Navicular Syndrome-related changes to collagen proportion of different cross-sections of the flexor tendons in equine distal forelimb

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ABSTRACT

The aim of this study was to quantify the distribution of aligned and non-aligned collagen in cross-sections of the superficial digital flexor tendon (SDFT) and deep digital flexor tendon (DDFT) in different levels of the distal forelimb of equines diagnosed with NS (Navicular Syndrome). Sixty equine forelimbs were collected. Was compared two groups (NA, Not affected vs. NS-group) by *t*-Student. Diagnosis of NS was based on clinical and lameness examination, diagnostic analgesia and radiological findings. The proportion of aligned and non-aligned collagens at 2 levels for the SDFT and 3 levels for the DDFT were measured by histochemical stains. The amount of aligned and non-aligned collagen in tendons were calculated using Colour-Based Segmentation function. Regarding collagen, there were significant differences in the amount of aligned collagen (NA: 21.2 $\pm$ 1.31; NS-group: 12.2 $\pm$ 4.67; $p = 0.0026$) and non-aligned collagen (NA: 21.8 $\pm$ 2.22; NS: 25.1 $\pm$ 1.73; $p = 0.0241$) at the DDFT insertion in the distal phalanx. We concluded that the flexor tendons of the forelimb in equines with NS have different proportions of collagen than those that do not present the diagnosis, indicated by histologically visible increased proportions of non-aligned collagen and decreased of aligned collagen in the extracellular matrix.

1. Introduction

Navicular Syndrome (NS) is a common cause of forelimb lameness in equines. It is a chronic, degenerative and progressive bilateral disease characterized by an increase in the strength that the deep digital flexor tendon (DDFT) exerts on the distal sesamoid bone (DSB) and consequently on the distal interphalangeal joint. Also involves the collateral sesamoid ligament, distal sesamoid ligament, podotrochlear bursa (Dyson et al., 2011). It is characterized by structural changes in the flexor tendons, such as fibrillation, adhesion formation and the presence of metaplastic fibrocartilaginous areas, rich in elastic fibers, particularly proximal to the bursa navicular. Numerous theories about the pathogenesis have been proposed (Colles, 1987; Dik and van den Broek, 1995; Wilson et al., 2001), however none of these completely explains all of the observed pathologic changes. One of them involves alterations in the biomechanics of the flexor tendons, particularly by the hyperextension observed, caused by abnormal flexion of the proximal metacarpophalangeal joint (Dyson et al., 2011).

Tendons consist of a tissue that is comprised of a dense network of collagen fibrils with relatively few fibroblasts. Proteoglycans make up less than 1% of the dry weight and are primarily in the form of low molecular weight decorin and biglycan. Since the large proteoglycan, aggrecan, is absent in the main substance of these structures, tendons have a lower water content and higher collagen content than fibrocartilage and cartilage. Water accounts for about 55% of the wet weight and aligned collagen (type I collagen) about 38%. Other constituents that may appear in small amounts include non-aligned collagen (type III collagen), elastin, glycoproteins, and other non-collagenous proteins (Wren et al., 1998). Histomorphologically, the collagen fibers form bundles that are oriented primarily in the direction of the long axis of the tendon. The predominant type of collagen in normal adult tendon is aligned collagen and a small portion consists of non-aligned collagen. The relative proportions of these two types of collagen therefore determine the mechanical properties of the tissue (Goodship et al., 1994).

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One of the interesting characteristics of tendons is that they are capable of transforming their architecture as a consequence of biomechanical local alterations. Therefore, the conformation of the flexor tendons plays a key role in regulating the adaptive response to the influence of mechanical factors on the modulation of the constituents of the extracellular matrix (ECM; Thompson, 2013).

According to our knowledge, there are no quantitative data in equine tendons diagnosed with NS. Blunden et al. (2006) described the histopathology of DDFT in lame equines, however, they did not provide quantitative data. The podotrochlear system (distal sesamoid bone, collateral and impar ligaments of the distal sesamoid bone, distal deep digital flexor tendon, and podotrochlear bursa) is highly adapted to dissipate forces away from the osteotendinous junction between the deep digital flexor tendon and the distal phalanx. Force analyses using 3D models constructed from CT scans of a forelimb indicate that an exaggerated dorsiflexion of the foot, a posture commonly observed in equines with NS, puts the deep digital flexor tendon under greater tension (Waguespack and Hanson, 2010; Dyson, 2011a, 2011b). With this in mind it is important to know how this tension influences the viscoelastic characteristics of the tendons and particularly to know the response of the tendon tissue, especially that which refers to the collagen present in the ECM. A central question is how exactly does the tendon respond to NS, and more specifically, how aligned and non-aligned collagens participate in this response. Our hypothesis is that the flexor tendons of the forelimb in equines with NS have different proportions of collagen than those that do not present the diagnosis, indicated by histologically visible increased levels of collagen in the ECM. The aim of this study was to quantify the distribution of aligned and non-aligned collagen in cross-sections of the superficial digital flexor tendon (SDFT) and DDFT in different levels of the distal forelimb of equines diagnosed with NS.

