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The deep fascia and retinacula of the equine forelimb – structure and innervation

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Abstract

Recent advances in human fascia research have shed new light on the role of the fascial network in movement perception and coordination, transmission of muscle force, and integrative function in body biomechanics. Evolutionary adaptations of equine musculoskeletal apparatus that assure effective terrestrial locomotion are employed in equestrianism, resulting in the wide variety of movements in performing horses, from sophisticated dressage to jumping and high-speed racing. The high importance of horse motion efficiency in the present-day equine industry indicates the significance of scientific knowledge of the structure and physiology of equine fasciae. In this study, we investigated the structure and innervation of the deep fascia of the equine forelimb by means of anatomical dissection, histology and immunohistochemistry. Macroscopically, the deep fascia appears as a dense, glossy and whitish lamina of connective tissue continuous with its fibrous reinforcements represented by extensor and flexor retinacula. According to the results of our histological examination, the general structure of the equine forelimb fascia corresponds to the characteristics of the human deep fasciae of the limbs. Although we did find specific features in all sample types, the general composition of all examined fascial tissues follows roughly the same scheme. It is composed of dense, closely packed collagen fibers organized in layers of thick fibrous bundles with sparse elastic fibers. This compact tissue is covered from both internal and external sides by loosely woven laminae of areolar connective tissue where elastic fibers are mixed with collagen. Numerous blood vessels running within the loose connective tissue contribute to the formation of regular vascular network throughout the compact layer of the deep fascia and retinacula. We found nerve fibers of different calibers in all samples analyzed. The fibers are numerous in the areolar connective tissue and near the blood vessels but scarce in the compact layers of collagen. We did not observe any Ruffini, Pacini or Golgi-Mazzoni corpuscles. In conclusion, the multilayered composition of compact bundles of collagen, sparse elastic fibers in the deep fascia and continuous transition into retinacula probably facilitate resistance to gravitational forces and volume changes during muscle contraction as well as transmission of muscle force during movement. However, further research focused on innervation is needed to clarify whether the deep fascia of the equine forelimb plays a role in proprioception and movement coordination.

Key words: equine; fascia; forelimb; innervation; retinaculum; structure.

Introduction

In recent years, research activity focusing on the structure, function, biomechanics as well as clinical pathologies of fasciae has increased greatly. Growing interest in this field has led to the introduction of a reconsidered nomenclature, which facilitates interdisciplinary communication between researchers and professionals of different areas of specialization. This terminology was proposed and developed at the Fascia Research Congresses (FRC) and subsequently published (Langevin & Huijing, 2009; Schleip et al. 2012). According to this comprehensive terminology, the term fascia defines the soft tissue component of the connective tissue system that permeates the human body. In other words, fasciae are fibrous collagenous tissues that are part of a body-wide tensional force transmission system (Schleip

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et al. 2012). In this sense, the fascial net includes not only dense plain tissue sheets such as muscle envelopes or retinacula, but also softer collagenous connective tissue such as superficial fascia. Consequently, fascial tissues are recognized as an interconnected tensional network adapting fiber arrangement and density to the local tensional demands (Schleip et al. 2012). Specifying, the term 'superficial fascia' currently describes the enveloping layer directly beneath the skin, which might contain areolar as well as dense connective tissue. The 'deep fascia', on the other hand, defines a continuous sheet of mostly dense, irregularly arranged connective tissue that limits the changes in shape of underlying tissues (Langevin & Huijing, 2009). The morphology and function of fasciae have been recently reviewed (Benjamin, 2009; Stecco et al. 2011). It was hypothesized that fasciae contribute to the mechanosensitive signaling system (Langevin, 2006), influence the biomechanics of the limbs, and facilitate the functional connection between the trunk and the limbs (Vleeming et al. 1995; Fairclough et al. 2006). It has become evident that the fascial network is involved in movement perception and coordination (Stecco et al. 2010b), as well as transmission of muscle force (Maas et al. 2005; Meijer et al. 2006; Yucesoy et al. 2006; Rijkelijkhuizen et al. 2007). Furthermore, deep fasciae of different topographical regions present diverse structures that seem to reflect specific functions of the lower and upper limbs and their connection to the trunk in the human subjects (Stecco et al. 2008, 2009). It was also concluded that the histological features of the ankle retinacula negate their classical role of pulleys, and should be considered morphological evidence of the integrative role of fascia and its derivatives (Stecco et al. 2010b).

