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Ultrasound tissue characterisation of the superficial digital flexor tendons in juvenile Thoroughbred racehorses during early race training

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Summary

Background: Injuries to the superficial digital flexor tendon (SDFT) are one of the leading causes of Thoroughbred (TB) wastage. Increasingly, the aim is to prevent injury rather than treat it. Conventional ultrasonography is not sufficiently sensitive to accurately monitor tendon and predict injury. Ultrasound tissue characterisation (UTC) is a relatively new technique, which improves tendon characterisation by providing a 3-dimensional (3D) SDFT reconstruction and objective calculation of fibre alignment by classifying fibres into one of 4 echo-types.
Objectives: To report a reference range of echo-types in a population of normal juvenile TB racehorses. It was hypothesised that: UTC would be easy to use on juvenile TB racehorses in a field setting; that results would be repeatable; that the UTC would demonstrate a physiologic response of the tendon and, finally, that the technique would allow monitoring of the SDFT for early detection of degenerative changes.
Study design: Prospective longitudinal cohort pilot study.
Methods: Thirty-two TB yearling racehorses were recruited. UTC measurements of bilateral forelimb SDFTs were taken every 60–90 days. The proportion of 4 echo-types were quantified as a relative percentage at specific zones over the length of the SDFT. Relationships were assessed by paired *T* tests or Wilcoxon signed rank tests.
Results: Mean percentage for echo-type I fibres were >85%; echo-type II fibres were <15%, with negligible echo-type III and IV. Significant right to left limb, zonal, and temporal differences in echo-type were identified.
Main limitations: No control group of untrained horses, limiting ability to differentiate whether findings were training-related as opposed to age-related changes.
Conclusions: Changes in SDFT characterisation over the first 6 months of training were identified. UTC may provide useful objective information when assessing juvenile SDFTs.

The Summary is available in Spanish – see Supporting Information

Keywords: horse; lameness; sports medicine; ultrasound tissue characterisation

Introduction

Overstrain injuries to the SDFT are amongst the most common musculoskeletal injuries [1–3] and account for a significant amount of wastage in the racehorse [4,5]. Regardless of the treatment chosen, it is often expensive and requires prolonged amounts of time out of training with no guarantee of success. It is desirable, therefore, to prevent injury [1,5,6].

Conventional ultrasonography has long been used to evaluate and monitor tendons and ligaments in the horse [7–9]. However, inconsistencies in imaging due to even minor changes in transducer angle, amplifier gain and displacement, as well as the inherent lack of ability of a 2D conventional ultrasound to fully decipher a 3D tendon structure have limited its usefulness as a predictor of injury and as a guide to rehabilitation [6,10,11]. Conventional ultrasound has been shown to not be sufficiently sensitive to accurately and unequivocally determine the type of tendon tissue under investigation [7,12], with Khan et al. reporting that a reduced area of hypoechogenicity on ultrasound did not correlate with an improved clinical outcome in human Achilles tendinopathy (12).

Ultrasound tissue characterisation (UTC) is a relatively new technique intended to improve objective tendon characterisation by standardising instrumental settings. UTC uses conventional ultrasonography to construct a 3D image of the tendon after capturing 600 transverse images over a 12 cm distance [13]. Dedicated algorithms map the tendon structure over multiple transverse images and quantify and classify structure related and non-structure related echoes into one of 4 echo-types (Fig 1). Histological studies have confirmed that these echo-types reflect the underlying structure and pathology in the tendon [3,11,14]. UTC has been used recently in both human and equine studies to identify and monitor tendon structure [12–16].

The main aim of the current study was to investigate tendon characterisation in juvenile horses and to report a reference range of echo types for a population of normal juvenile TB racehorses. Additionally, it was hypothesised that UTC would be easy to use on juvenile TB racehorses in a field setting; that results would be repeatable; that the UTC would be able to demonstrate a physiologic response of the tendon and finally, that the technique may assist monitoring of the SDFT in detecting early degenerative changes and clinical injury.

Materials and methods

Horses

This was a prospective longitudinal cohort pilot study. Thoroughbred yearling racehorses within, approximately, their first 2 weeks of training were recruited from a single training facility. Horses not destined to go to public auction ('sales') were included in the study. Horses intended for future sale were excluded, as it was considered likely they would be lost to follow up prior to completion of the study. All horses were classed as 'untrained' at the start of the study.