2. Materials & methods

This study was conducted at the Institute of Biology of Pontificia Universidad Católica de Valparaíso, Chile. Sixty equines (*Equus ferus caballus*) destined for slaughter were used. 30 equines with NS were chosen through a convenience sampling and a clinical examination pre-mortem and then compared with 30 equines selected based on clinical history, without antecedents, clinical signs or radiographic findings of NS. The selection of equines, diagnosis, obtaining of tendon samples and the conformation of groups was carried out in different stages: pre-mortem on abattoir and at laboratory (Fig. 1).

2.1. Ethical statements

The physical examination of equines was carried out in accordance with the Chilean Law 20,380 about protection of animals for scientific purposes.

2.2. Pre-mortem clinical examination

A pre-mortem clinical examination at the slaughterhouse was performed in equines with a history of uni- or bilateral forelimb lameness of at least 2 months' duration. An evaluation of the conformation of the foot and lameness were performed. In addition, complementary tests such as diagnostic analgesia and radiography were performed. In foot were recorded findings such as: subjective assessment of conformation, digital pulse amplitudes, response to hoof testers, distension of the distal interphalangeal joint capsule or digital flexor tendon sheath and other swellings, response to palpation and digital manipulation and posture. Lameness was graded on a scale (Dyson, 2011a) of 0–8 (0 = sound; 2 = mild; 4 = moderate; 6 = severe; 8 = non weight- bearing) at both walk
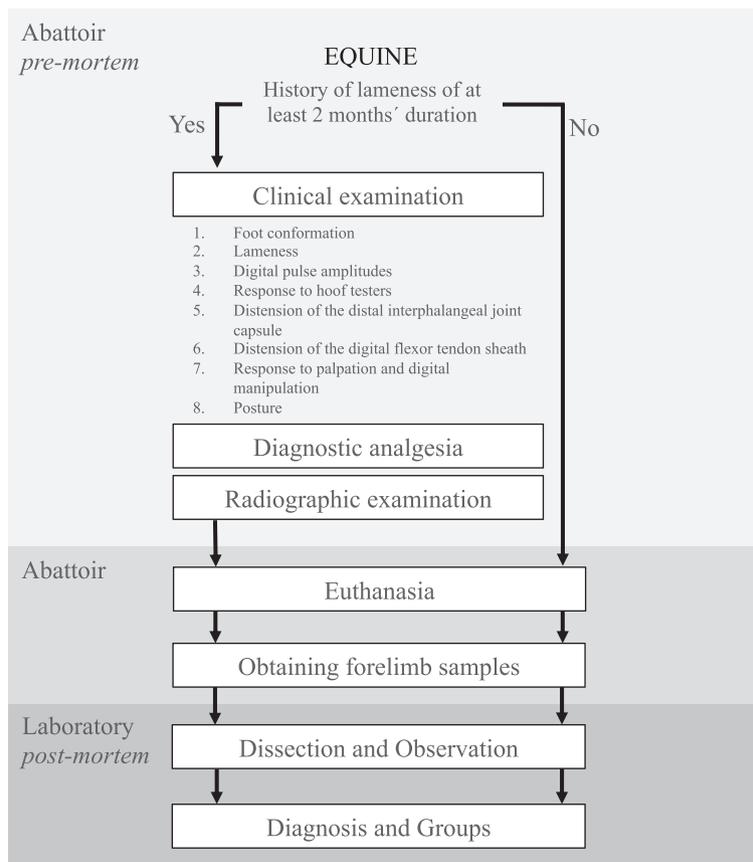
Fig. 1. Describes the selection of equines, diagnosis, obtaining samples of tendons and conformation of groups in different stages of the study: pre-mortem in the slaughterhouse and in the laboratory.

