, it was recommended that repeated maximal exercise
417 should be avoided for three days after competitive racing (Docking et al., 2012).

418

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

434

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

449

450 In summary, the authors regard ultrasonographic imaging as a vital tool for the
451 assessment of equine flexor tendons and routinely use Doppler ultrasonography for lesion
452 monitoring. In the authors' experience the reproducibility of elastography is currently still
453 hampered by difficulties in application of uniform pressure distribution using the ultrasound
454 transducer. The use of dynamic shear-wave elastography might overcome some of the
455 limitations of strain elastography in the future, as the technique additionally offers
456 quantitative values for the characterisation of tendon tissues. However, dynamic elastography
457 has not been developed specifically for the use in equine patients so far. The clinical
458 application of UTC is gaining popularity in equine practice. Future longitudinal studies with

459 larger case numbers should show whether UTC is a reliable tool not only for early detection,
460 monitoring and prognostication but also for the prediction of tendon lesions.

461

462 **Conclusion**

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

476

477 **Conflict of interest statement**

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

480

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1179 **Table 1**

1180 Reference values for the tendon cross-sectional area (CSA) as detailed for the superficial
 1181 digital flexor tendon (SDFT) and deep digital flexor tendon (DDFT) of foals, young horses
 1182 and adults including several specific horse breeds. The flexor tendon CSA is smallest in the
 1183 mid-metacarpal region and gradually enlarges again towards the level of the fetlock. Mean
 1184 values are provided, and the reader is referred to the original literature to find reference
 1185 values for the different tendon levels.

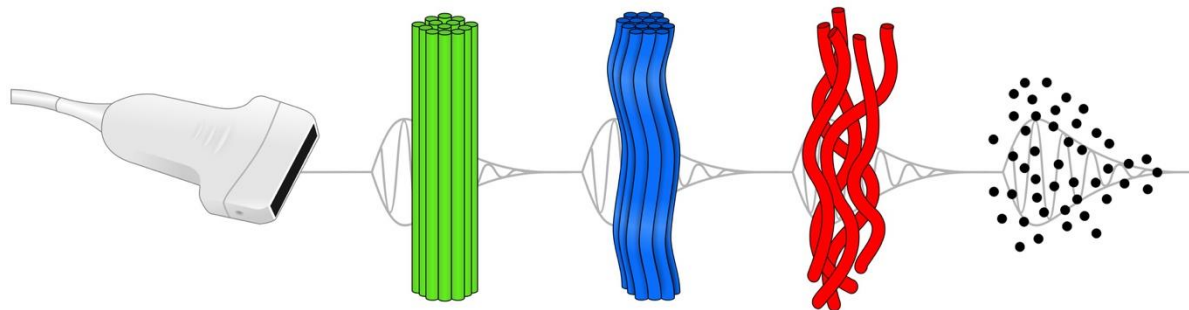
Horse - breed	CSA SDFT	CSA DDFT	Total No. horses	Literature source
Foal 1-6 months	37 – 84 mm ²	31 – 54 mm ²	7	(Korosue et al., 2015; Spinella et al., 2015; Spinella et al., 2016)
Foal 7-24 months	62 – 146 mm ²	57 – 85 mm ²	SDFT = 40 DDFT = 7	(Moffat et al., 2008; Korosue et al., 2015)
Thoroughbred	80 – 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)

1187 **Table 2**

1188 Following acquisition of three-dimensional ultrasound data, ultrasound tissue characterisation
 1189 (UTC) algorithms can distinguish between four ultrasound echo-types that correlate with
 1190 histo-morphological characteristics of the tendon (van Schie et al., 2013). The echo-types
 1191 represent the integrity of the tendon and the level of fibrillar organisation (van Schie et al.,
 1192 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

1193

1194 **Figure legends**

1195 Fig. 1. Flow chart detailing the systematic analysis and selection of scientific literature
1196 available on equine flexor tendon imaging. The following search terms were used for review
1197 part 1 without restrictions: ‘tendon’ AND ‘ultrasonography’ OR ‘elastography’ OR
1198 ‘ultrasound tissue characterisation’ AND ‘equine’ OR ‘horse’. Additional search terms for
1199 review part 2: ‘magnetic resonance imaging’ OR ‘computed tomography’.

1200

1201 Fig. 2. Longitudinal contrast-enhanced ultrasound image of the flexor tendons at the level of
1202 the digital flexor tendon sheath in a horse without evidence of orthopaedic disease. The intra-
1203 thecal injection of the digital flexor tendon sheath with 10 ml of ultrasonographic contrast
1204 medium (2 ml of room air in 8 ml of saline, shaken for 10 seconds prior to injection) aids the
1205 delineation of the superficial digital flexor tendon (SDFT) and the deep digital flexor tendon
1206 (DDFT) (black arrow) as well as the *manica flexoria* (white arrows). The limb is in a flexed
1207 position, distal is to the left (Image courtesy of Nadine Ogden, University of Liverpool 2020).

1208

1209 Fig. 3. (A) Colour Doppler transverse ultrasonographic image of the deep digital flexor
1210 tendon (DDFT) of an 18-year-old Pony mare with chronic DDF tendinopathy. There is
1211 positive Colour Doppler signal and focal mineralisation of the DDFT (white arrow). (B)
1212 Power Doppler longitudinal ultrasonographic image of the superficial digital flexor tendon
1213 (SDFT) of a 16-year-old Warmblood gelding six weeks after acute injury of the SDFT. Note
1214 the reduced fibre alignment and hypoechoic appearance of the SDFT with diffuse Power
1215 Doppler signal indicative for neovascularisation.

1216

1217 Fig. 4. Transverse elastogram (compression elastography) on the left and corresponding gray-
1218 scale ultrasonographic image (on the right) of the flexor tendons of a 14-year-old Warmblood

1219 gelding with deep digital flexor tendinopathy. The green line (bottom left) indicates the
1220 application of adequate pressure using the ultrasound transducer. The relative stiffness of the
1221 tissue follows the colour scale (top left) where red is soft, and blue is hard. Note the diffusely
1222 heterogenous fibre pattern with small hypoechoic areas throughout the deep digital flexor
1223 tendon on the ultrasonographic image that correspond to discrete areas of decreased tissue
1224 stiffness on the elastogram.

1225

1226 Fig. 5. Ultrasound tissue characterisation examination showing the colour-coded pixel
1227 stability of a core lesion (cross hairs) of the superficial digital flexor tendon (SDFT) in
1228 transverse (A), longitudinal (B) and frontal plane (C) with the associated gray scale frontal
1229 plane image (D) at the same level. The intact tendon fibres of the superficial and deep digital
1230 flexor tendons appear in green colour (echo type I). The SDFT core lesion is characterised by
1231 some red (echo type III = fibrillar tissue) and mainly black colour (echo type IV = amorphous
1232 tissue) illustrating the loss of integrity of the affected tendon fibres.