Interest in fasciae is also growing in the field of veterinary medicine, but there is still a lack of detailed and precise anatomical and histological description of particular fasciae and fascial structures in many animals. In a comparative context, a broad analysis of the fascial architecture and composition in different species might provide valuable data for comparison and understanding of the functional meaning of fascial tissue structure according to different types and specializations of limbs in particular vertebrates. Additionally, from a veterinary point of view, knowledge of the structure and physiology of the deep fasciae of limbs and trunk seems to be especially important in horses. Due to different evolutionary strategies of terrestrial habitation, horses developed as cursorial quadrupeds, being primarily a prey species with gastrointestinal tract adapted to continual grazing. Survival mechanisms of these precocial animals exhibiting fight-or-flight response to external stimuli include the ability to run fast at short distances to flee predators, and to cover long distances at slower speeds for food migration (Wilson & Weller, 2011). The anatomical features that provide the energy-efficient and fast locomotion comprise a reduction of a distal limb mass, i.e. a concentration of locomotor muscles in proximal limb regions, a reduction of the number of digits, as well as an increase in limb length by an elongation of an autopodium and lateral position of the scapula on the thorax (Payne et al. 2005a,b; Biancardi & Minetti, 2012). Locomotor effectiveness of horses also depends on the release of kinetic energy stored in tendinous and fascial tissues during eccentric contraction of muscles, which is then reused and added to the energy produced in the concentric phase (Rivero, 2009). Elasticity of the fascial tissues reduces the engagement of muscle fibers in movement (Heiduk, 2015) that might be implicated in training programs. Due to the nature and specificity of the equine industry as well as the role of horses as companion animals, their musculoskeletal apparatus is under the scope of many investigations. Physiotherapy has recently arisen as a complementary rehabilitation tool to the traditional, strictly evidence-based orthopedic treatment in equine medicine (Paulekas & Haussler, 2009), with manual therapy receiving growing attention from practitioners and animal owners. Techniques such as stretching, massage, trigger point therapy, myofascial release or kinesio taping are supposed to address the impairments in the neuromusculoskeletal and myofascial system (Goff, 2009; Haussler, 2009, 2010; Scott, 2009; Molle, 2016). Although in humans the effectiveness of such therapies was reported, the evidence of their therapeutic potency in horses is mostly anecdotal (Haussler, 2009). Introduction of the veterinary module to the FRC in 2015 as well as translating bipedal human myofascial kinetic lines into quadruped horses (Elbrønd & Schultz, 2015) indicate new research directions to investigate equine fasciae. Fundamental anatomical studies would be essential for the understanding of the roles and functions of fascial structures in musculoskeletal disorders in horses, which could be further translated into the therapy and rehabilitation of specific conditions.

The aim of this study was to investigate the histological characteristics of the deep fascia and its derivatives in the equine forelimb, concerning the antebrachial fascia, the carpal flexor retinaculum, the flexor retinaculum branches in the metacarpal region and the carpal extensor retinaculum. Additionally, we sought to investigate histological differences between structures commonly referred to as retinacula and the antebrachial fascia.

Materials and methods

Postmortem dissection of seven equine forelimbs was conducted within 24 h after the horses were euthanized or slaughtered for reasons unrelated to forelimb pathologies. Animals were of different ages, breeds and sexes (Table 1). Only the skin was removed in order to display the superficial and deep fasciae, before 49 samples were obtained *en bloc* for histological and immunohistochemical (IHC) analysis. Seven samples were collected from each forelimb representing the following tissues: the antebrachial fascia, the carpal extensor retinaculum, the carpal flexor retinaculum and the metacarpal flexor retinaculum (Fig. 1). All samples were immediately fixed in 4% buffered formalin. After sample collection the

Table 1 Description of the horses included in this study.