and trot gaits in straight lines, and in circles on both soft and firm surfaces (gravel ± modified asphalt). A diagnostic analgesia was performed to localize the experienced pain. First, an intra-articular analgesia of the distal interphalangeal joint via a dorsal approach was performed (5 ml bupivacaine). Second, intra-thecal analgesia of the navicular bursa under radiographic control was performed (3 ml bupivacaine; Schumacher et al., 2001) and third, the innervation of the distal forelimb was blocked from distal to proximal levels (Nagy et al., 2009; Parkes et al., 2015). Specifically, medial and lateral palmar digital nerves (*N. digitalis palmaris lateralis et medialis*) blocks were performed immediately proximal to the ungular cartilages (2 × 2 ml bupivacaine [0,5%, BupinexVet, Pharmavet]). Medial and lateral palmar nerves (*N. palmaris medialis at lateralis*) blocks were performed at different levels: at the junction of the proximal three-quarter and distal-quarter of the metacarpus, at distal to the second and fourth metacarpal bones and at the base of the proximal sesamoid bones (2 × 2 ml bupivacaine). Lameness was reassessed 10 min after each perineural nerve blocks and 5 min after intra-synovial blocks in both straight lines and circles and graded as no change, slightly improved (<50% improved), improved (>50% < 80% improved), or sound according Bassage and Ross (2010). The response to diagnostic analgesia was recorded for the circumstances under which the equine was most lame. Finally, radiographs in both distal forelimbs were obtained (to compare between both distal forelimbs and for diagnostic purposes only). Three radiologic views were used: lateromedial, dorsoproximal-palmarodistal oblique and palmaroproximal-palmarodistal oblique 45°. Obtaining and interpreting radiographic images was performed as described by Dyson (2011b). The images were obtained by means of indirect digital radiology (Orange 9020 HF X-ray generator, EcoRay Co., LTD., Seoul, Korea). The digitalization of the images was conducted through the portable veterinary radiography (CR) system (Portable Veterinary Radiography System, iCRcoTM, model iCR CHROME VET, United States). The radiological findings and criteria used to diagnose NS are described in Tables 1 and 2. Each equine examined was assigned an ID.

2.3. Tendon sampling and dissection

Once the equines previously examined were euthanized (for reasons unrelated to this investigation), the distal forelimbs were obtained, fixed and transported to the laboratory for the cleaning, disinfection, labelling and dissection procedures. In the next stage of the analysis, simple observation was used to compared gross morphological changes in both flexor tendons of the distal forelimbs. Subsequently, the SDFT and DDFT were dissected. Tendon samples were obtained of cross sections of the DDFT and SDFT (1 cm thick) at different levels (sp: proximal section; sm: middle section; sd: distal section) for histochemical study, according to the following (Fig. 2): DDFTsp: half of metacarpus III; DDFTsm: metacarpophalangeal joint, DDFTsd: level of DSB (distal interphalangeal joint); SDFTsp: half of metacarpus III and SDFTsm: metacarpophalangeal joint. Subsequently, the sections were photographed and fixed in 10% formalin in order to collagen study. Finally, a dissection was performed to observe and evaluate the DSB.

Table 1
Radiological grading in distal sesamoid bone.

Condition	No-pathology	Slight	Moderate	Marked	Severe
Cortical – trabecular bone interface	0	1	2	3	4
Medullary sclerosis	0	1	2	3	4
Radiolucent zones (cystic)	0	1	2	3	4
Alteration of the middle sagittal crest (ridge)	0	1	2	3	4
Elongation of flexor cortex	0	1	2	3	4

Table 2
Presentation of radiological findings in podotrochlear system (DSB: distal sesamoid bone).

	Presentation	
Fragments in the distal edge in DSB	Absent	Present
Thickening of the flexor cortex in DSB	Absent	Present
Thinning of the flexor cortex in DSB	Absent	Present
Osteophytes or enthesophytes in the proximal border in DSB	Absent	Present
Bone neoformation in the flexor face in DSB	Absent	Present
Dystrophic collateral sesamoid ligament mineralization	Absent	Present
Joint space	Absent	Present
Asymmetry in DSB	Absent	Present