1 **Review**

2

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

5

6

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8

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20

21 **Abstract**

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

36

37 *Keywords:* Elastography; Horse; Imaging; ~~Tendon~~; Tendinopathy; -Ultrasound

38

39 **Introduction**

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

44
45 The majority of tendon injuries (75-93%) [occurring in the equine athlete](#) involve the
46 superficial digital flexor tendon (SDFT), with the mid-metacarpal region of the forelimb
47 being affected in 97-99% of cases (Ely et al., 2004; Kasashima et al., 2004; Lam et al., 2007).
48 The prevalence of SDF tendinopathy is particularly high in racing Thoroughbreds (11-30%)
49 and the recovery often includes a prolonged period of rehabilitation and is associated with a
50 poor prognosis for return to high-level exercise (Lam et al., 2007; Ely et al., 2009; O'Meara
51 et al., 2010; Kalisiak, 2012; Witte et al., 2016).

52
53 A growing body of evidence suggests that not only acute overstrain can lead to tendon
54 injury but that the accumulation of tendon micro-damage as an effect of ageing and repetitive
55 strain commonly precedes the development of clinical tendinopathy (Thorpe et al., 2013;
56 Birch et al., 2016; Godinho et al., 2017; Thorpe et al., 2017; O'Brien et al., 2020). In the
57 rapidly advancing field of diagnostic imaging, a specific focus is set on the non-invasive
58 analysis of tendon viscoelastic properties in order to identify subclinical tendon damage and
59 prevent injury (Docking et al., 2012; Lustgarten et al., 2015; Tamura et al., 2017a; Plevin et
60 al., 2019).

61
62 The aim of this literature review is to provide evidence detailing the commonly used
63 and recently developed [ultrasonographic](#) imaging techniques for the assessment of equine

64 tendons. The current state of knowledge including implications, pitfalls and future directions
65 of ultrasonography, elastography and ultrasound tissue characterisation for equine flexor
66 tendon imaging will be discussed. A systematic PubMed, Medline and Google Scholar search
67 with the following search terms was performed without restrictions: 'tendon' AND
68 'ultrasonography' OR 'elastography' OR 'ultrasound tissue characterisation' AND 'equine'
69 OR 'horse'. Additionally, studies were identified by searching the reference list of eligible
70 articles (Fig. 1). Articles were excluded if they were not available in English language or if
71 they were not published in peer reviewed journals. Additionally, references were not
72 discussed in the manuscript where the authors did not regard them as recent or their content
73 had already been discussed in detail in previous reviews.

74

75 **Ultrasonography**

76 Brightness-mode (~~B-brightness~~-mode) sonography displays the difference in acoustic
77 impedance within a two-dimensional tissue cross-section and remains the modality most
78 widely used for the assessment of tendon and ligament injuries in equine practice (Rantanen,
79 1982; Gaschen and Burba, 2012; Palgrave and Kidd, 2014; Berner, 2017). Tendon lesions are
80 generally classified as core lesions, border lesion, ~~or~~ tendon splits, or diffuse decrease in
81 echogenicity with loss of long linear parallel echoes with transverse as well as
82 longitudinal ~~sagittal~~ plane ultrasonography required to determine the extent of an injury
83 (Smith, 2008; Alzola et al., 2018).

84

85 *Tendon cross-sectional area*

86 The tendon cross-sectional area (CSA) as well as the CSA and length of a tendon
87 lesion can be calculated with reasonable accuracy using ultrasonography (Genovese et al.,
88 1990; Smith et al., 1994; Gillis et al., 1995a; Alzola Domingo et al., 2017; Kojah et al.,

89 2017). Other measurements including latero-medial or dorso-palmar/plantar dimensions of
90 tendon lesion are considered less sensitive (Reef, 2001; Smith and Cauvin, 2014).

91
92 The flexor tendon CSA may be influenced by acute changes in hoof angulation. With
93 marked elevation of the toe, flexor tendons can appear smaller whereas significant heel
94 elevation might lead to an increase in tendon CSA. However, a consistent impact on CSA
95 measurements could only be demonstrated with heel or toe elevations in excess of 10° in one
96 study (Hagen et al., 2018). Despite individual variations, conventional remedial farriery
97 rarely alters hoof angulation greater than 5° and should therefore have limited impact on the
98 calculation of the flexor tendon CSA (Riemersma et al., 1996; Lawson et al., 2007; Hagen et
99 al., 2017; Hagen et al., 2018).

100
101 The age, breed and type of horse further influence the flexor tendon CSA. Reference
102 values for the tendon CSA and echogenicity have been described for foals, young horses and
103 adults as well as for several specific horse breeds (Table 1). Additionally, reference values
104 are available for the proximal flexor tendons in the tarsal region (Vilar et al., 2011). It is
105 interesting to note that besides the differences in CSA and echogenicity, breed specific
106 differences in tendon biochemical and mechanical properties have been demonstrated
107 recently (Ploeg et al., 2017; Verkade et al., 2019). Biomechanical testing identified a
108 significantly higher stiffness of the SDFT in Thoroughbreds when compared to Warmblood
109 horses which might in part explain the predisposition to SDFT injury in certain breeds like
110 Thoroughbreds (Verkade et al., 2019).

111
112 Based on variations in functional adaption, the flexor tendon CSA does not increase
113 in a proportional rate during maturation (Birch et al., 1999; Korosue et al., 2015). A small

114 number of studies describe a tendon hypertrophy and reduction in echogenicity with training,
115 but results are inconsistent and tend not to demonstrate statistically significant alterations
116 unless clinical tendinopathy is present (Gillis et al., 1993; Birch et al., 1999; Kasashima et al.,
117 2002; Perkins et al., 2004; Moffat et al., 2008; Avella et al., 2009; Matos Santiago Reis and
118 Arantes Baccarin, 2010). Despite some conflicting reports, overall age, sex, height at the
119 withers or body mass index do not appear to have a strong influence on the equine flexor
120 tendon CSA in the adult horse (Smith et al., 1994; Gillis et al., 1995b; Gillis et al., 1995c;
121 Moffat et al., 2008; Agut et al., 2009; Avella et al., 2009; Boehart et al., 2010a; Köster et al.,
122 2014). Additionally, most studies have not identified a difference between limbs (left vs right
123 forelimb), which implies that ultrasonographic examination of the contralateral limb can
124 serve as a valid comparison provided bilateral tendinopathy can be excluded (Smith et al.,
125 1994; Gillis et al., 1995b; Perkins et al., 2004; Moffat et al., 2008; Agut et al., 2009; Avella et
126 al., 2009; Köster et al., 2014). A 20% increase in tendon CSA when compared to the
127 contralateral side is regarded as a sign for tendinopathy by several authors (Smith et al., 1994;
128 Reef, 2001; Smith, 2008; Smith and Cauvin, 2014). In case of bilateral tendinopathy, it has
129 been suggested to analyse the SDFT:DDFT ratio to assess for tendon enlargement (Berner,
130 2017).

131
132 Tendon lesions with a maximal injury zone of more than 25% of the total transverse
133 tendon CSA are usually classed as severe lesions (Reef, 2001; Smith, 2008). A recent study
134 showed a probability of 29-35% for a successful return to racing after SDFT injury in core
135 lesions that involved less than 50% of the tendon CSA, with a decrease to 11-16% in lesions
136 ≥50% CSA. The study additionally found that the prognosis in cases of tendinopathy without
137 core lesion reduced from 49-99% to just 14% if at the maximal injury zone 75% or more of
138 the longitudinal fibre pattern was disrupted (Alzola et al., 2018).