Horse No.	Age	Sex	Breed	Weight (kg)	Cause of death
1	6 years	Gelding	Lusitano	540	Neoplastic tumor in the thorax
2	4 years	Mare	Quarter horse	422	Renal insufficiency
3	17 years	Gelding	Hungarian Warmblood	633	Neoplastic tumor in the reproductive tract
4	6 years	Mare	Warmblood mix	608	Laryngospasm in the post-surgery period
5	7 months	Stallion	Noriker	300	Slaughter
6	14 years	Mare	Haflinger	400	Slaughter
7	2 years	Stallion	Lusitano	450	Septic tendonitis in the hindlimb

specimens were further dissected and the superficial fascia was removed to facilitate the anatomical description of the deep fascia. The antebrachial fascia was sampled with the underlying muscle at the medial side of the proximal half of the antebrachium, caudal to the cephalic vein. The carpal extensor retinaculum was sampled above the extensor carpi radialis tendon at the level of the carpus. The carpal flexor retinaculum was sampled together with the radial artery and vein to the depth until the flexor carpi radialis tendon in the carpal canal was exposed. Metacarpal flexor retinaculum was collected in four locations, at the lateral and medial sides of the palmar metacarpal region. Proximal samples were collected at the level directly distal to the base of the II and IV metacarpal bones. Distal samples were collected at the level of the mid metacarpal region, where the branches of the metacarpal flexor retinaculum become thinner and gradually end at the shafts of the splint bones. Samples were post-fixed for 24–72 h in 4% buffered formalin and embedded in paraffin. The embedding process was extended to 20 h to assure adequate penetration of the paraffin into fibrous and dense tissue samples. Sections 4 μm thick were cut and stained with hematoxylin and eosin, Weigert's resorcin-fuchsin for elastic fibers, and Heidenhain's AZAN trichrome stain for connective tissue. For immunohistochemical staining of general nervous tissue markers the following primary antibodies were used: anti-S-100 protein (DAKO, 1 : 5000) and anti-protein gene product 9.5 (Thermo Scientific, 1 : 3000). Sections were deparaffinized, rehydrated and peroxidase activity was removed with 0.6% H_2O_2 in methanol. Subsequently, sections were washed in tap water, mounted in phosphate-buffered saline (PBS; pH 7.4) and treated with normal 1.5% goat serum for 30 min at room temperature. Then sections were incubated overnight with the primary antibody at 4 °C, washed in PBS and incubated with secondary antibody (BrightVision Poly-HRP-anti-rabbit, ImmunoLogic, Duiven, The Netherlands) for 30 min at room temperature. Next, sections were washed with PBS and peroxidase activity was detected with diaminobenzidine (DAB) for 10 min at room temperature. Finally, sections were rinsed, counterstained with hematoxylin, dehydrated and mounted with DPX (Fluka, Buchs, Switzerland). For anti-PGP 9.5 staining, pretreatment with 0.01 M citrate buffer for 2 h in a water bath at 65 °C was required. Negative controls were obtained by omitting the primary antibody. Sections of the equine nerve fibers and blood vessels from the region of the fetlock joint *(a.v.n. digitalis palmaris)* served as positive controls. Sections were examined in terms of general structure and stratification. Collagen fiber arrangement and direction were evaluated as well as the presence and distribution of elastic fibers. The presence of the muscle fibers was investigated in all samples from all sites of collection. Blood vessels and nerve fibers were described according to their presence, caliber and distribution within the tissues evaluated.

Results

Anatomical dissection

The deep fascia of the equine forelimb appeared as a dense, glossy and whitish lamina of connective tissue, easily separable from the underlying muscles. Underneath the antebrachial fascia the muscles were surrounded by their epimysium, which was clearly thinner and had a more delicate structure than the deep fascia. On the medial side of the antebrachium, caudal to the cephalic vein, the macroscopically visible fibers of the antebrachial fascia coursed towards the vein, in a craniodistal direction. Cranial to the vein a long band of fibers was present, running along the long axis of the limb in the distal direction, from the distal third of the antebrachium to the base of the second metacarpal bone. The cephalic vein and the medial cutaneous antebrachial nerve were bundled up with the areolar connective tissue and coursed through the medial surface of the antebrachial fascia. At the level of the carpal canal, on the caudomedial side, the antebrachial fascia was continuous with the carpal flexor retinaculum, and there was no visible anatomical separation between those two structures. Superficial to the radial artery and vein, macroscopically visible crisscrossing fibers were identified. On the lateral side these fibers originated from the accessory carpal bone, and on the medial side from the distal end of the radius, the radial and the second carpal bones. The carpal flexor retinaculum extended further distally on the proximal metacarpal region and continued as the metacarpal flexor retinaculum, which appeared as a reinforcement of the deep fascia. The metacarpal flexor retinaculum extended between the II and IV metacarpal bones in an upside-down V-shape manner. The lateral and medial branches of the metacarpal flexor retinaculum inserted along the shafts of the splint bones, surrounding the flexor tendons from both sides. Coursing distally, the branches became thinner and finally ended at the level of the communicating branch between medial and lateral palmar nerves. Between branches of the metacarpal flexor retinaculum, the deep fascia was very thin, without visible fibers, and continued in the direction of the digit. The branches of the metacarpal flexor retinaculum were much thicker than the