All examinations were performed by primary and secondary authors, both experienced ultrasonographers but relatively new to using UTC technology. Sampling took place approximately every 60–90 days for a total of 3-time sampling points: T = 0, T = 1, T = 2. The first measurement

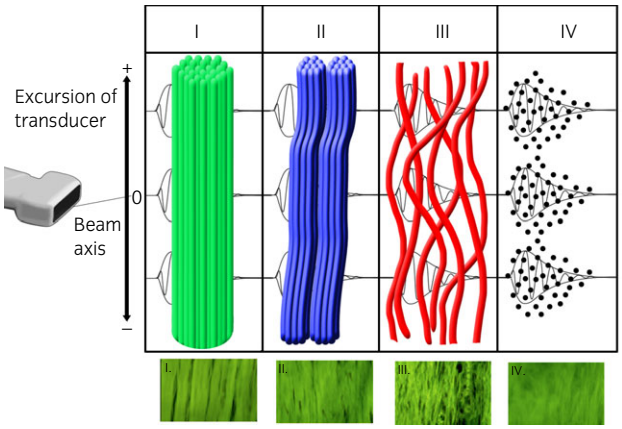

Fig 1: Representation of different echo-type classification using ultrasound tissue characterisation. Echo-Type I: generated by intact and aligned tendon bundles coloured green. Echo-Type II: generated by discontinuous, swollen and wavy tendon bundles, coloured blue. Echo-Type III: mainly fibrillar tissues, coloured red. Echo-Type IV: amorphous tissue with mainly cellular components and fluid, coloured black. Echo-type I and II are generated by structural reflections from larger structures with axial diameter above spatial resolution (>0.35 mm), while echo-types III and IV are interfering echoes from smaller entities below the limits of spatial resolution (<0.35 mm). These echo types are quantified as relative percentages of the tendon in the region of interest (ROI). The image below each schematic demonstrates the histological fiber composition for each echo type.

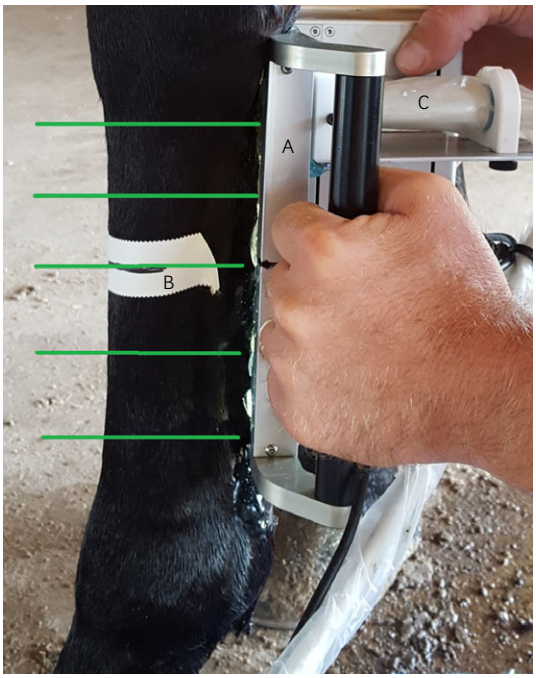

Fig 2: The UTC cradle A) is placed against the palmar metacarpal region of the SDFT with a midpoint line on the cradle matching a line placed 15 cm distal to the accessory carpal bone B). By travelling distally, the 12 mHz linear transducer C) then automatically obtains still transverse images of the SDFT every 0.2 mm over the 12 cm cradle length. The resulting 600 images permit 3D reconstruction and assessment of fibre composition. The superimposed horizontal green lines represent the anatomic locations of the region of interest (ROI) frames 50, 175, 300, 425, and 550 which were used for echo-type analysis.

(T = 0) was taken in October at the start of the Thoroughbred breaking and training process and the final measurement (T = 2) was taken between March and April as horses were beginning to disperse for racing around the country. Each SDFT was palpated prior to each scan and once horses were in sufficient training to trot in hand they were assessed for lameness prior to data collection. Any horse exhibiting clinically enlarged or painful tendons was documented. The trainer was consulted prior to each data collection point to establish presence of lameness or absence from training or other relevant history. All horses were trained in an anti-clockwise direction on a ¾ mile dirt track. After 2 weeks of stall and barn ridden exercise, horses were trotted ¾ mile on the racetrack daily for 1 month. Following this, they cantered approximately 1½ miles in addition to trotting for ¾ miles 6 days a week for another 2 months prior to commencing once weekly fast speed work (FSW) (FSW = <15 s/1/8th mile) in addition to trotting ¾ mile and cantering 1½ miles 5 days per week. Horses had approximately 2 h small pasture turn out per day. Horses were not scanned within 3 days of fast speed work i.e. breezing [17] and, to ensure consistency, horses were not scanned on any day following a day out of training. Images were reviewed at the time of examination for quality control by the primary and secondary authors and examinations were repeated when necessary. Images were processed and assessed by a single investigator (S.P.). A subset of the images from each data collection point were assessed by another investigator (H.V.S.) for the purposes of quality control.

