First Application of Axial Speed of Sound to Follow up Injured Equine Tendons

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Abstract

Ultrasonography is an established technique to follow up injured tendons, although the lesions' echogenicity tends to become normal before the tendon is ready to sustain the stresses imposed by exercise. Normalized axial speed of sound (SOS) has been found to correlate with an injured tendon's stiffness; therefore, the purpose of this study was to establish whether SOS would be a useful tool in tendon injury follow-up. Axial SOS was measured in 11 equine superficial digital flexor tendons during a 15 weeks follow-up period, and compared with an ultrasonographic grading system. SOS significantly decreased 2 weeks after the surgical induction of a core lesion, showing a minimum between 7 and 10 weeks; ultrasonographic grade showed a minimum at 3 weeks and increased thereafter. The ultrasonographic grading at 15 weeks was correlated to normalized SOS. These results suggest that axial SOS provides complementary information to ultrasonography that could be of clinical interest.

Keywords: Soft tissue; Tendon; Injury; Quantitative ultrasound; Speed of sound; Ultrasonography.

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1. Introduction

Tendon lesions are the most investigated and most common equine musculoskeletal injuries (Thorpe et al. 2010); they present a high rate of recurrence and their outcome is often negative (Dyson 2004; Marr et al. 1993; van den Belt et al. 1994). Training and competing are both responsible of these injuries (Ely et al. 2004; Murray et al. 2006; Pinchbeck et al. 2004). They often require long periods without full training and they represent one of the major reasons of horse athletes retirement (Lam et al. 2007).
Ultrasonography is a widely spread technique for tendon lesions diagnosis, follow-up and a tool to establish a prognosis (Denoix et al. 1990; Genovese et al. 1986). Although qualitative in nature, the signs characterizing the ultrasonographic images of a tendon lesion (e.g. size, echogenicity and architecture) can be semi-quantitatively assessed by grading (Genovese et al. 1990; van den Belt et al. 1993). This operator-dependant technique relies on the clinician's experience; this aspect is critical during the follow-up, when the echogenicity of healing tendons progressively increases up to its normal level (Fig. 1). In order to stimulate collagen production and optimize fibres alignment (Alves et al. 2001; Kingma et al. 2007) without applying unnecessary stresses to a still fragile tendon, a reliable prognosis is required to determine the most appropriate time to start controlled exercise and its intensity (Dowling et al. 2000; Gillis 1997).
Quantitative ultrasound is being applied to non-invasively evaluate tendon load (Crevier-Denoix et al. 2009; Pourcelot et al. 2005; Roux and Defontaine 2005), since it has been demonstrated that axial speed of sound (SOS) in tendon varies with the tendon loading (Pourcelot et al. 2005). Recently, SOS has been measured in healthy equine superficial flexor tendons (SDFT) and 3.5 months after the induction of a core tendon lesion (Vergari et al. In Press). SOS significantly decreased after the induction and, while SOS values were not correlated to injured tendon's elastic modulus, a correlation was observed between the latter and normalized SOS (the ratio of injured on normal tendon SOS). However, the progression of SOS during the 3.5 months follow-up was not reported in this previous study, and the clinical relevance of this technique has not been investigated yet.
The objectives of the present study were (i) to report the axial SOS measured during a 15 weeks follow-up of 11 injured tendons, (ii) to compare these SOS values with the semi-quantitative ultrasonographic assessment of the same tendon lesions made by clinicians and (iii) to verify if SOS could provide complementary information for the evaluation of tendon status.

2. Materials and methods

2.1 Subjects and timing

Eleven French Trotters (2-4 years old) were included in the present study. They were participating in a clinical trial testing the efficiency of a regenerating agent on SDFT lesions. Six horses were thus bilaterally treated with this molecule, while a placebo was administered to the other 5. The evaluation of this molecule, however, is beyond the scope of the present paper.
The trial (approved by the Ethical Committee ComEth Afssa/Ecole Nationale Vétérinaire d'Alfort/Université Paris-Est Créteil) required the bilateral surgical induction (SI) of a tendon core lesion in the middle metacarpal area of the forelimb SDFT. The lesion was induced with a specially designed amagnetic pin, 30 cm long and ending with a 4 edged arrowhead of 10 mm, using a previously described surgical technique (Schramme et al. 2010; Vergari et al. In Press). The lesions were about 7 cm in length and included about half of the tendon cross-section. Ultrasonographic images of the SDFT were acquired before the SI and 3, 7, 10 and 15 weeks after it (subscripts from 0 to 4). Axial tendon SOS was measured before SI and 2, 7, 10 and 15 weeks after it (subscripts from 0 to 4). Horses were weighted before the SI (437 ± 27 kg average body mass) and 15 weeks after it (437 ± 28 kg), finding a non-significant variation.