2.4. Diagnosis of NS and groups

Diagnosis of NS was based on history, clinical examination *pre-mortem*, lameness examination, diagnostic analgesia and radiological findings observed in podotrochlear system which included the DSB, ligaments and articular cavity of the distal interphalangeal joint (Table 1). The *post-mortem* findings obtained from the DSB dissection and observation procedure were used to complement and confirm the diagnosis according criteria described by Komosa et al. (2014). The equines and their corresponding distal forelimbs without a history of lameness and features suggestive of NS were labeled as NA-group (Not Affected group). The equines whose forelimbs were collected had suggestive characteristics of NS such as lameness, low angulation of the metacarpophalangeal joint, long walls and concavity in the *solear face* of the hoof, and distal forelimbs without corrective management that were labeled with the pre-diagnosis of NS (Dyson et al., 2011). The criteria
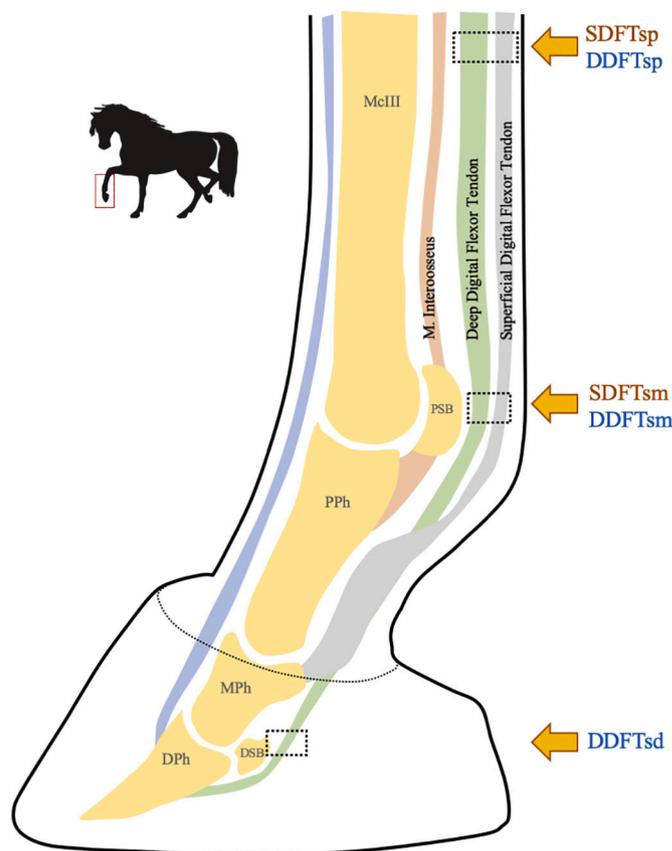
Fig. 2. Illustration of the site for obtaining tendon samples for histochemical studies. Superficial (SDFT) and deep (DDFT) digital flexor tendon. DDFT sp.: half of metacarpus III; DDFT sm: metacarpophalangeal joint, DDFT sd: tendon insertion in the distal phalanx; SDFT sp.: half of metacarpus III and SDFT sm: metacarpophalangeal joint.

used in radiological interpretation are described by Dyson (2008). Then, the groups were formed using Tables 1 and 2 according to: Not Affected (NA-group; $n = 30$) and Navicular Syndrome (NS-group; n = 30). Not Affected hands (NA-group) were considered, those that evidenced ≤2 findings with graduation ≤2 described in Table 1 and also evidenced ≤2 categorical data described in Table 2, and hands with NS (NS-group) those that evidenced ≥3 findings with graduation ≥3 described in Table 1 and also evidenced ≥3 categorical data described in Table 2.

2.5. Histological processing

Sections of SDFT and DDFT were obtained and fixed in 10% formaldehyde for 7 days at room temperature (22 °C). They were dehydrated in a series of alcohols, rinsed in xylol, and included in Paraplast (Paraplast Plus embedding medium, melting point: 54 °C, Sigma-Aldrich Chemical Co., St Louis, MO, USA). Serial cuts of 5 μm thickness were made. Subsequently, the sections were rehydrated, immersed in xylol, exposed to a descending battery of ethanol and finally to distilled water. For differential observation of aligned collagen vs non-aligned collagen, Red Picrosirius staining was performed. The collagen present in the ECM exhibits different interference colours and birefringence spectra, which is useful for a study of the proportion of aligned and non-aligned collagen (Lattouf et al., 2014). Two trained observers performed all measurements, each using his own microscope and computer software, unaware to the identity of the groups to avoid bias.

2.6. Quantification of collagen

Sections of SDFT and DDFT stained with Red Picrosirius were observed in a polarized light microscope (Leica® DM750 polarized light microscope, Leica Microsystems, Switzerland) equipped with polarized circular light consisting of a simple polarizer and an analyser filter. Non-aligned collagen (fibrillar-immature) was identified by green birefringence, while aligned collagen (dense-mature) was identified by yellow-red birefringence (Södersten et al., 2013). Digital images (40×) of tendon sections were obtained using digital camera (Leica® MC170HD digital camera, Leica Microsystems, Switzerland) associated with image capture software (Leica Aquire v3.2 software for Mac OS X, Leica Microsystems, Switzerland). Five random fields were evaluated. Two digital images of each field were made, one with normal light and the other with polarized light. Images were analyzed with the software ImageJ v1.49 (Fig. 1B). The collagen content was determined using the Colour-Based Segmentation function and expressed as the area fraction (%) of each image (Salinas et al., 2018; Schindelin et al., 2015).