139
140 Image acquisition during consecutive ultrasonographic examinations can be
141 performed by different operators but CSA measurements should ideally be obtained by the
142 same person in order to avoid inaccuracy (Pickersgill et al., 2001). An increase of a lesion
143 CSA in excess of 10% during re-assessment is most likely indicative for a degree of re-injury
144 and requires adjustment of an existing rehabilitation program (Reef, 2001; Smith, 2008;
145 Smith and Cauvin, 2014; Kümmerle et al. 2019).

146
147 *Tendon echogenicity*

148 The ultrasonographic echogenicity will provide some information about the collagen
149 density of the tendon tissue and multiple scoring systems estimating the tendon intensity
150 (brightness), homogeneity and the degree of axial alignment of tendon fibres (seen as long
151 linear parallel echoes) have been proposed for the grading and documentation of tendon
152 lesions (Reef, 2001; Rantanen et al., 2011; Smith and Cauvin, 2014; Alzola Domingo et al.,
153 2017). It has been well established that the qualitative or semiquantitative assessment of the
154 tendon echogenicity is highly subjective and operator dependent (Crass et al., 1988;
155 Genovese et al., 1990; van Schie et al., 1999). Good intra- and inter-rater agreement has been
156 described for parameters including type of injury, maximal injury zone, location, tendon
157 cross-sectional area and longitudinal fibre pattern (echo alignment), but not for echogenicity
158 (Alzola Domingo et al., 2017) (~~Table 1~~).

159
160 In order to increase objectivity, it has been suggested to perform first-order gray level
161 statistics where different shades of gray are quantified within a defined region of interest
162 (Wood et al., 1993; Tsukiyama et al., 1996; Micklethwaite et al., 2001; Crevier-Denoix et al.,
163 2005). It has since been demonstrated that the use of such first-order gray level analysis,

164 where the distribution of the shades of gray is quantified without determining the relative
165 position of the various gray levels within the image (second-order statistics), has a low
166 sensitivity for the accurate quantification of tendon tissue (van Schie et al., 1999; van Schie et
167 al., 2000; ~~Aggarwal and Agrawal, 2012~~). The tendon echogenicity is additionally influenced
168 by the amplifier gain output and the position of the transducer. Tilting the transducer by only
169 3° can change the mean gray level of the tissue by as much as 40%, as the returning echo is
170 directed away from the transducer (anisotropy phenomenon). Displacement of the transducer
171 (2 mm) may account for a 20% difference in the gray level histogram of an acute tendon
172 lesion (van Schie et al., 1999). As research in this area progresses, the use of ultrasound
173 tissue characterisation (UTC) was developed to quantify the architecture of the tendon matrix
174 more accurately (see below) (van Schie et al., 2001; 2003).

175
176 Besides the described untoward effects, tilting the transducer purposely
177 (approximately 10°) might be useful to further assess the different components of the tendon
178 structure. The so-called angle contrast ultrasonography (ACUS) has been introduced mainly
179 for the evaluation of the suspensory ligament but can also be used for the assessment of the
180 flexor tendons (Werpy and Axiak, 2013; Werpy et al., 2013; Denoix and Bertoni, 2015).
181 Scanning a tendon off-incidence can be helpful to clearly distinguish the outline of the tendon
182 and to differentiate between tendon fibres, ~~fat, muscle~~ and scar tissue (Bubeck and Aarsvold,
183 2018). Angle contrast ultrasonography may also aid the identification of tendon lesions,
184 especially longitudinal tears, which can be difficult to detect on standard ultrasonographic
185 images (Edinger et al., 2005; Smith and Wright, 2006; Arensburg et al., 2011; Bertuglia et
186 al., 2014).

187

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

195
196 Contrast-enhanced ultrasonography (CEUS) (Fig. 2) has been suggested for the
197 identification of longitudinal DDFT tears. The injection of 10 ml of ultrasound contrast
198 medium containing stabilised sulphur hexafluoride microbubbles ($2-3 \times 10^8$
199 microbubbles/ml), into the digital flexor tendon sheath, increased the sensitivity of angle
200 contrast ultrasonography in an experimental study (Bertuglia et al., 2014). Contrast-enhanced
201 ultrasonography was further validated for intra-venous and intra-arterial use in sound horses.
202 The intra-arterial application of stabilised microbubble contrast ($0.5-1 \text{ ml}; 5-6 \times 10^{10}$
203 microbubbles/ml) in the lateral palmar digital artery, at the level of the metacarpophalangeal
204 joint, resulted in visible contrast enhancement in the soft tissues of the distal limb, without
205 evident adverse reactions (Seiler et al., 2016). Further research is required to show whether
206 the technique is useful for the characterisation of tendon injuries and how it compares with
207 Doppler ultrasonography (~~Fig. 2~~).

208
209 *Doppler ultrasonography*

210 Doppler imaging for the assessment of musculoskeletal pathology was initially
211 described in human patients with rotator cuff, Achilles, or patellar tendon injury (Newman et
212 al., 1994; Hollenberg et al., 1998; Weinberg et al., 1998). The Colour Doppler signal

213 indicates the direction and velocity of blood flow, based on the interference of the moving
214 blood cells with the wavelength (and hence frequency) of ultrasound waves (Naredo and
215 Monteagudo, 2014; Palgrave and Kidd, 2014).

216

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

228

229 Doppler signal that occurs in relation to a tendon injury should decline after 3-6
230 months, as tendon healing progresses. A study evaluating the healing process of tendon
231 lesions found that surgically induced SDFT lesions in the hindlimb showed significantly less
232 Doppler signal when compared to forelimb lesions, 6 months post injury (Estrada et al.,
233 2014). During sequential examination, continuous or re-occurring Doppler signal in
234 association with a flexor tendon lesion is suggestive of a delayed healing response or re-
235 injury (Alfredson et al., 2003; Sharma and Maffuli, 2005; Smith and Cauvin, 2014; Hatazoe
236 et al., 2015). For optimal visualisation of blood flow, the limb should be kept in a non-weight

237 bearing position when Doppler ultrasonography is performed (Kristoffersen et al., 2005;
238 Boesen et al., 2007; Rabba et al., 2018).

239

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

247

248 Areas of focal mineralization are occasionally found in equine flexor tendons,
249 especially in the DDFT. Tendon mineralization can be an incidental finding or may
250 potentially be associated with previous corticosteroid injection ~~as well as~~ clinically
251 ~~apparent or subclinical~~ tendon injury (Ross et al., 2011; O'Brien et al., 2012; Zhang et al.,
252 2013; O'Brien and Smith, 2018; [Ali et al., 2021](#)). Doppler ultrasonography is regarded as a
253 useful indicator for the detection of clinically relevant and painful tendon mineralization in
254 human patients (Chiou et al., 2002; Le Goff et al., 2010). Similar observations have been
255 described in a small number of horses in a recent report (Fig. 3A) (O'Brien and Smith, 2018).