2.2 Ultrasonographic images

Trained clinicians acquired ultrasonographic images (with an Aloka Alpha-10 Prosound, using a 7.5 MHz linear probe with a standoff pad) of both forelimbs SDFT of each horse in the metacarpal area. Both longitudinal (with the ultrasound beam parallel to the tendon fibres) and transverse images (with the ultrasound beam perpendicular to the tendon fibres) were acquired. The ultrasonographic machine's magnification, contrast and luminosity were standardised, while for each image the gain was adapted, in order to optimize the brightness of the SDFT, and the focus was placed in the middle of the lesion.

The ultrasonographic examinations included the entire metacarpal area, where the lesions were targeted. Three weeks after the SI, a region of interest (ROI) for a given horse was defined as the tendon cross-section where the maximal lesion severity was observed.

2.3 Ultrasonographic scoring

Different ultrasonographic scoring systems have been previously used to semi-quantitatively evaluate injured tendons in veterinary practice (Genovese et al. 1986; Saini et al. 2002; Van den Belt et al. 1993). In the present study, four semi-quantitative ultrasonographic criteria were defined (score 0 – 4): a. lesion echogenicity (0 sane echogenic tissue - 4 pathologic anechogenic tissue), b. transversal lesion extent (0 no extension - 4 lesion area > 50% of the cross-sectional area), c. transversal lesion architecture (0 normal - 4 pathologic hypoechogenic tissue) and d. longitudinal lesion architecture (0 sane tendon - 4 complete disorganization of fibres pattern). Criteria a to c were defined on the images corresponding to the ROI. Although a score was assigned to each ultrasonographic criterion on each examination, the definitive scoring was reassessed for each horse during a longitudinal blind collegial review of all images. The clinicians who examined the ultrasonographic images did not have access to the SOS measured values.

The four scores were summed (yielding scores from 0 to 16) and normalized to give a final ultrasonographic grade (UG) from 0 for normal tendon to 10 for maximal lesion severity, according to: UG = 10 – score*10/16. Each tendon was independently graded at each stage (UG_0 to UG_4). A reduced ultrasonographic grade (RUG) was also calculated by summing and normalizing only the two scores relative to the lesion's echogenicity and transversal architecture (RUG = 10 – score*10/8).

2.4 Speed of sound measurements

SOS in the right SDFT of each horse was measured with a previously described technique (Pourcelot et al. 2005) and protocol (Crevier-Denoix et al. 2009; Vergari et al. In Press). The probe was composed by a 1 MHz broadband pulse emitter and two receivers, which are 1 cm spaced. The received ultrasonic signals (400 per second) were digitized at 10 MHz and the time of flight of the first arriving signal was estimated using the first zero crossing criterion (Bossy et al. 2002); the speed of this first arriving signal was calculated as the distance between the two receivers divided by the corresponding signal time-of-flight difference.

SOS measurements were performed during 6 series of walk (about 5 strides each) on an asphalt pavement. The maximal SOS value measured in each stride, corresponding to the tendon's maximal load, was selected, then these maximal values were averaged to obtain the mean maximal SOS. This quantity was measured before the SI (SOS_0) and 2, 7, 10 and 15 weeks after it (SOS_1 to SOS_4). SOS_0 was measured in the middle palmar metacarpal area (the expected location of the lesion) while SOS_1 through SOS_4 were measured in the ROI. Considering SOS variability among sane tendons (Crevier-Denoix et al. 2009) and the influence of the initial severity of the lesion on its evolution, two normalized values of SOS were calculated to quantify the impact of the lesions on SOS: $SOS_{1\text{-}4}$ / SOS_0 (i.e. relative to the SOS in normal tendon) and $SOS_{2\text{-}4}$ / SOS_1 (i.e. relative to the SOS measured in the recently injured tendon).

2.5 Statistical analysis

Normality was tested with the Lilliefors test. Pearson's correlation coefficient was calculated for normally distributed variables while Spearman's rank correlation coefficient was used for the non-normally distributed ones. Differences between measurements at different stages were statistically analyzed with Wilcoxon signed-rank test. Significance level was set at $p < 0.05$.

The short term precision of the SOS measurement, as defined by Gluer et al. (1995), was calculated separately for each stage. For this precision evaluation, it was considered that the average of each series of walk (about 5 strides each) corresponded to one SOS measurement; thus, SOS measurement was repeated 6 times for each horse and each exam.