2.7. Statistical analysis

Statistical analyses were performed with a commercial software package (GraphPad Prism version 8.00 for iOSX (GraphPad Software, La Jolla California USA). Descriptive statistics were generated for each variable. The data distributions of continuous variables were tested for normality by means of the D'Agostino Pearson Test. This study was inferential, the proportion of aligned and non-aligned collagen was reported as mean ± SD (%). To ensure that the groups are comparable in terms of age and weight, a *t*- Student test was performed. Also, the mean values of aligned and non-aligned collagen present in the same tendon levels were compared between distal forelimbs with and without a diagnosis of NS with the *t*-Student test. Statistical significance for all tests was established for $p < 0.05$ and 95% Confidence Interval.

3. Results

In the present study, male equines were used, ages 4–10 years (mean: 8.7 ± 2.4 years) and 350 ± 52.5 kg (range: 298–431 kg; condition score: 3–4 condition; Carroll and Huntington, 1988). NS-group mean ± SD body weight and age were 341 ± 47.2 kg and 9 ± 3.7 years respectively.

NA-group mean ± SD body weight and age were 341 ± 47.2 kg and 9 ± 3.7 years respectively. There were no differences between groups in age ($p > 0.8427$) or weight ($p = 0.5666$). The dissection of the tendons, there were difficulties due to the hardness of the tissue. We decided to dissect thin cross sections to facilitate penetration of the fixator into the tissue. Regarding the lameness observed in equines that were diagnosed with NS, these were generally mild to moderate and observed only in equines whose ages were in the range of 7–9 years. In general, in the pre-mortem clinical examination the young and senile equines did not present lameness. The most prevalent radiographic findings in the distal forelimbs of equines with NS were loss of cortico-medullary definition, associated with sclerosis and osteolysis of compact bone tissue on the flexor face and remodeling of the proximal and distal margins of the DSB. Gross changes seen only in NS equines included DDFT surface fibrillation, DDFT core lesions and adhesions between the DDFT and DSB. Also, partial and full thickness fibrocartilage loss and partial and full thickness palmar cortex erosion in DSB. During the dissection of DSB, gross anatomical findings in NS-group were observed such as: loss and thinning of fibrocartilage from the flexor face even with exposure of subchondral bone, central and distal adhesions of the DDFT, a depression into the subchondral bone. Furthermore, adhesions, periarticular osteophytes and distal border fragments were observed in lower prevalence among others. Most of these gross features were observed in the distal region of the finger, and they were absent in the proximal region.

In each distal forelimb, 5 images were analyzed, id est. three images in the DDFT and 2 images in the SDFT (Fig. 3.)). In total, 150 images were analyzed per group (2048 × 1536 pixels) per group. Normality test showed the data was normally distributed. Therefore, a parametric test was used for statistical analysis. Post mortem evidence suggests that their differences between the proportion of aligned collagen (NA-group: 21.2 ± 1.31; NS-group: 12.2 ± 4.67; $p = 0.0026$) and non-aligned collagen (NA-group: 21.8 ± 2.22; NS-group: 25.1 ± 1.73; $p = 0.0241$) collagen at the level of insertion of DDFT in the distal phalanx, but not in other levels (Fig. 2). Table 3 describes proportion of aligned and non-aligned collagen measured at different distal forelimb levels in equines with and without NS.

4. Discussion

This study provides further insight towards the changes in flexor tendons of distal forelimbs on a microscopic level that can be attributed to NS in equines. Different sections of DDFT and SDFT at different levels were studied in equines with and without NS. For this, a histochemical study was performed to observe evidence of structural and molecular variations, using collagen as a model. The result of this study supported the hypothesis that distal forelimbs diagnosed with NS have different amount of aligned and non-aligned collagen at the DDFT insertion level only.

This difference, moreover, is evident only in the distal region of the DDFT, which demonstrated the influence of mechanical factors on the modulation of ECM in pathological conditions. These observations indicate that the amount of collagen in the tendons correlate with the mechanical environment produced by NS, such as the intermittent compressive loads (Carter and Beaupre, 2001) present in the region of the podotrochlear system, at the level of the DSB, especially between the DDFT and the flexor face of the DSB. Compared to the NA group, the DDFTsd of equines diagnosed with NS demonstrated a loss of the normal alignment of collagen fibers. This suggests that the mechanical properties, including elastic modulus and maximum stress, decrease in response to NS.