Ultrasound tissue characterisation

Horses were sedated with butophanol[a] and detomidine[a], the dosage was determined for the individual, and ranged from 0.01 to 0.04 mg/kg butorphanol i.v. and 0.02 to 0.04 mg/kg detomidine i.v. The palmar aspects of the limbs were clipped before examination using a number 40 blade. The limbs were scrubbed with a sponge soaked in alcohol to remove gross dirt and then wiped down with alcohol soaked 4 × 4 gauzes. Ultrasound coupling gel was applied liberally to the skin overlying the tendon and between the transducer and built in standoff pad ensuring no air bubbles were present.

A 5–12 MHz linear array ultrasound transducer[b] mounted onto a tracking device with a motor drive and built in acoustic coupling standoff[c] was used for all measurements (Fig 2).

Horses were required to stand with all 4 limbs square and bearing weight equally. A white tape marker was placed over the dorsal aspect of the limb 15 cm distal to the base of the accessory carpal bone to enable comparable transducer placement at each data collection point. A permanent mark was made on the frame of the transducer half way between the top and bottom. The tracking device was placed on the palmar aspect of the limb, parallel to the long axis with the marker on the limb lining up with the half way marker on the tracker frame to ensure repeatability of placement (Fig 2).

Once an appropriate grey scale transverse image of the SDFT was identified, image acquisition was initiated.

The transducer moved automatically over the length of the palmar aspect of the tendon by means of a motor drive, allowing for transducer tilt, gain, time-gain compensation (TGC) curve, focus and depth to be standardised (12 MHz, focus = 1.3 cm, depth = 3 cm, window size = 17). Window size representing the number of contiguous transverse images used to calculate fibre composition was set at 17. Window size 17 refers to the correlation of 17 contiguous transverse images collected at precise steps of 0.2 mm, thus 16 steps of 0.2 mm. Window size 17 is ideal for equine applications. It affords measurement of small scale ultra-structural changes and is more sensitive for detection of small-scale waviness. It may provide a better representation of the actual tendon structure, whilst decreasing the risk of movement artifact incurred when using lower window sizes.

During the acquisition, transverse images were collected at even distances of 0.2 mm over a 12 cm proximal to distal scanning distance and sent in real time to a dedicated high capacity laptop computer for processing. Compilation of these images resulted in a 3D data block containing ultra-structural information of the entire tendon volume [11] (Fig 3). This data-block was used for tomographic visualisation in transverse, sagittal and coronal views, and for quantification of ultra-structural integrity. Dedicated UTC algorithms[c] quantified the dynamics of the grey levels of corresponding pixels in contiguous images over 3.2 mm or 17 scans (window size 17). Previous fundamental research has revealed that there is a close relationship between the degree of stability of echo-

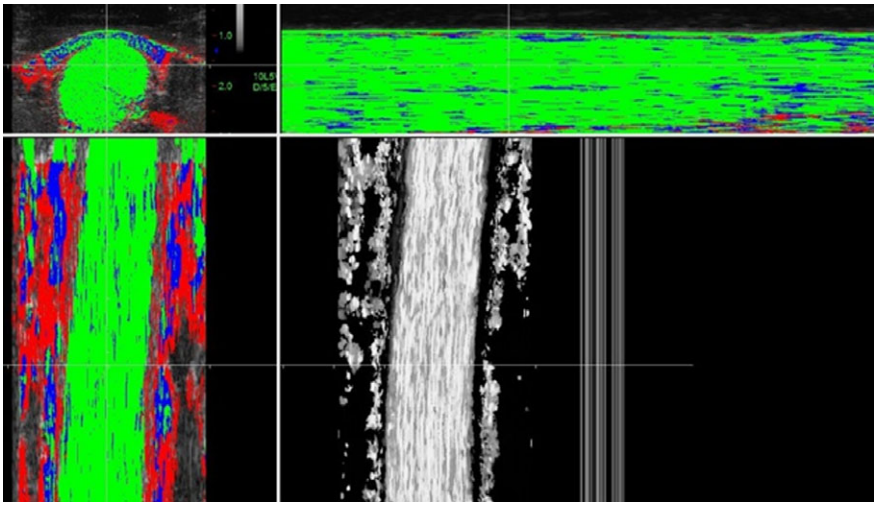

Fig 3: Representative screenshot from UTC examination. Transverse (top left), longitudinal (top right) and frontal/coronal (bottom left: following analysis; bottom right: grey scale coronal image)

patterns in contiguous images and the ultra-structural organisation of exactly matching tendon specimens [1,3,13] (Fig 1). Compilation of this information over the entire length of the scan resulted in complete information regarding the composition of the tendon. The scanning acquisition over 12 cm took 45 s. Sampling was repeated if there was horse or operator movement during this acquisition period. Scans were also repeated if the images were questionable at the time of review, which took place within the sampling period. Intra and inter- observer variability was assessed on randomly selected horses at each data collection time point.