3. Results

3.1 Ultrasonographic grade

Figure 1 shows an example of transverse ultrasonographic images in normal tendon and during its lesion follow up. Table 1 reports the average value for each variable and each exam. UG_0 was 10 for all horses (since all the tendons were initially healthy), while grades from UG_1 to UG_4 were significantly lower. The grade was at its lowest 3 weeks after the SI (average $UG_1 = 1.6 \pm 0.7$). It started increasing thereafter up to half of its original value ($UG_4 = 5.2 \pm 0.6$) 15 weeks after the SI (Fig. 2). Horses #5 and #9 were the only two horses whose grade remained constant between 3 and 7 weeks ($UG_1 = 2.5$ and 3.1, respectively). Average UG in each measurement session was significantly different from the previous one. RUG presented a similar variation in time (i.e. a minimum 3 weeks after the SI followed by a steady increase), but showed a higher range of variation (from $RUG_1 = 0.9 \pm 1.0$ to $RUG_4 = 6.1 \pm 0.9$).

3.2 Speed of sound

Table 1 reports the average SOS of each measurement session. Averaged SOS in healthy tendons was 2178.8 ± 32.8 m/s (before SI). SOS significantly decreased after two weeks (2096.6 ± 49.7 m/s) and seven weeks (2041.8 ± 62.6 m/s) after SI. Then, a stabilization was observed from week 7 to 10 after SI (2041.5 ± 67.5 m/s). Finally, a significant increase was measured at week 15 after SI (2072.4 ± 66.2 m/s, Fig. 2). While at the end of the study no tendon had regained its initial SOS, two tendons (#3 and #10) reached similar or higher SOS values than those measured 2 weeks after SI.

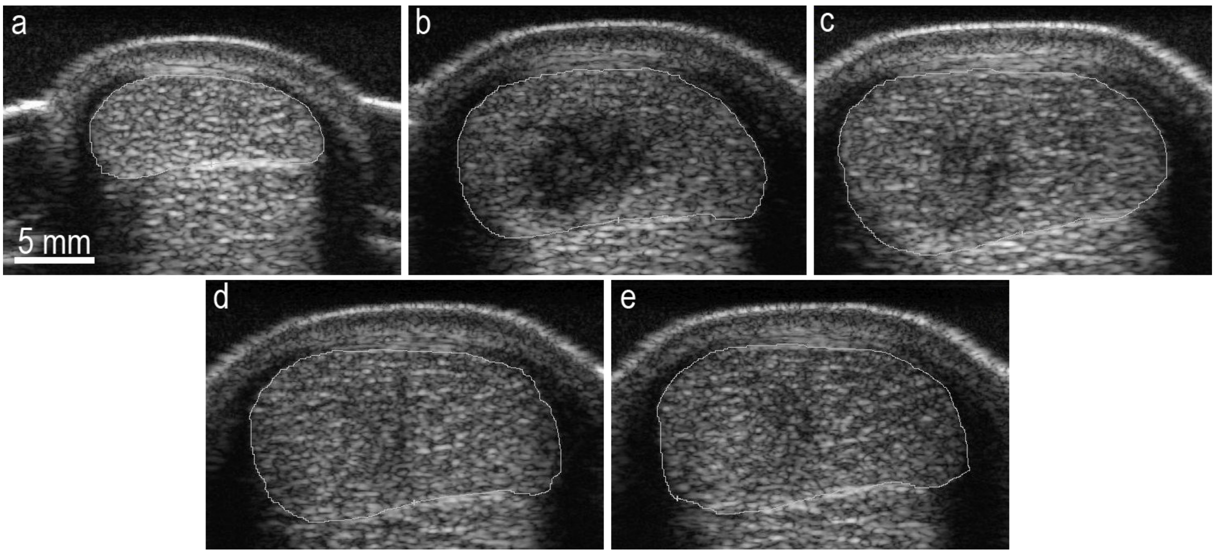

Figure 1. Transverse ultrasonographic images of tendon #5, acquired in normal tendon (a), 2 (b), 7 (c), 10 (d) and 15 weeks (e) after the surgical induction of a core lesion.

Table 1. Ultrasonographic evaluation and speed of sound measured before and during the injured tendons' follow-up

Parameter	Value				
	Normal tendons	2-3 weeks from SI	7 weeks from SI	10 weeks from SI	15 weeks from SI
UG	10	1.6 ± 0.7	3.4 ± 0.7	4.6 ± 0.7	5.2 ± 0.6
RUG	10	0.9 ± 1.0	3.6 ± 0.9	5.2 ± 1.1	6.1 ± 0.9
SOS [m/s]	2178.8 ± 32.8	2096.6 ± 49.7	2041.8 ± 62.6	2041.5 ± 67.5	2072.4 ± 66.2

UG: ultrasonographic clinical grade; RUG: reduced ultrasonographic clinical grade; SOS: speed of sound.