256

257 In contrast to the commonly utilized Colour Doppler, Power Doppler ultrasonography
258 analyses the total intensity of returning signal without characterising the direction of flow
259 (Fig. 3B). The technique is independent of the ultrasound probe being aligned to the direction
260 of blood flow and is particularly sensitive for imaging of low-velocity flow from small
261 vessels (Martinoli et al., 1998; Anderson and McDicken, 2002; Naredo and Monteagudo,

262 2014; Palgrave and Kidd, 2014). Power Doppler ultrasonography has been shown to be
263 useful for the detection of microvascularity in both Achilles and patellar tendons in man
264 (Newman et al., 1994; Terslev et al., 2001; Peers et al., 2003; Richards et al., 2005). Initial
265 reports describing the application of Power Doppler ultrasonography for the assessment of
266 the equine suspensory ligament branches and SDF tendinopathy show promising results;
267 however, a study directly comparing Colour- and Power Doppler ultrasonography in horses is
268 not currently available (Rabba et al., 2018; Lacitignola et al., 2020; Rabba et al., 2020).

269

270 **Elastography**

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

278

279 Depending on the stress applied to the tissue and the measured physical quantity,
280 elastography can be divided into quasi-static and dynamic imaging (Bamber et al., 2013;
281 Sigrist et al., 2017; Washburn et al., 2018). During quasi-static strain elastography
282 (compression elastography), the operator applies stress to the tissue manually, by applying
283 pressure using the ultrasound transducer. The resulting tissue deformation (relative stiffness
284 of the tissue) is measured by radiofrequency echo correlation-based tracking and
285 subsequently transformed into a colour-coded elastogram (Fig. 4) (Klauser et al., 2014; Winn
286 et al., 2016; Prado-Costa et al., 2018). Dynamic elastography relies on the detection of shear-

287 wave propagation. Dynamic stress is applied to the tissue with mechanical vibrating devices
288 or acoustic radiation force and the shear-waves created by the excitation are recorded (Ooi et
289 al., 2014; Sigrist et al., 2017; Taljanovic et al., 2017).

290

291 An initial study investigating the use of strain elastography for the assessment of
292 equine flexor tendons demonstrated a moderate reproducibility (68%, Kappa agreement (κ) =
293 0.46) and good repeatability (83%, κ = 0.78) when different operators performed
294 elastography in horses without evidence of tendinopathy (Lustgarten et al., 2014). There was
295 no significant difference in [elastography readings of](#) tissue stiffness when tendons were
296 imaged in various leg positions, but the flexor tendons appeared softer in the longitudinal
297 plane compared to the transverse plane. The same technique was subsequently applied in
298 clinical cases of tendon and ligament injury and compared with gray-scale ultrasonography
299 [\(SDFT, DDFT, accessory ligament of the DDFT, suspensory ligament\)](#) and high-field MRI
300 (1.5T) [\(DDFT, suspensory ligament\)](#) (Lustgarten et al., 2015). Elastograms were assessed
301 subjectively using a qualitative colour-grading system. Additionally, an algorithm was
302 developed for the quantitative analysis of the percentage of each colour within a specific
303 region of interest. A significant correlation was found between lesions detected during strain
304 elastography, gray-scale ultrasonography, [short tau inversion recovery-fast spin echo \(STIR-](#)
305 [FSE\)](#) and [proton density \(PD\) weighted](#) MRI sequences. Chronic / [subacute](#) tendon lesions (>
306 2 weeks post trauma) appeared stiffer than acute lesions when quantitative and qualitative
307 elastographic evaluation was performed.

308

309 Further research focused on the use of strain elastography as a monitoring tool for the
310 recovery period of Thoroughbred racehorses that had sustained an SDFT injury (Tamura et
311 al., 2017a; Tamura et al., 2017b). A prospective longitudinal study that followed a group of

312 horses (n = 7) with SDFT tendinopathy showed that the lesion echogenicity changed within
313 the first two months post injury using gray-scale ultrasonography. However, evidence for a
314 significant increase in tendon stiffness was ~~only~~ detected 4 to 7 months later on elastograms
315 of the affected SDFTs (Tamura et al., 2017b). A second study by the same group confirmed
316 that a significant difference in tendon stiffness can be detected using sonoelastography during
317 the later stages of tendon healing (5 vs 9 months post injury), where gray-scale
318 ultrasonographic findings remain largely unchanged (Tamura et al., 2017a).

319

320 In human athletes, the use of elastography as a monitoring tool for the identification
321 of Achilles tendons with an increased injury risk showed promising results (Ooi et al., 2015;
322 Balaban et al., 2016; Ooi et al., 2016). Additionally, age-related changes in Achilles tendon
323 stiffness have been characterised with elastography in man (Klauser et al., 2014; Turan et al.,
324 2015). Since the equine SDFT is considered functionally equivalent to the human Achilles
325 tendon, monitoring protocols for the prevention of flexor tendon injury may be adaptable for
326 equine athletes (Patterson-Kane and Rich, 2014; Washburn et al., 2018). Whilst current
327 reports describe a good repeatability and interobserver agreement for strain elastography in
328 horses, the variability of the manually applied tissue compression is a limitation that is yet to
329 overcome (Klauser et al., 2010; Lustgarten et al., 2014; Tamura et al., 2017a; Tamura et al.,
330 2017b). Due to an uneven pressure distribution, colour artefacts can additionally occur,
331 particularly on transverse images of the SDFT. A standoff pad reduces artefacts at the tendon
332 margins and can additionally be used as an external standard reference for quantitative
333 sonoelastographic evaluation but may occasionally result in reverberation artefacts
334 (encountered as red lines) (Lustgarten et al., 2014; Tamura et al., 2017a; Tamura et al.,
335 2017b).

336

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

347
348 *Acoustoelastography*

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

357
358 The method was initially described in porcine and canine tendons and has since been
359 used for the evaluation of the equine SDFT and DDFT in groups of clinically normal horses
360 (Duenwald-Kuehl et al., 2012a; Ellison et al., 2013; Ellison et al., 2014; Berger et al., 2018).
361 Good intra- and inter-observer repeatability with no significant effect of age, sex or limb was

362 demonstrated (Ellison et al., 2013; Ellison et al., 2014). It is, however, important to note that
363 the stiffness may not be homogeneous along the length of the tendon and can vary amongst
364 individual horses (De Gasperi et al., 2017; Berger et al., 2018). Additionally, sedation
365 (detomidine-butorphanol combination) appears to significantly decrease the stiffness gradient
366 of the equine SDFT (De Gasperi et al., 2017).

367

368 In contrast to strain elastography, acoustoelastography is thought to more accurately
369 mimic the natural situation, where tendons are exposed to longitudinal strain rather than
370 compression (Ellison et al., 2014). Initial reports of the use of acoustoelastography for the
371 detection of strain-induced damage or tendon tears, as well as for monitoring purposes after
372 tendon injury are available for other species (Duenwald-Kuehl et al., 2012b; Frisch et al.,
373 2014; Hans et al., 2014). Further research assessing the feasibility of acoustoelastography for
374 the detection of tendon injury and monitoring of tendon healing in equine patients is
375 warranted before this technique might be more widely used in the field of equine
376 orthopaedics.