Tendon structure was quantified by manually selecting regions of interest (ROI) along the length of the SDFT during post collection processing of images. ROI were located at frames 50 (most proximal), 175, 300, 425 and 550 (most distal) to represent 5 equidistant zones along the tendon. These zones were assigned the classification: Z1; Z2; Z3; Z4; Z5 from proximal to distal. Contours, collected as freehand silhouettes of the SDFT at each of the ROI frames, were assessed for the percentage of each of the four echo-types [3,11].

Data analysis

Objective echo-types (echo-type I to IV) were calculated at each of the 5 scanning zones in each forelimb for each horse at each time point (T = 0, T = 1, T = 2) using proprietary UTC software[c]. Data was recorded in terms of percentage of grade 1–4 fibres present at each zone. Mean and standard deviations were calculated for percentage distribution of each fibre type at each sampling time to generate a reference range in normal juveniles.

Data was assessed to determine the presence of differences between forelimbs of an individual and differences in fibre pattern at each zone over time (Z1–Z5). Using a commercially available software package[d] data was assessed for homoscedasticity using the Shapiro-Wilks test and, where normally distributed, paired *T*-tests were used for comparisons and Wilcoxin signed-rank tests were used for comparison of any non-normally distributed data.

To determine if fibre composition differed between scanning zones (Z1–Z5) within each limb, a Kruskal-Wallis rank test was used with subsequent paired *T*-tests to identify specific scanning zones that differed from each other. Bland-Altman analysis was used to determine the level of intra-operator variability and inter-operator agreement in image acquisition.

For all statistical analyses significance was set at $P \leq 0.05$.

Results

32 horses were initially enrolled in this study (21 males, one gelding, 10 females). Data was available for all 3 sampling times (T = 0, T = 1, T = 2) for 18 horses (13 males, 5 females). Six horses were excluded after the first data collection point due to a change in classification from racing to sale prospects. One horse was excluded at this stage due to an injury that precluded further training. Their data was not used due to the single sampling point. A further 7 horses were lost to follow up after the second data collection point due to early relocation to the racetrack. The average interval from T = 0 to T = 1 was 91.16 (±5.47) days left forelimb (LF) and 90.76 (±5.21) days right forelimb (RF). The average interval from T = 1 to T = 2 was 65.55 (±5.55) days LF and 65.62 (±5.91) days RF. For all samples, echo-type I and echo-type II were very close to reciprocal: as one increased, the other decreased proportionately. Echo-types III and IV were too few to perform statistical analyses. The mean percentages and standard deviations of echo-type I and II at each zone and time are shown in Table 1.

At T = 0 a significant difference existed between left and right limbs in zones 4 and 5 of the SDFT with an increased percentage of echo-type I in the right forelimb compared to the left forelimb (88% right vs. 83% left, P = 0.001). At T = 1 a significant difference existed between percentage of echo-type I in the left and right forelimbs at zone 2, with more echo-type I fibres present in the left limb than the right (88% right vs. 90% left, P = 0.05).

In the left forelimb SDFT, between T = 0 and T = 1, there was a significant increase in echo-type I in zone 1 (P = 0.006), zone 2 (P = 0.0001) and zone 3 (P = 0.04) with 85% echo-type I at T = 0 and 90% echo type I at T = 1. In the right forelimb SDFT, between T = 0 and T = 1, there was also a significant increase in echo-type I in zone 1 (P = 0.008).

In the left forelimb SDFT, between T = 1 and T = 2, there was a significant decrease in echo-type I in zone 2 with 90% echo type I at T = 1, compared to 86% at T = 2 (P = 0.03). In the right forelimb SDFT, between T = 1 and T = 2, there was also a significant decrease in echo-type I in zone 1, with 90.4% echo type 1 at T = 1 compared to 87.2% at T = 2 (P = 0.03). The temporal changes in percentage of echo-type I and II for the RF and LF SDFT are shown graphically in Fig 4.

At T = 0, there was no significant differences in percentage of echo-type I and II between sampling zones (Z1–Z5) within individual tendons in either right or left limb. At T = 1, a significant difference existed between zones in the left forelimb (P = 0.003) and the right forelimb (P = 0.04).