The short term precision of the technique for each stage was inferior or equal to 0.3 % (corresponding to about 6 m/s).

3.3 Comparisons

UG_4 was found correlated to several SOS relative variations (Table 2); in particular, the best predictor of UG_4 was SOS_2/SOS_0 ($r = 0.78$, $p = 0.005$, Fig. 3). A similar correlation was found when considering RUG_4 ($r = 0.79$, $p = 0.004$). SOS values at other stages were not correlated to their corresponding ultrasonographic grades, but SOS_3/SOS_0 and SOS_4/SOS_0 were both correlated to UG_4 (Table 2). The ratios SOS_2/SOS_1 and SOS_3/SOS_1 were also significantly correlated both to UG_4 ($r = 0.72$, $p = 0.01$) and RUG_4 ($r = 0.63$, $p = 0.04$). However, the ratio SOS_4/SOS_1 was not correlated to the clinical ultrasonographic grade.

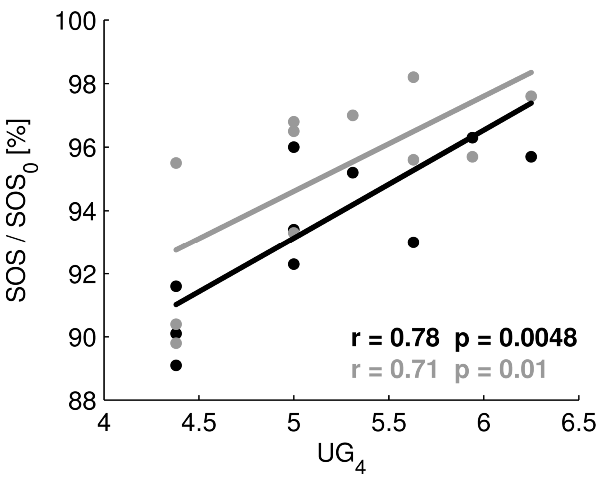

Figure 2. Relations between the ultrasonographic clinical grade (UG4, measured 15 weeks after lesion induction) and the speed of sound (SOS) measured 7 (black dots) and 15 weeks (gray dots) after lesion induction, both normalized on the initial speed of sound, in 11 equine superficial digital flexor tendons.

4. Discussion

This study represents the first application of axial SOS in the monitoring of surgically induced tendon lesions. SOS was compared with the ultrasonographic assessment all along the tendons healing process, performed by trained clinicians. Six horses were bilaterally treated with a regenerating agent, while the other 5 were bilaterally administered a placebo; the evaluation of this regenerating agent, however, was beyond the scope of the present paper. The precision error of the technique was found lower than 6 m/s at all stages; Crevier-Denoix et al. (2009) previously evaluated the precision of SOS measurements in normal tendons during *in-vitro* tension cycling, finding an error inferior to 1 m/s for loads between 600 and 4050 N (the loads expected at walk being in this range). Although six times higher than the error found *in vitro*, the precision error evaluated in this study was still below 0.3%; the difference is likely due to the variability of *in-vivo* dynamic (i.e. at walk) measurements contrary to controlled laboratory testing.

SOS is clearly showing an important interindividual variability (Crevier-Denoix et al. 2009), most likely because of the different

Table 2. Correlation coefficient describing the relation of normalized speed of sound values with the ultrasonographic clinical grade and reduced clinical grade († $p < 0.05$; ‡ $p < 0.01$).

	SOS_2/SOS_0	SOS_3/SOS_0	SOS_4/SOS_0	SOS_2/SOS_1	SOS_3/SOS_1
UG_4	0.78‡	0.74‡	0.71†	0.72†	0.65†
RUG_4	0.79‡	0.66†	0.64†	0.70†	0.63†

SOS: speed of sound; UG: ultrasonographic clinical grade; RUG: reduced ultrasonographic clinical grade; subscript 0: measurements in normal tendon; subscripts from 1 to 4: measurements performed 2, 7, 10 and 15 weeks, respectively, after the surgical induction of core tendon lesions.

mechanical properties that affect ultrasound propagation (i.e. elastic modulus, density and Poisson's ratio). Normalized SOS values were expected to account for this variability and thus better reflect the effects induced by the tendon injury, so it is not surprising that those values were significantly correlated with the clinical assessment by ultrasonography. The correlation between SOS_4/SOS_0 and UG_4 suggests that normalized SOS at a late stage (relatively to the present study) is capable of quantifying the clinical ultrasonographic assessment, which relies on the examiner's experience. Normalized SOS measured 7 and 10 weeks after induction were already correlated with this final clinical assessment, which was performed several weeks later, suggesting that normalized SOS values may be capable of predicting the tendon status a few weeks in advance. This result was unexpected and the predictive capacity of normalized SOS values should be confirmed with a larger cohort and, possibly, on a longer term study.