Our results confirm what was described by authors, who affirm that NS is an exclusive condition of the podotrochlear system, and therefore it would not affect the gross anatomy of the flexor tendons, especially in proximal regions (Salinas et al., 2014a; Ferraro et al., 2003). It has been reported that the majority of macroscopic lesions attributed to NS occur in the distal segments of the thoracic member, affecting mainly the

NOT AFFECTED   NAVICULAR SYNDROME
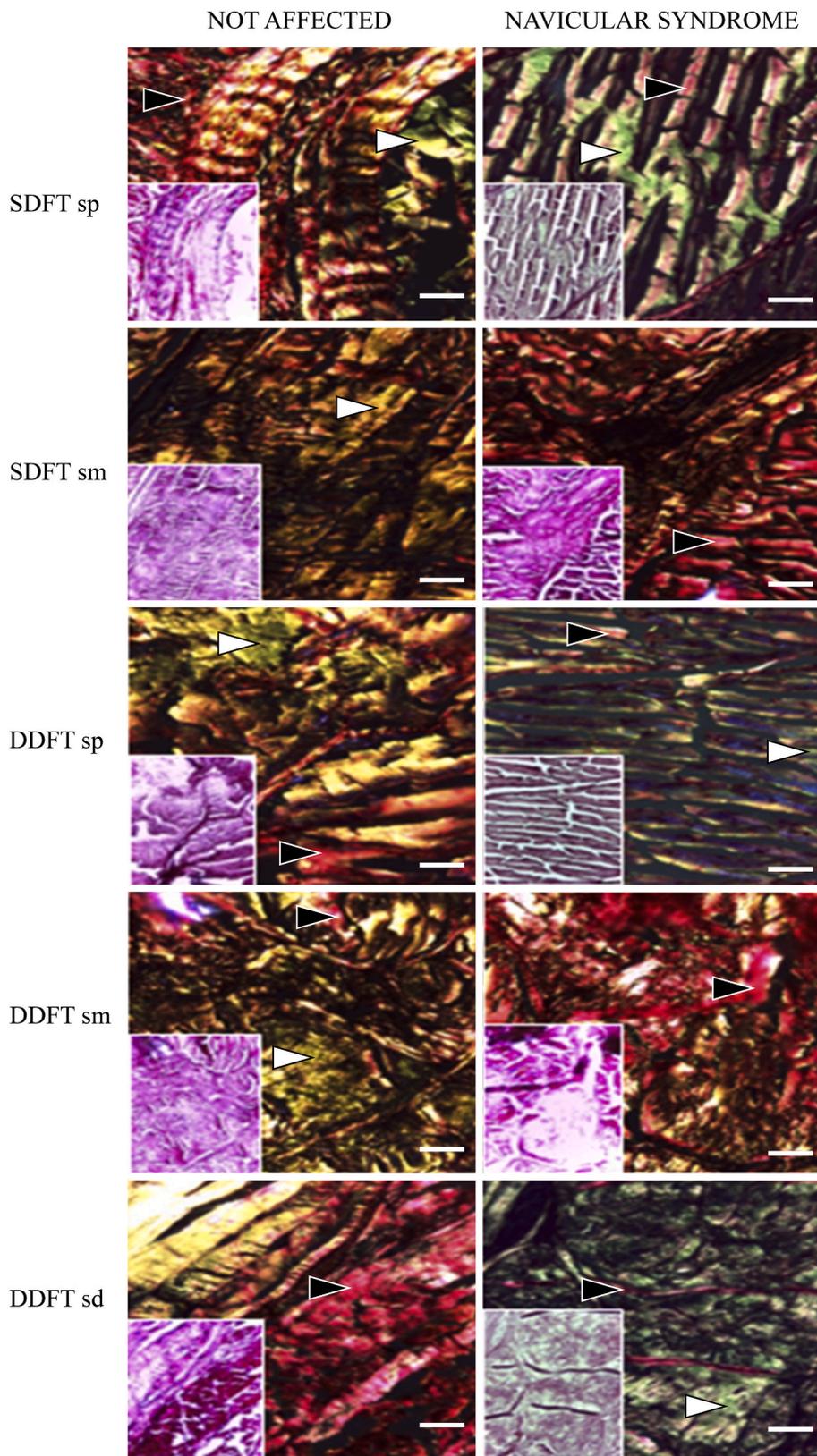
Fig. 3. Histological sections of the superficial (SDFT) and deep (DDFT) digital flexor tendon stained with Red Picrosirius, observed under the microscope equipped with circularly polarized light. Non-aligned collagen (type III): green birefringence (white arrow). Aligned collagen (type I): yellow-red birefringence (black arrow). The images in the inset correspond to tendon tissue without polarized light. DDFT sp.: half of metacarpus III; DDFT sm: metacarpophalangeal joint, DDFT sd: tendon insertion in the distal phalanx; SDFT sp.: half of metacarpus III and SDFT sm: metacarpophalangeal join (bar = 50 μm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