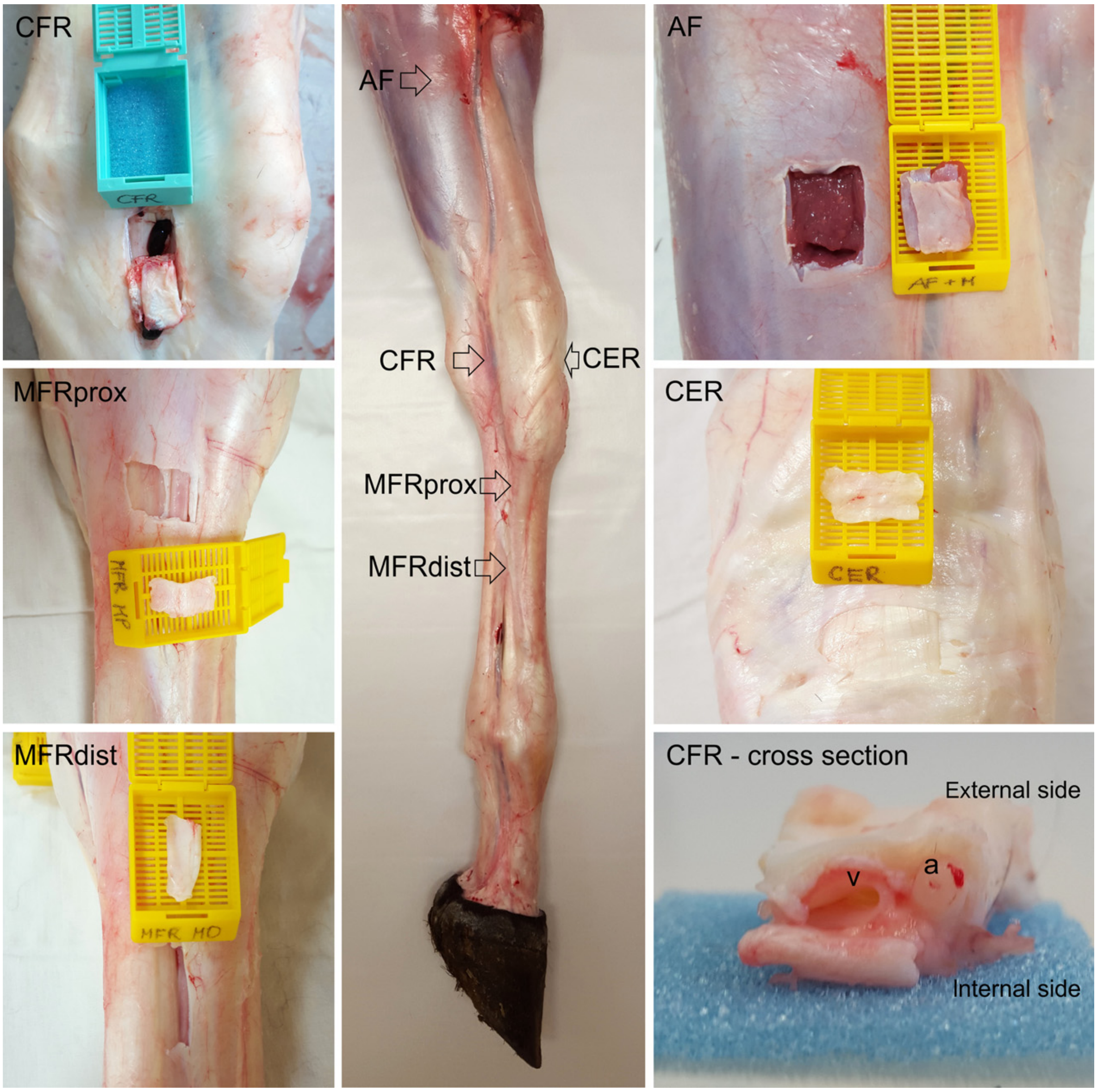

Fig. 1 Sites of sample collection for the fascial tissues evaluated in this study. The photograph in the middle presents the equine forelimb separated from the cadaver at the level of the elbow joint after skin removal, medial view. CFR, the carpal flexor retinaculum, MFRprox, the metacarpal flexor retinaculum at the proximal site of collection, MFRdist, the metacarpal flexor retinaculum at the distal site of collection, AF, the antebrachial fascia with underlying musculature, CER, the carpal extensor retinaculum. The picture in the right lower corner shows the transverse cross section of the sample representing the carpal flexor retinaculum. v, radial vein, a, radial artery. The samples of the lateral branch of the metacarpal flexor retinaculum were collected correspondingly to the presented medial branch.

continuation of the deep fascia between them. Layers of fibers were visible to the naked eye. In each layer, the fibers ran parallel to each other but they showed different orientation in the adjacent layers. At the level of the bases of the splint bones the fibers of the lateral and medial sides crossed each other in the sagittal plane of the limb. The lateral branch of the metacarpal flexor retinaculum was visibly thicker than the medial. Additionally, a fibrous band originating from the distal end of the accessory carpal bone contributed to the lateral branch. At the level of the carpal canal and the proximal metacarpal region in both carpal and metacarpal flexor retinacula, there were visible foramina for the perforating vessels and nerves. In two horses, of Haflinger and Noriker breeds, a thin muscle band was present under the lateral branch of the metacarpal flexor retinaculum, between the IV metacarpal bone and the III interosseous muscle. This muscle band was recognized as a remnant of the IV interosseous muscle. At the cranial side of the limb, the carpal extensor retinaculum superficial to the extensor carpi radialis tendon appeared as a broad band of fibers of transverse orientation to the long axis of the limb.

Microscopic analysis

General composition of equine fascia