Analysis of intra-operator repeatability revealed the mean difference in echo-types was only 0.007 (P = 0.6). The mean difference between operators (inter-operator agreement) was only 0.008 (P = 0.23).

Discussion

The main aim of this study was to investigate normal tendon characterisation using UTC and to provide, for the first time, a reference

TABLE 1: Mean percentages, and standard deviations for echo-type I and II fibres at each of the 5 scanning zones at sampling times T0, T1, T2 for left and right forelimb SDFT. Values are expressed as % of each echo type observed

	Echo-type I fibres (%)						Echo-type II fibres (%)					
	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Combined mean	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Combined mean
Left front												
T = 0												
Mean	86.38	84.89	85.09	83.14	83.61	84.62	13.25	14.37	14.24	15.91	15.54	14.66
s.d.	4.57	5.38	6.44	6.73	5.74	5.77	4.24	5.55	6.54	6.97	5.65	5.79
1.96 s.d.	8.95	10.55	12.63	13.18	11.25	11.31	8.31	10.89	12.83	13.65	11.07	11.35
T = 1												
Mean	89.87	90.04	88.64	86.02	85.76	88.07	9.85	9.43	10.47	12.69	13.36	11.16
s.d.	5.11	3.52	4.43	5.25	6.04	4.87	4.79	3.41	3.80	4.44	5.98	4.48
1.96 s.d.	10.01	6.89	8.68	10.29	11.84	9.54	9.38	6.68	7.45	8.71	11.73	8.79
T = 2												
Mean	88.26	86.08	87.82	87.65	87.34	87.43	11.64	13.44	11.98	11.94	12.14	12.23
s.d.	4.59	5.62	4.14	3.69	4.43	4.49	4.58	5.25	4.04	3.68	4.27	4.36
1.96 s.d.	9.00	11.02	8.11	7.22	8.67	8.80	8.97	10.30	7.93	7.20	8.38	8.55
Right front												
T = 0												
Mean	87.59	86.79	87.39	88.41	87.65	87.57	12.33	12.72	12.00	10.95	12.04	12.01
s.d.	4.70	5.20	6.59	5.37	5.25	5.42	4.64	5.17	6.36	4.69	5.05	5.18
1.96 s.d.	9.21	10.19	12.92	10.53	10.29	10.63	9.10	10.13	12.46	9.20	9.89	10.16
T = 1												
Mean	90.36	88.22	88.11	88.83	87.57	88.62	9.51	11.45	11.49	10.47	11.83	10.95
s.d.	3.69	3.87	4.24	3.84	3.47	3.82	3.61	3.83	4.25	3.96	3.51	3.83
1.96 s.d.	7.23	7.58	8.32	7.52	6.80	7.49	7.08	7.50	8.33	7.77	6.88	7.51
T = 2												
Mean	87.21	85.93	89.01	87.43	85.57	87.03	12.63	13.58	10.40	11.85	13.85	12.46
s.d.	3.67	4.38	2.47	5.78	3.93	4.05	3.65	4.45	2.54	5.67	3.99	4.06
1.96 s.d.	7.19	8.58	4.84	11.34	7.71	7.93	7.15	8.72	4.97	11.10	7.81	7.95

range of echo types in a population of juvenile TBs in pre-race training. In this normal population of training juvenile TBs, at all temporal and anatomical sampling points, the percentage of echo-type I was 85% or greater. Similarly, the percentage of echo-type II was no greater than 15%. There was a negligible proportion of echo-types III and IV throughout the study. These percentages are different than those previously documented by Docking *et al.* [17] when they studied tissue characterisation in a population of mature racehorses. In that study the normal percentage of echo-type I was >90% and echo-type II was <5%. The higher percentage of echo-type I in combination with the lower percentage of echo-type II, in that study, was attributed to mature tendon containing more aligned and organised fascicles (generating echo-type I) and lower percentages of swollen and less aligned fascicles (generating echo-type II). Furthermore, they reported a negligible amount of echo-type III and <5% echo-type IV. The higher percentage of echo-type IV reported by Docking et al. may be indicative that 'healthy' mature tendon already contains matrix degradation, although still without clinical signs. Conversely, the juvenile tendon, in the current study, showed a higher percentage of echo-type II, generated by swollen and less aligned fascicles and negligible amounts of echo-type III and IV. This finding supports the theory that juvenile SDFT echo-type distribution, more specifically the relative increase of echo-type II and the lack of echo-type IV, may be adaptive in nature.