Values of SOS normalized on SOS_1 offer an advantage on those normalized on SOS_0, since in normal clinical practice a SOS reference of normal tendon (i.e., before the lesion's insurgence) is rarely available; nevertheless, the lack of a significant correlation between SOS_4/SOS_1 and UG_4 casts doubts on the relevance of this normalization. A reference for normal tendon SOS might be obtained in the contralateral limb. However, as a SOS difference of about 10 m/s between two normal tendons coming from the same horse has been previously reported (Crevier-Denoix et al. 2009), normalization on the contralateral limb should be investigated further before being applied in the clinical context.

The clinical grading by ultrasonography evaluated four lesion features: echogenicity, transversal extent, transversal architecture and longitudinal architecture. While normalized SOS values were in agreement with the clinical assessment by ultrasonography at 15 weeks, SOS measurements gave different but complementary information. In fact, the latter show a minimum between 7 and 10 weeks after the induction, while the clinicians observed that the ultrasonographic characteristics of the lesions started recovering from 3 weeks. This difference between ultrasonographic evaluation and SOS might reflect two different aspects of the injured tendon; while the former evaluates its structure and architecture (i.e. lesion extent, alignment of newly formed fibres), the latter is related to tendon elastic modulus (Vergari et al. In Press). Still, normalized SOS is probably affected by the tendon's architecture, as suggested by its correlation with the reduced ultrasonographic grade. In fact, the latter is based on the lesion's echogenicity (injured tendon's mean echogenicity has been reported to be correlated to tendon's elastic modulus (Crevier-Denoix et al. 2005)), and transversal architecture; other combinations of grading were not correlated with normalized SOS.

Tendon healing can be divided in three or four overlapping phases (Patterson-Kane and Firth 2009; Sharma and Maffulli 2005). The first reaction to the injury, lasting about 4 days and often accompanied by haemorrhage, is an inflammatory process characterised by swelling and infiltration of inflammatory cells, which after a few days are replaced by new blood vessels and fibroblasts. This early repair tissue is gelatinous and contains randomly oriented fibres (Watkins et al. 1985). After 6 weeks, the remodelling phase (consolidation and maturation) commences and, between 8 and 12 weeks, the newly-produced collagen fibres start aligning along the stress direction. Ten weeks after the injury, the fibrous tissue is gradually substituted by scar-like tendon tissue. The SOS in injured tendon seems to roughly follow these phases, with a decrease that begins with the injury occurrence and continues until the beginning of the remodelling phase. A significant SOS increase was then observed during the supposed formation of scar tissue (after 10 weeks from induction). The follow up ended 15 weeks after the lesion induction, so it is not known how the SOS would have changed later; however, since the mechanical properties of healed tendons rarely match their original quality (Crevier-Denoix et al. 1997), it can be supposed that eventually SOS would have not regained its original value.

It can be hypothesized that the SOS decrease observed during the first weeks was due to the swelling induced by the inflammatory state and to the decreased injured tendon's elastic modulus (Crevier-Denoix et al., 1997). The SOS increase observed between 10 and 15 weeks probably coincided with the beginning of the fibres realignment. Numerical simulations could be used to assess the factors affecting the SOS progression in healing tendon, as it was recently done for healing bones (Machado et al., 2010). However, this would require more information on the mechanical and acoustic local properties of injured tendon.

5. Conclusions

The present study did not last enough to thoroughly test the ability of SOS measurements to help in the establishment of a reliable prognosis of tendon lesions. Moreover, the tested lesions were not spontaneous, and the sample was too small to positively define technique's power to characterise them. Still, it was observed that SOS is affected both by the presence of a lesion and by its evolution in time, and that SOS variations (i.e., the normalized values) are related to the lesion status as assessed by trained clinicians. While the functional meaning of axial SOS has yet to be investigated, the presented results suggest that SOS measurements, as indicative of tendon's stiffness, could provide complementary information to the ultrasonographic exam. These results confirm the potential clinical interest of axial SOS measurements in the follow-up of tendon lesions.

Conflict of interest statement

The authors have no conflicts of interest to disclose.

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