377

378 **Ultrasound Tissue Characterisation**

379 Computerised ultrasound tissue characterisation (UTC) uses a high-resolution
380 ultrasound probe mounted in a tracking device with a built-in standoff pad. With standardised
381 settings including transducer tilt angle, gain, focus and depth, approximately 600 consecutive
382 transverse images of the tendon are acquired at a set distance (every 0.2 mm over 12 cm) and
383 concatenated to create a three-dimensional ultrasound data-block. By correlating the pixel
384 stability across a number of contiguous transverse images, specific algorithms can distinguish
385 between four different structure- and non-structure-related echo-types, which reflect the
386 integrity of the tendon matrix (Table 2). Based on this data a colour code is generated, where

387 green displays a good pixel correlation (uniform tissue), blue \leq 10% difference in pixels
388 (irregular tissue), red \geq 10% difference in pixels (fibrillar tissue) and black = no correlation
389 (amorphous tissue) (Table 2). Additionally, longitudinal information (sagittal and frontal
390 planes) can be reconstructed, and the percentage of each echo-type can be quantified for a
391 specific tendon region (Fig. 5) (van Schie and Bakker, 2000; van Schie et al., 2001; 2003;
392 van Schie et al., 2010; van Schie et al., 2013; Cook and Purdam, 2014).

393

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

400

401 Ultrasound tissue characterisation was further used as a monitoring tool for tendon
402 healing in several experimental studies with surgically created tendon lesions in horses.

403 Based on this research, UTC is currently considered the most objective tool for longitudinal
404 *in vivo* assessment of tendon healing (van Schie et al., 2009; Bosch et al., 2011; David et al.,
405 2012; Cadby et al., 2013; Geburek et al., 2017). Treatment options for equine tendinopathy
406 including cast immobilisation, platelet-rich plasma and adipose-derived mesenchymal
407 stromal cells have been investigated with the aid of this technique (Bosch et al., 2011; David
408 et al., 2012; Geburek et al., 2016; Geburek et al., 2017).

409

410 Besides the application for lesion monitoring, it has been shown that UTC can be
411 employed to illustrate the tendon's response to exercise. When UTC examination was

412 performed in a group of racing Thoroughbreds, it was demonstrated that the SDFT shows a
413 significant reduction of aligned tendon bundles (echo-type I) with an increase in
414 discontinuous secondary fascicle bundles (echo-type II) and fibrillar components (echo-type
415 III), for two days post racing (Docking et al., 2012). The described decrease in the tendon's
416 structural integrity appeared to be reversible and returned to baseline within approximately
417 72 hours. Due to this observation, it was recommended that repeated maximal exercise
418 should be avoided~~the authors recommend avoiding repeated maximal exercise~~ for three days
419 after competitive racing (Docking et al., 2012).

421 Ultrasound tissue characterisation~~TC~~ was subsequently used in a longitudinal field
422 study, where the SDFT of yearling Thoroughbreds was evaluated as they started race training
423 (Plevin et al., 2019). The study showed that the ultrasonographic characteristics of the SDFT
424 changed significantly within the first 6 months of training, which is thought to reflect the
425 tendon's response to altered loading patterns as race training commences. ~~The authors report~~
426 ~~a good feasibility for the use of~~ the UTC equipment was considered easy to use with~~n~~
427 young horses under field conditions. However, the technique was susceptible to movement
428 artefacts, which usually present as blue lines and necessitated repeated examination. The
429 reference values identified within this young group of horses were $\geq 85\%$ echo-type I and \leq
430 15% echo-type II with a negligible proportion of echo-type III and IV for all tendons at all
431 time points. In the ~~previously described racing~~ group of Thoroughbreds (mean \pm SD age 3.8
432 ± 0.6 years) described in the previous paragraph, values were somewhat different with $> 90\%$
433 echo-type I, $< 5\%$ echo-type II and up to 5% of echo-type IV for mature horses in training
434 (Docking et al., 2012). The increased percentage of echo-type IV might be explained by
435 transient intra-fascicular fluid accumulation as well as subclinical tendon matrix degradation
436 (Docking et al., 2012; Plevin et al. 2019).

437

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

452

453 In summary, the authors regard ultrasonographic imaging as a vital tool for the
454 assessment of equine flexor tendons and routinely use Doppler ultrasonography for lesion
455 monitoring. In the authors' experience the reproducibility of elastography is currently still
456 hampered by difficulties in application of uniform pressure distribution using the ultrasound
457 transducer. The use of dynamic shear-wave elastography might overcome some of the
458 limitations of strain elastography in the future, as the technique additionally offers
459 quantitative values for the characterisation of tendon tissues. However, dynamic elastography
460 has not been developed specifically for the use in equine patients so far. The clinical
461 application of UTC is gaining popularity in equine practice. Future longitudinal studies with

462 larger case numbers should show whether UTC is a reliable tool not only for early detection,
463 monitoring and prognostication but also for the prediction of tendon lesions.

465 **Conclusion**

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

480 **Conflict of interest statement**

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

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1189 **Table 1**

1190 Reference values for the tendon cross-sectional area (CSA) ~~and echogenicity~~ as detailed for
 1191 the superficial digital flexor tendon (SDFT) and deep digital flexor tendon (DDFT) of foals,
 1192 young horses and adults including several specific horse breeds. The flexor tendon CSA is
 1193 ~~smallest~~ ~~decreasing~~ in the mid-metacarpal region and gradually enlarges again towards the
 1194 level of the fetlock. Mean values are provided, and the reader is referred to the original
 1195 literature to find reference values for the different tendon levels.

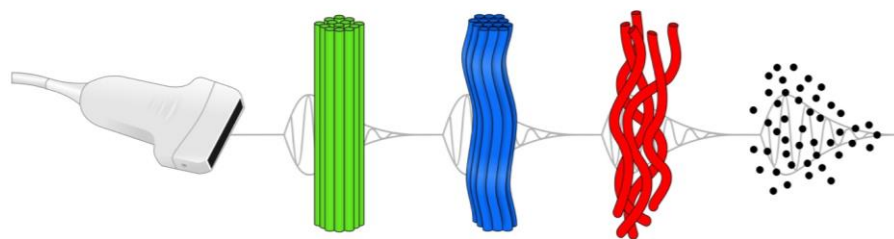
Horse - breed	CSA SDFT	CSA DDFT	Total No. horses	Literature source
Foal 1-6 months	37 – 84 mm ²	31 – 54 mm ²	7	(Korosue et al., 2015; Spinella et al., 2015; Spinella et al., 2016)
Foal 7-24 months	62 – 146 mm ²	57 – 85 mm ²	SDFT = 40 DDFT = 7	(Moffat et al., 2008; Korosue et al., 2015)
Thoroughbred	80 – 146 mm ²	72 – 213 mm ²	SDFT = 268 DDFT = 86	(Gillis et al., 1993; Smith et al., 1994; Gillis et al. 1995 b ; 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., 2010 ^a)
Haflinger	52 – 85 mm ²	64 – 143 mm ²	30	(Boehart et al., 2010 ^b)
Pony	71 – 83 mm ²	65 – 149 mm ²	15	(Smith et al., 1994)

1197 **Table 2**

1198 Following acquisition of three-dimensional ultrasound data, ultrasound tissue characterisation
 1199 (UTC) algorithms can distinguish between four ultrasound echo-types that correlate with
 1200 histo-morphological characteristics of the tendon (van Schie et al., 2013). The echo-types
 1201 represent the integrity of the tendon and the level of fibrillar organisation (van Schie et al.,
 1202 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

1203

1204 **Figure legends**

1205 Fig. 1. Flow chart detailing the systematic analysis and selection of scientific literature
1206 available on equine flexor tendon imaging. The following search terms were used for review
1207 part 1 without restrictions: 'tendon' AND 'ultrasonography' OR 'elastography' OR
1208 'ultrasound tissue characterisation' AND 'equine' OR 'horse'. Additional search terms for
1209 review part 2: 'magnetic resonance imaging' OR 'computed tomography'.