In all samples analyzed, the deep fascia or retinacula were represented by a continuous sheet of mostly dense, closely packed collagen fibers. This dense connective tissue was covered from the external and internal sides by the areolar connective tissue (Fig. 2). On the external side it represents the superficial fascia, and on the internal side it corresponds to the connective tissue filling the space between the deep fascia and the adjacent structures underneath it. Both layers showed similar features independent of the site of sample collection, and there were only minor differences observed in particular sample types. They appeared as loosely woven laminae of variable thickness, composed of undulated collagen fibers, arranged in an irregular, loose, wavy manner. Within these layers, collagen fibers were oriented without any specific direction and created a reticular net of areolar connective tissue sometimes with a tortuous organization (Fig. 2). An exception to this characteristic was the internal layer underneath the antebrachial fascia, visible between the fascia and the epimysium (Fig. 3); this was organized in a very regular, multilayered way. Collagen fibers were also oriented irregularly, but thicker bundles were perpendicular to the epimysium. At regular distances this layer connected with the muscle through the gaps in the epimysium. At those points of connection, blood vessels coursed between the muscle and the antebrachial fascia (Fig. 3B). The other exception was the internal layer visible in samples representing the carpal flexor retinaculum. Due to the specific site of collection, the internal layer corresponded to the synovial membrane of the flexor carpi radialis tendon sheath. In samples of the metacarpal flexor retinaculum taken from lateral and medial branches at the distal point of collection, internal and external layers joined each other at the axial border of the dense middle layer. These areolar connective tissue layers were richly vascular, and capillaries, artrioles, venules as well as arteries and veins could be identified. Vessels coursed between the collagen in the direction of the fibers, often in a tortuous and wavy fashion (Fig. 2). These blood vessels provided branches to the middle, compact layer and contributed to the vascular network throughout the fascial tissue. With Weigert's resorcin fuchsin stain, elastic fibers were detected in the areolar connective tissue in large amounts, distributed throughout the whole thickness of the tissue. They could be identified around the blood vessels, followed the vascular network into the middle layer and were also distributed between and along the collagen fibers (Fig. 3C,D).