A speculative physiological response of the tendon in young training horses was identified in the current study. At the commencement of the study, the first data collection point demonstrated a difference in fibre echo-type within the lower third of the SDFT between the left and right tendons, with the right tendon demonstrating a significantly higher proportion of echo-type I compared to the left tendon. At the time of the first UTC measurement the horses were classed as untrained yearlings. Variations in the composition of the tendons could not, therefore, be attributable to the effects of training and were presumed to be due to the innate state of the tendons. Horses have been shown to exhibit an inherent 'sidedness' with kinematic differences between the left and right limbs [18–20]. A predominance of left sidedness in the horse has been documented [20]. A preference to advance one forelimb is thought to reflect greater agility on that side of midline, with the non-advanced limb thought to bear more weight [20]. In this current population it is plausible that the greater proportion of echo-type I, aligned 'normal' fibres in the bottom third of the right limb is due to an inherent or manmade laterality in these horses.

By the time of the second data collection point, approximately 3 months after the start of formal training, the difference in echo-type I composition in the distal third of the tendon between left and right SDFTs was no longer apparent. Instead a significant between limb difference in the proportion of echo-type I fibres in one of the upper zones of the tendon (Z2) was seen, with the LF SDFT exhibiting more echo-type I, aligned normal fibres than the RF SDFT. Horses at this collection point were in full training and most of the population had completed at least one fast speed work ('breeze'). Changes in temporal and linear kinematics induced by training have been previously documented [21]. Differences in kinematics between limbs, especially the leading and trailing forelimbs have been recorded. Studies using treadmills and force plate analyses have shown that at the canter (3–11 m/s) higher vertical forces are seen in the trailing forelimb [22,23]. In this current study all horses were trained in an anti-clockwise direction. The differing forces and loads assigned to each limb could account for physiological changes in the tendon and would be a logical explanation for the differing fibre patterns documented between limbs 3 months into training in this juvenile population of horses. Regardless of the cause, the changing fibre composition validates the hypothesis that UTC can demonstrate a physiologic response of the SDFT in juvenile racehorses.

Between time points T0 and T1 a significant increase in the number of echo-type I fibres was observed in the upper zones of both the left and right SDFTs. One explanation for this may be found in the changes in biochemical composition known to occur in exercising tendons.

It has previously been demonstrated that the biochemical composition of SDFT is continually shifting and that up to maturation at 2 years of age, the SDFT is capable of a change in biochemical make up and structure, with transitions being seen in response to ageing and exercise [23–26]. Whilst it has been shown that all biochemical components change early in

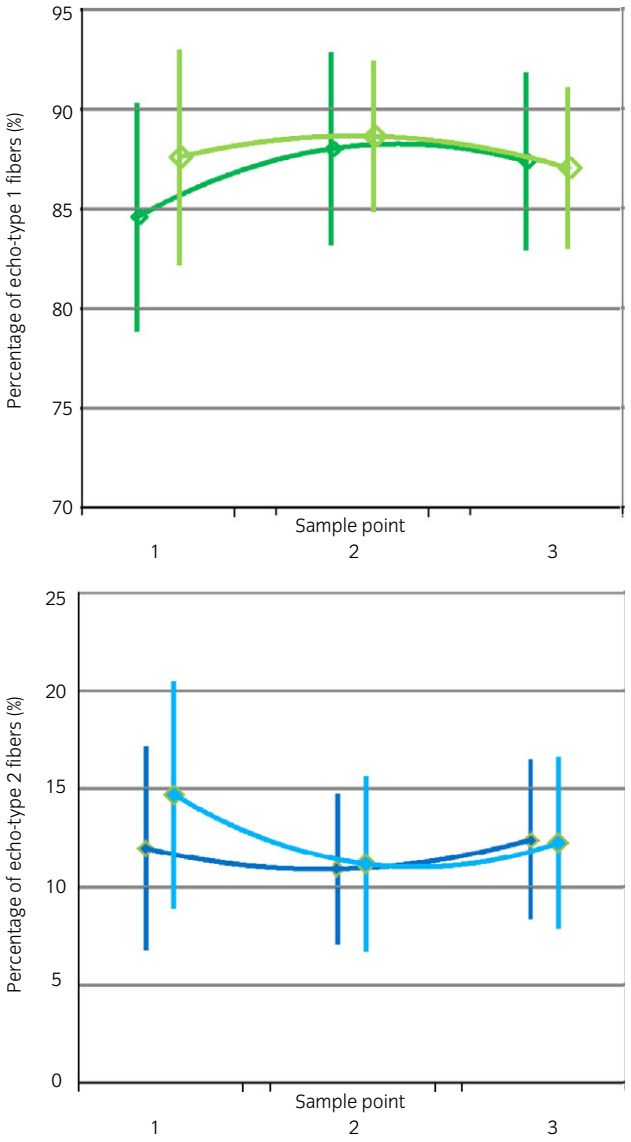

Fig 4: Mean (and s.d.) % of echo-type I (green) and II (blue) for left and right forelimb SDFT at each sampling point. Dark green and blue represent left fore SDFT, light green and blue represent the right fore SDFT.