structures located in the distal interphalangeal joint (Salinas et al., 2014b; Komosa et al., 2014), the insertion site of the flexor tendons and the flexor face of the DSB (Dyson et al., 2011; Salinas et al., 2014b; Waguespack and Hanson, 2010). Our data show that there are gross morphological changes in DDFT of the distal forelimbs, which agree that

with was reported previously (Murray et al., 2006; Ely et al., 2004; Pinchbeck et al., 2004). These gross alterations observed in the clinical cases of NS, such as DDFT fibrillation, partial and full thickness fibrocartilage loss and partial and full thickness palmar cortex erosion in DSB, would have a marked effect in the limb conformation. Several reports

Table 3

Collagen organization. Data are reported as mean (%) ± SD (95% CI). Tendon fraction area and quantification of aligned and non-aligned collagen (%) in the tendon of the flexor muscles of the distal forelimb NA and NS; DDFT: deep digital flexor tendon; SDFT: superficial digital flexor tendon. sp.: half of metacarpus III; sm: metacarpophalangeal joint; sd: tendon insertion in the distal phalanx ($n = 60$).

		Mean % ± SD (CI)		
		NA-group n = 30	NS-group n = 30	*p*-Value
SDFT sp	Aligned collagen	13.2 ± 7.16 (6.85–20.01)	11.3 ± 8.42 (2.87–19.87)	0.9599
	Non-aligned collagen	29.7 ± 3.94 (25.91–33.07)	31.3 ± 23.98 (8.07–53.73)	0.0786
SDFT sm	Aligned collagen	16.6 ± 8.84 (8.83–24.34)	20.8 ± 9.18 (10.77–29.41)	0.3105
	Non-aligned collagen	25.2 ± 4.99 (20.79–29.13)	26.7 ± 3.93 (22.83–29.96)	0.3233
DDFT sp	Aligned collagen	8.7 ± 1.54 (7.76–9.03)	18.0 ± 15.46 (2.72–33.38)	0.2118
	Non-aligned collagen	23.6 ± 3.20 (20.84–26.17)	26.0 ± 8.70 (17.86–32.11)	0.4354
DDFT sm	Aligned collagen	23.7 ± 9.68 (13.88–31.81)	23.9 ± 8.24 (14.84–32.03)	0.9613
	Non-aligned collagen	26.6 ± 3.29 (23.58–29.41)	27.1 ± 4.49 (23.55–32.49)	0.9211
DDFT sd	Aligned collagen	21.2 ± 1.31 (19.79–23.91)	12.2 ± 4.67 (7.84–17.02)	0.0026[a]
	Non-aligned collagen	21.8 ± 2.22 (19.05–24.11)	25.1 ± 1.73 (23.47–27.31)	0.0241[a]

[a] Significant differences, $p < 0.05$.

indicate that tendon damage is one of the most frequent presentations observed in racing Thoroughbreds (Ely et al., 2004; Pinchbeck et al., 2004), reaching almost 90% prevalence in the SDFT (Salinas et al., 2014b). The effect that NS would have on the appearance of these damages and how the tendon tissue responds to stress would include changes in the proportion of collagens. It is well known that aligned collagen grants tensile strength and that non-aligned collagen is related to tissue remodeling processes, so that a domain of non-aligned over aligned collagen would result in a loss of resistance in the structures involved.