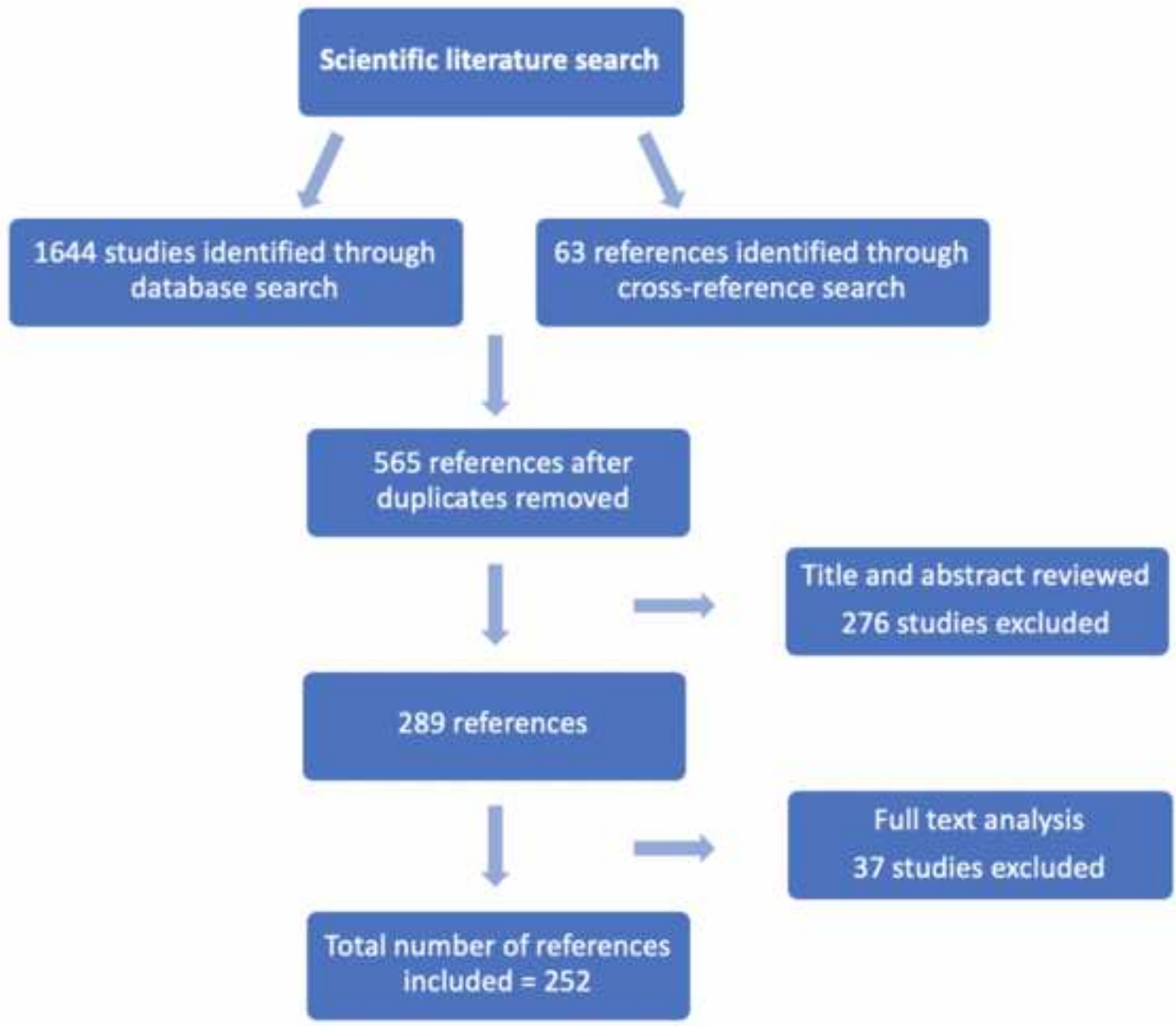
1210
1211 Fig. 2. Longitudinal contrast-enhanced ultrasound image of the flexor tendons at the level of
1212 the digital flexor tendon sheath [in a horse without evidence of orthopaedic disease](#). The intra-
1213 thecal injection of the digital flexor tendon sheath with 10 ml of ultrasonographic contrast
1214 medium (2 ml of room air in 8 ml of saline, shaken for 10 seconds prior to injection) aids the
1215 delineation of the superficial digital flexor tendon (SDFT) and the deep digital flexor tendon
1216 (DDFT) (black arrow) as well as the *manica flexoria* (white arrows). The limb is in a flexed
1217 position, distal is to the left (Image courtesy of Nadine Ogden, University of Liverpool 2020).

1218
1219 Fig. 3. (A) Colour Doppler transverse ultrasonographic image of the deep digital flexor
1220 tendon (DDFT) of an 18-year-old Pony mare with chronic DDF tendinopathy. There is
1221 positive Colour Doppler signal and focal mineralisation of the DDFT (white arrow). (B)
1222 Power Doppler longitudinal ultrasonographic image of the superficial digital flexor tendon
1223 (SDFT) of a 16-year-old Warmblood gelding six weeks after acute injury of the SDFT. Note
1224 the reduced fibre alignment and hypoechoic appearance of the SDFT with diffuse Power
1225 Doppler signal indicative for neovascularisation.