In the dense middle layer the collagen fibers were closely packed and arranged in thick fibrous bundles. Between them, artrioles, venules and capillaries created a regular network continuous with the vascular branches penetrating from the external and internal areolar tissues.

Nerve fibers, revealed by staining for general markers of nervous tissue, were best evidenced by anti-S-100 but were also positive for PGP 9.5 (Fig. 4). Nerve fibers were identified in all specimens analyzed, they were present in large numbers in the areolar connective tissue and were numerous close to the blood vessels (Fig. 4). They were also present in the dense components of fascial tissues but in a smaller number and only between collagen fiber bundles following the vascular network throughout the tissue; however, they were not present between individual collagen fibers. The nerve fibers were of diverse caliber, from bundles of nerve fibers between radial artery and vein or at the external side of the metacarpal flexor retinaculum to single axons (Fig. 4). We did not observe any Ruffini, Pacini or Golgi-Mazzoni corpuscles.

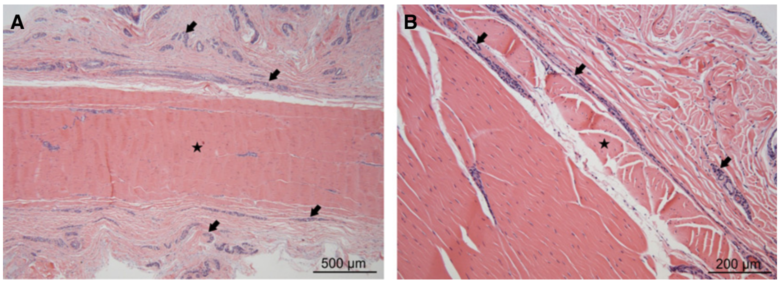

Fig. 2 The metacarpal flexor retinaculum. (A) Middle, compact layer of densely packed collagen fibers (asterisk) is surrounded internally and externally by the areolar connective tissue. This reticulate net of collagen fibers is rich in numerous blood vessels (arrows) sometimes presenting a wavy and tortuous course. (B) In the main, dense fibrous component of the retinaculum the collagen fibers show an almost homogeneous orientation, although in the adjacent thinner layers (asterisk) the fibers might be oriented at some angle to the main layer. Multiple blood vessels of different caliber penetrate through the tissue (arrows). (A,B) H&E staining.