In general, there is a decrease and an increase in the proportion of aligned and non-aligned collagen were observed in DDFT, respectively. However, the DDFT shows an increase in aligned collagen at the level of the middle of the metacarpus III and a decrease in the distal area at the level of distal interphalangeal joint in NS compared to NA. This difference between groups would reflect a chronic underlying inflammatory process. It is known that DDFT as such can regulate the connective tissue remodeling as a response to mechanical loading, as well as diseases. Thus, it is striking that changes in the proportions of collagen were located close to the site of the lesion and not in more proximal regions, which we could consider as an adaptive tendon response (Dowling and Dart, 2005). On the other hand, there is the central role that matrix metalloproteinases (MMPs) play in the inflammation and adaptation of tendon tissues to mechanical loading, injury, and disease, especially when they affect to fibrillar collagen. These collagens make up a important fraction of the total mass tendon ECM, and they serve as a critical link between fibroblasts and their surrounding environment (Davis et al., 2013). A frequent observation in many musculoskeletal injuries is an upregulation of MMPs and disordered collagen expression. We hypothesize that an MMPs-mediated degradation of fibrillar and network collagens impairs the ability of fibroblasts to properly sense forces transmitted through the ECM, disrupts their potential to respond to mechanical loading, and leads to the failure of fibroblasts to repair sites of injury causing a chronic inflammatory lesion.

What is striking are the proportions observed in the aligned and non-aligned collagens in the DDFT in the NS group. In general, a decrease and an increase in the proportion of aligned and non-aligned collagen

were observed, respectively. This suggests a probable differential effect on the activity of MMPs. Apparently there is an activation of MMPs that degrade complete collagen molecules, especially fibrillar aligned collagens, such as MMP-1, -8 and -13, and at the same time an inhibition of gelatinases that they include MMP-2 and -9 which function to degrade non-aligned collagen (smaller fibrillar and network collagens and the pieces of fibrillar collagens) left over from collagenase activity (Bramono et al., 2004; Pasternak and Aspenberg, 2009). Targeted, temporal inactivation or overexpression of various MMPs using fibroblast-specific promoters would allow for the testing of this hypothesis. Gaining a greater understanding of MMP biology will help in the design and selection of specific MMP inhibitors that could substantially advance the NS treatment. Also, this differences in the proportion of aligned and non-aligned collagen present in DDFTsd, suggesting a possible effect of the NS on synthesis, expression, collagenase activity and/or distribution of the tendon tissue in the ECM. In this regard, different authors agree that the greatest amount of morphological effects induced by NS are observed at the level of the distal interphalangeal joint, due to the excessive tension that DDFT exerts on DSB, the proximity between DDFT and podotrochlear system, and the potential inflammation, our study coincides with these authors (Blunden et al., 2006; Komosa et al., 2014).

Finally, according to the results of the present study, it is possible hypothesized that the differences in collagen content observed in DDFTsd may reflect a differential role of MMPs in clinical cases of NS. This phenomenon is explained by Sharma and Maffulli (2006) who state that excessive tension in the tendon would increase the synthesis non-aligned collagen under the influence of MMPs that control collagen proteolysis. Likewise, it has been described that tendons of the flexor muscles of the distal forelimbs subjected to an increase in tension forces have shown signs of inflammation in the structures involved, an increase in the stimulation of tenocytes, an increase in MMPs as a response damage and consequently a decrease in aligned collagen (Spiesz et al., 2015). The reference article adds that the function of MMP in tendons exposed to severe damage is affected and therefore some type of deficiency in their regulation and production would occur. We believe that it is important to understand how DDFT specializes to withstand continuous application of high stresses and whether it responds differently to increases in tensile forces produced by NS.

5. Conclusions

We conclude that there are differences in the proportion of aligned and non-aligned collagen present in DDTF at the level of the distal interphalangeal joint in clinical cases of NS. This appears to be part of a process driven by excessive tension and compression forces described in the distal forelimbs diagnosed with SN. These results suggest that the differentiating effects of Navicular Syndrome are present in DDFT at the level of the distal interphalangeal joint, in aligned and non-aligned collagens. Considering that both collagens confer viscoelastic properties to the tendon, the SN would influence the biomechanical properties of tendon tissue, particularly in DDTF and, therefore, increases susceptibility to injury. This effect does not involve the proximal levels of the DDTF and SDTF. Further investigation should be performed to identify the biomechanical risks that could be involved in the development of this syndrome, determine which factors trigger the progression of NS, analyse the structures adjacent to the podotrochlear system and investigate the composition and structure of the interfascicular matrix of the muscle whose tendon is compromised.

Financial disclosure

Authors'contribution

P.S. contributed to conceptualization; P.S. and C·S contributed to methodology; P.S., D.L, A.B and C·S. contributed to formal analysis; P.S., D.L, A.B and C.S. contributed to writing original draft; P.S. and C.S contributed to writing review and editing; P.S. was responsible for supervision of study.

Declaration of Competing Interest

None.

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