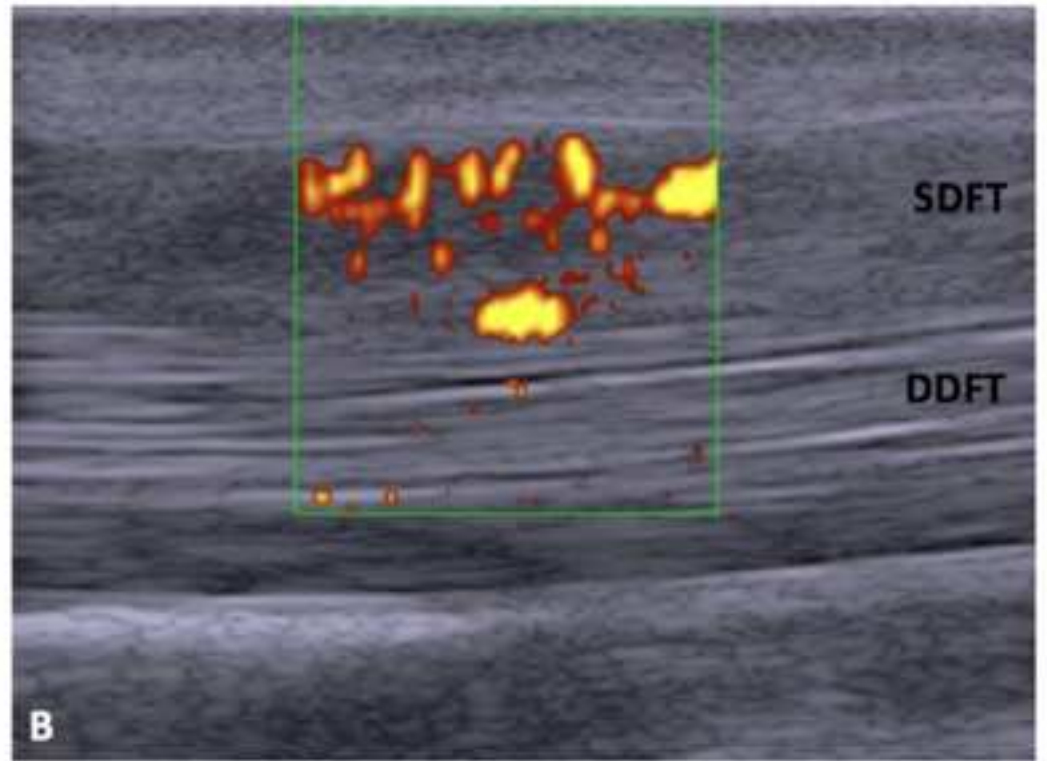
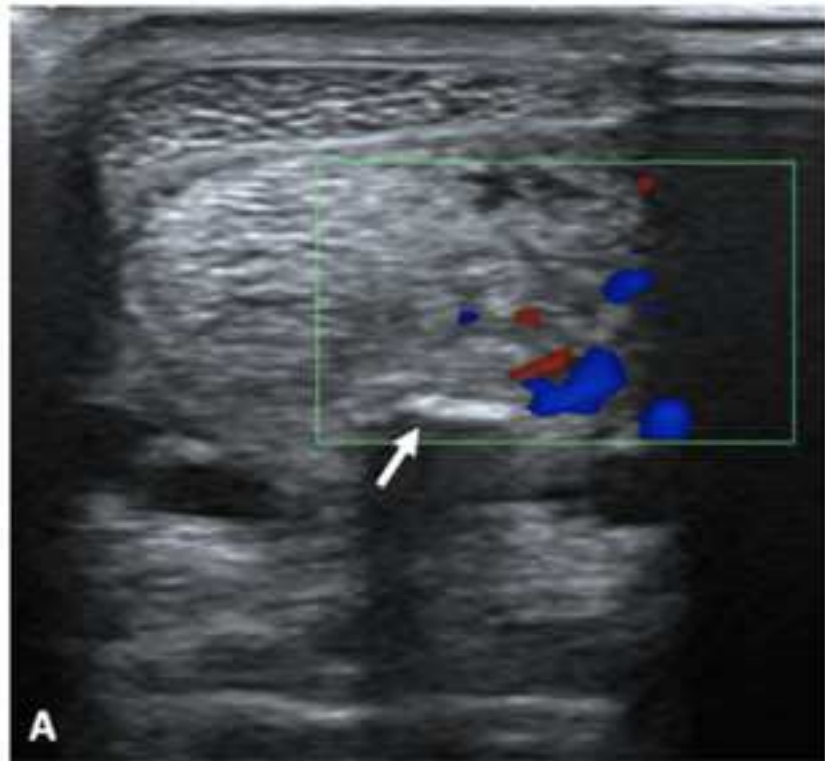
1226
1227 Fig. 4. Transverse elastogram (compression elastography) on the left and corresponding gray-
1228 scale ultrasonographic image (on the right) of the flexor tendons of a 14-year-old Warmblood

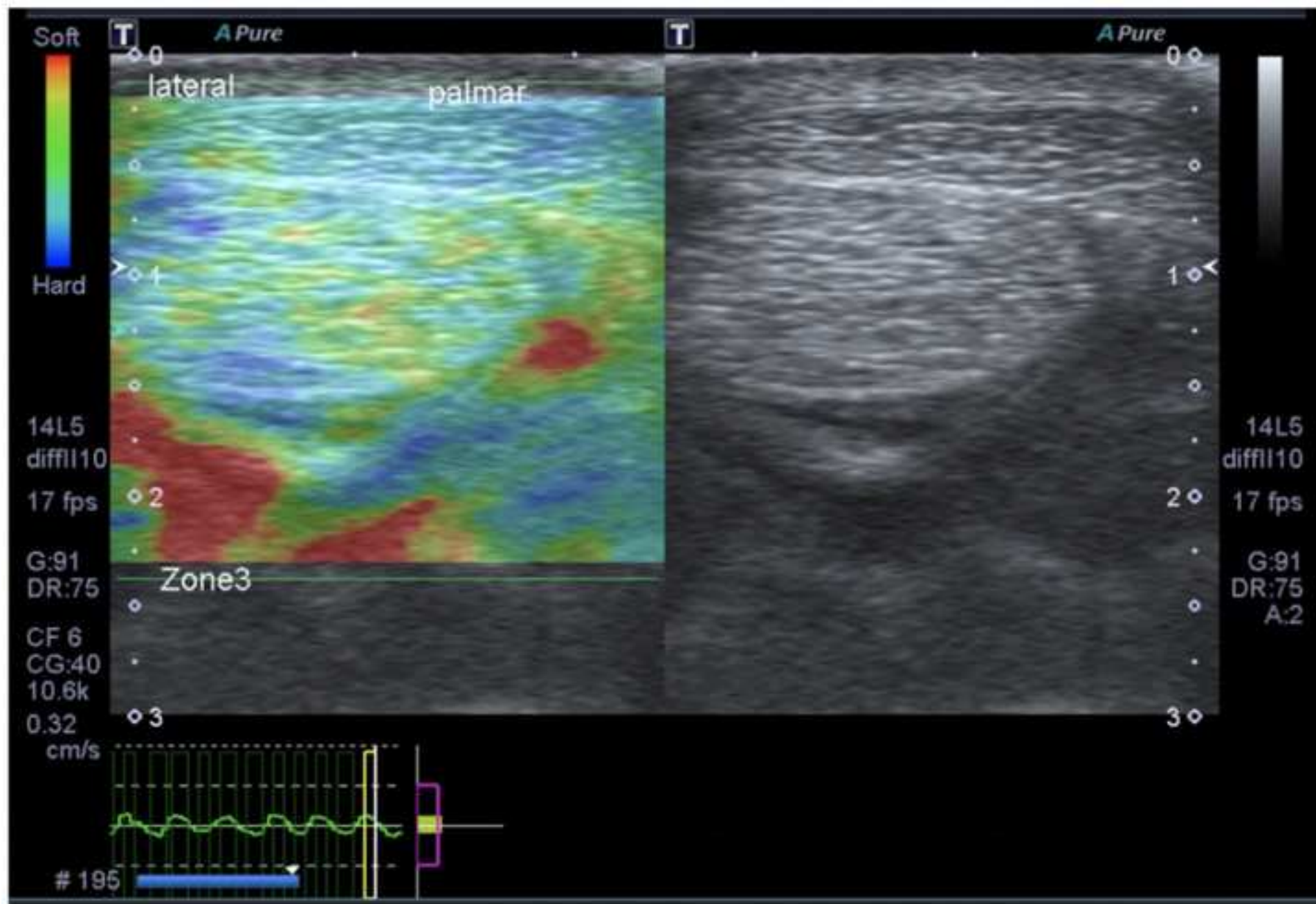
1229 gelding with deep digital flexor tendinopathy. The green line (bottom left) indicates the
1230 application of adequate pressure using the ultrasound transducer. The relative stiffness of the
1231 tissue follows the colour scale (top left) where red is soft, and blue is hard. Note the diffusely
1232 heterogenous fibre pattern with small hypoechoic areas throughout the deep digital flexor
1233 tendon on the ultrasonographic image that correspond to discrete areas of decreased tissue
1234 stiffness on the elastogram.