life in the absence of a specific exercise regimen [22], it has also been shown that differing exercise regimes cause differing biochemical changes [23–25]. Biochemically the SDFT, therefore, demonstrates a fluidity in composition and structure [26–31]. The increase in the number of echo-type I fibres in the upper zones of the left and right SDFTs between time point T0 and T1 in the current study, along with only negligible amounts of type III and IV, is speculated to be indicative of adaptive physiological changes, with improved fibre alignment in response to appropriate training regimens. These differences in fibre type are, most likely, in response to the differing loads and tensions experienced during the initial training period, similar to the impetus responsible for the biochemical changes already described in the literature [27,28]. Further research, including concurrent histological studies are needed to substantiate this and to investigate the regionality of this change within the metacarpal region of the SDFT.

Between time points T1 and T2 (from 3 to 5 months training), a decrease in the levels of echo-type I (normal aligned fibres) and a reciprocal increase in the proportions of echo-type II fibres in the upper zones of both the left and right tendons was seen. This finding is suggestive of further changes in the tendon ultrastructure. It is unknown, however, whether this change represents an adaptive or degenerative response. Cook *et al.* [32] proposed a cell driven model for the development of tendon pathology, in mature horses, stating that a tenocyte driven reactive non-inflammatory response is the first response of the tendon to mechanical stimuli. Tenocytes respond to mechanical stimuli by up-regulating the production of large proteoglycans, such as aggrecan and versican, which bind water into the tendon matrix, increasing the total amount of ground substance. The diffuse thickening of the tendon is a short-term attempt to reduce tendon stress, by decreasing the load per square mm [17,32,33]. Increasing water content of the ground substance, as described, would inevitably lead to tendon fibres becoming wavy and swollen; the definition of UTC echo-type II fibres. One may surmise, therefore, that the increase in echo-type II fibres in the current population of adolescent TBs represents tendon maturation and an adaptive response to the increasing intensity of training between the second and third sampling points. A study performed by Docking *et al.* [17] also supports the thought that an increase in the proportion of echo-type II fibres in clinically normal tendon can represent non-degenerative adaptation. In that study, UTC was used to analyse SDFTs pre- and post-race in a population of mature TB racehorses. A temporary increase in echo-type II fibres post-race was found. This shift resolved within 72 h. The transition in echo-type was attributed to a temporary swelling of the secondary tendon bundles due to an increase in water content of the ground substance, as described by Cook *et al.* [32], following speed work and was not considered degenerative or pathologic. Conversely, both van Schie *et al.* [13] and Rosengarten *et al.* [15] have suggested that in mature tendon a decrease in the percentage of echo type I fibres with a reciprocal increase in the percentage of echo type II fibres suggests a degenerative effect on the normal tendon integrity. Whilst disagreement exists regarding the nature of change in the mature tendon, based on the known ability of young tendon to adapt, the lack of clinical development of tendinitis and the lack of an increase of echo-type III and IV seen in the current study, it is suggested that the increase in echo-type II fibres, in this population of juvenile TBs, represents maturation and a normal adaptive response to increasing training intensity. In support of this theory, in adolescent humans it was found that the percentage of echo type II fibres in healthy Achilles tendons was 43–58% and that in fact the presence of type II fibres may not indicate inferior tendon quality but a necessary morphological, histological and functional requirement in juveniles [34]. However, as T2 was our last collection point it is unknown if horses in the current study subsequently developed tendon injury. If a significant proportion of horses did develop clinical tendonitis, the increase in echo-type II fibres may indeed represent an early indicator of degenerative change within the juvenile SDFT. Future longitudinal clinical and histological studies should aim to provide explanation for type II echo development in training horses.

Regional variations in tendon composition were also identified in the current study. At T1, significant differences existed between the upper zones of the SDFTs and the lower zones in both the LF and RF limbs. This is the first time that regional differences specific to the metacarpal SDFT have been reported using UTC. Historically, the biochemical composition of the SDFT has been shown to vary considerably between regions over the entire tendon, not specifically the metacarpal area, reflecting differences in loading [27]. In contrast, a previous histological study of young juvenile SDFTs demonstrated no significant differences in any of the biochemical parameters of the proximal, middle and distal parts of the metacarpal SDFT [24].

In the current population, the regional variations in the tendon were only apparent after the commencement of training, suggesting that within the metacarpal SDFT regional physiological differences in the tendons response to training are present: something that has not been previously identified. Further investigation is needed to fully understand the significance of this information.

Intra observer variation was low and inter observer repeatability was high in the present study. These findings agree with previous UTC studies [11,17]. The UTC equipment was relatively easy to use in the field. At the start of the study period most of the horses were not only untrained but to a certain extent unhandled daily. This made data collection difficult, especially, at this first collection point. Even small movements of the limb

can produce iatrogenic discrepancies, notably an artificial increase in the proportion of blue fibres. These movement artifacts, seen as completely blue lines in the sagittal processed image, with experience, can clearly be discriminated from non-artificial type II fibres, which are much more diffusely distributed. In this, previously undocumented, population it was necessary to repeat and verify scans that showed a high percentage of echo-type II fibres to ensure reliable results. This was time consuming but necessary for the accuracy of this pilot study. This requirement decreased as the study progressed, as horses became more accustomed to human interaction and as researchers became more familiar with the equipment.

One of the major limitations of this study was the relatively small end sample size of 18 horses. Additionally, due to early unexpected population loss the time interval between T1–T2 was less than between T0–T1. Ideally all assessment periods would have been identical. Future studies using larger populations would be beneficial, as would studies over a longer time frame, perhaps encompassing more frequent sampling.

Due to the absence of a control group of untrained horses in the current study it is not possible to conclusively prove that findings are training related as opposed to age-related changes and this is a major limitation. However, obtaining such a control population of yearling TB horses not in training is very challenging. Due to industry standards and demands, healthy and uninjured Thoroughbred yearlings are, typically, in active training and not available to be used as an untrained control. As the primary objective of the current study was to describe reference values for juvenile horses that are in training, however, it was felt appropriate to report these results independent of an untrained control group.

In the current study, no horses developed clinical injury to the SDFT during the study period. All horses were lost to follow up, due to lack of trainer compliance, thus the reliability of UTC to predict clinical injury could not be determined. This will be an important area of research for future studies.

A further limitation of the current investigation was the lack of concurrent histology. In this very new arena, comparison of the UTC fibre composition analysis to histology may increase our understanding of the transitional state of the SDFT during training. Although UTC has been shown to correlate well with histological studies in mature horses [11], concurrent histological evaluation of the juvenile tendon is lacking and would help to confirm if changes were adaptive, degenerative or inflammatory.

One final limitation was the reliance on the trainer for information regarding training records and injuries. A cursory examination of the tendons was made before each sampling point, however, the trainer was responsible for reporting any abnormalities observed during training and any variations in individual training schedules and protocols.

In conclusion, this study allowed a reference range of normal UTC values for a juvenile TB (<2-year-old) population to be generated. Echo-type I should be >85% and echo-type II should be <15%. Negligible echo-types III and IV should be present. This differs slightly from that previously reported for mature racing TBs [17].

For the first time using UTC, a presumptive physiological response was observed in juvenile SDFTs, over the first 6 months of training, and observation of the maturation of tendon tissue in adolescent horses was demonstrated non-invasively.

This pilot study also established that, similar to mature populations, the reproducibility of information is high, and the operator error is low, making this an ideal objective tool for examining the ultrastructure of the juvenile SDFT. Future long-term studies to determine the ability of UTC to accurately predict subsequent clinical injury of the SDFT in a juvenile population are required. If UTC can be shown to accurately monitor changes in the SDFT, as has been eluded to by many researchers [11,19], the technology could provide invaluable information, allowing early modifications to training and potentially preventing clinical injury to the SDFT. This could be of great importance in controlling one of the leading causes of injury and loss of use in the racehorse.

Authors' declaration of interests

The primary and secondary authors have no competing interests. Dr van Schie was fundamental in developing the UTC machine used in this study and is a director of the company. He provided guidance and support during the study. Dr van Schie was not involved in study design, data collection, analysis or interpretation of results. He did review a sample of studies throughout the investigation period for quality control. His input did not affect study findings in any way.

Ethical animal research

Research ethics committee oversight not required by this journal: descriptive clinical report using non-invasive techniques. Owners gave consent for their animals' inclusion in the study.

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Authorship

S. Plevin and J. McLellan were jointly responsible for study design and data collection. S. Plevin analysed and interpreted all data. H. van Schie reviewed data for quality control. J. McLellan and T. Perkins performed all statistical analysis. S. Plevin prepared the manuscript and approved the final version.

Manufacturers' addresses

[a]Zoetis, Parsippany-Troy Hills, New Jersey, USA.
[b]Teratech, Burlington, Massachusetts, USA.
[c]UTC Imaging, Stein, the Netherlands.
[d]SPSS software, IBM, Armonk, New York, USA.

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Supporting Information

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