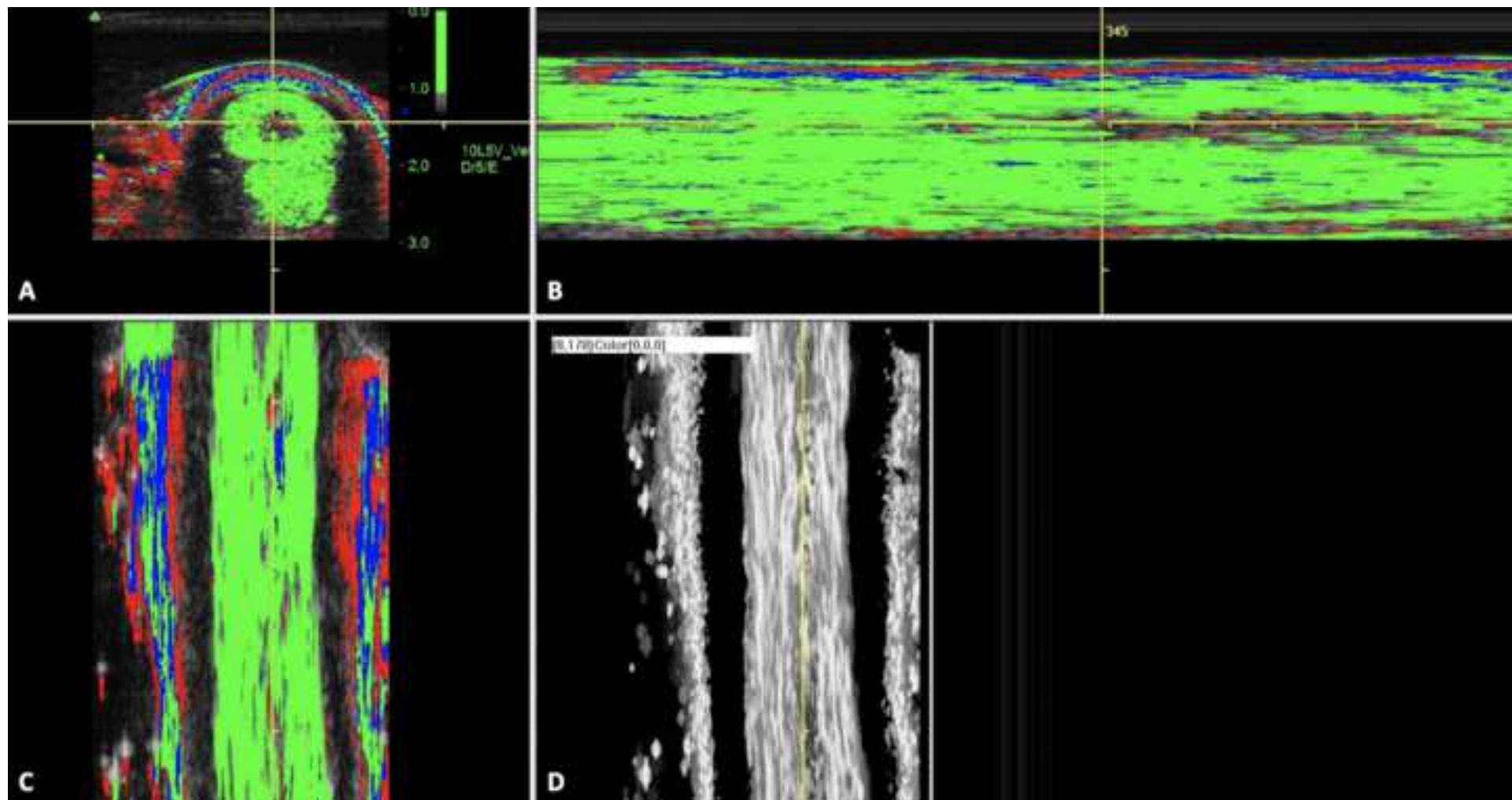
1235
1236 Fig. 5. Ultrasound tissue characterisation examination showing the colour-coded pixel
1237 stability of a core lesion (cross hairs) of the superficial digital flexor tendon (SDFT) in
1238 transverse (A), longitudinal (B) and frontal plane (C) with the associated gray scale frontal
1239 plane image (D) at the same level. The intact tendon fibres of the superficial and deep digital
1240 flexor tendons appear in green colour (echo type I). The SDFT core lesion is characterised by
1241 some red (echo type III = fibrillar tissue) and mainly black colour (echo type IV = amorphous
1242 tissue) illustrating the loss of integrity of the affected tendon fibres.











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).

Reviewer comment: 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?

Authors response: 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.

Reviewer comment: 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?

Authors response: 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.; Miyamoto, 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.

Reviewer comment: 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.

Authors response: 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.

Reviewer comment: 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.

Authors response: 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.

Reviewer comment: 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.

Authors response: The authors agree and both bullet points were rephrased accordingly.

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

Authors response: 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 lesions 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 lesions 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 reference 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 reference 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 have 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 occurring tendon and ligament injuries of the equine distal limb. *Veterinary Radiology & Ultrasound* 56, 670-679.

Reviewer comment: 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).

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

Reviewer comment: 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.

Authors response: 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.

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

Authors response: 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?

Authors response: 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.

Reviewer comment: *Line 73 or diffuse decrease in echogenicity with loss of long linear parallel echoes in longitudinal images*

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

Reviewer comment: *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*

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

Reviewer comment: *Line 85 Tendon CSA measurements have been shown to be user-dependent in previous studies*

Authors response: 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.

Reviewer comment: *Line 86 the reference to Table 1 belongs next to CSA in line 85*

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

Reviewer comment: *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.*

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

Reviewer comment: 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

Authors response: 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.

Reviewer comment: Line 121 adhesions, as well as or adhesion formation, as well as

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

Reviewer comment: Line 133 Is there any published evidence that this invasive technique provides useful clinical information?

Authors response: 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.

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

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

Reviewer comment: 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?

Authors response: 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.; Miyamoto, 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 from equine research is still sparse).

Reviewer comment: 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

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