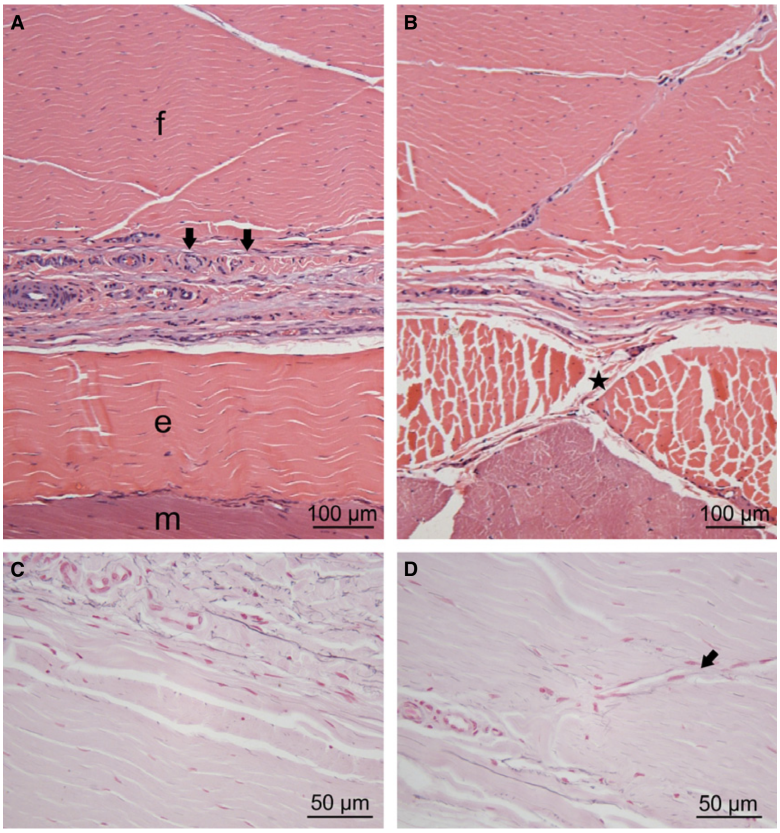

Fig. 3 The antebrachial fascia. (A) The areolar connective tissue (arrows) separates the dense collagen bundles of the antebrachial fascia (f) from the epimysium (e). (B) This loose connective tissue contacts with the skeletal muscle (m) through the epimysial gaps (asterisk), allowing the penetration of the vascular branches. (C) Numerous elastic fibers are present in the areolar connective tissue (right, upper corner), in contrast to a few visible at the periphery of the dense layer of collagen fibers. (D) Elastic fibers follow the vascular network into the fibrous tissue of the fascia (arrow). (A,B) H&E staining. (C,D) Weigert's resorcin fuchsin staining.

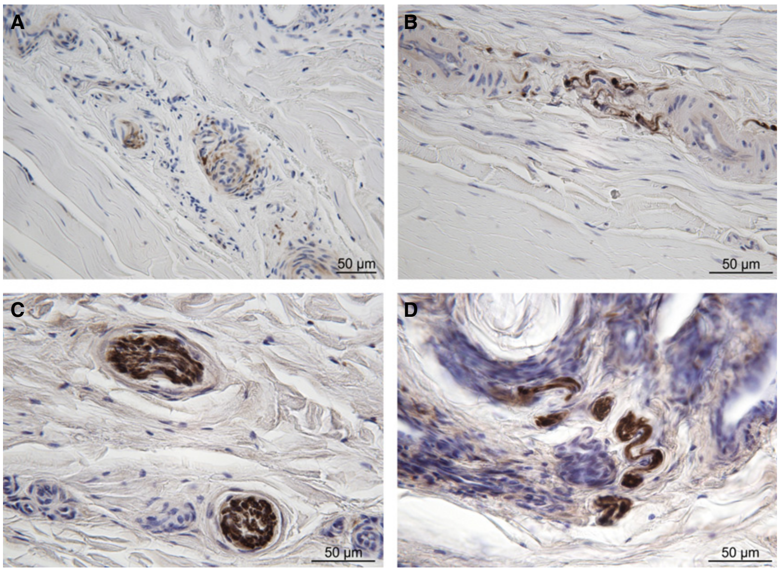

Fig. 4 Nerve fibers positive for general nervous tissue markers. (A) PGP 9,5 – nerves accompanying blood vessels in the areolar connective tissue between compact bundles of collagen in the metacarpal flexor retinaculum. (B) S-100 – nerve fibers in the loose connective tissue between the antebrachial fascia and the epimysium. (C,D) S-100 – nerve fibers of different caliber present in the areolar connective tissue of the metacarpal flexor retinaculum.

Specific characteristics of different fascial tissues

The antebrachial fascia is a homogeneous tissue of constant thickness (Fig. 3). It is composed of compact layers of collagen bundles and, according to orientation, it could be divided into a main middle layer and thin internal and external layers. In the main, thickest, layer the collagen fibers were orientated in an oblique manner to the long axis of the limb in a craniodistal direction. In the thin external and internal layers, fibers were oriented at at angle to the adjacent middle layer. Elastic fibers could be identified mostly at the peripheries but they were also visible in the middle layer following the blood vessels (Fig. 3C,D).

Compact bundles of collagen fibers in the carpal extensor retinaculum were almost transverse to the long axis of the limb and, in contrast to the antebrachial fascia, they were separated by thin sheaths of loose connective tissue (Fig. 5A). A few elastic fibers accompanied blood vessels and were also identified between collagen fibers (Fig. 5B). However, they were present in higher numbers at the peripheries of the retinaculum.

In both medial and lateral branches of the metacarpal flexor retinaculum, collagen bundles became thinner and gradually ended distally (Fig. 6A). The layer of bundles was also thicker abaxially, next to the attachment along the splint bones, than axially. At the axial periphery of both metacarpal retinaculum branches, the collagen bundles formed a smooth, oval end where the external and internal areolar connective tissue layers joined (Fig. 7A). The main, thickest component of the metacarpal flexor retinaculum was composed of collagen bundles with fibers oriented in an almost longitudinal direction to the long axis of the limb. As these fibers coursed in the direction of the splint bones to their attachment side, the orientation was more oblique, i.e. craniodistal. Thin layers of collagen bundles

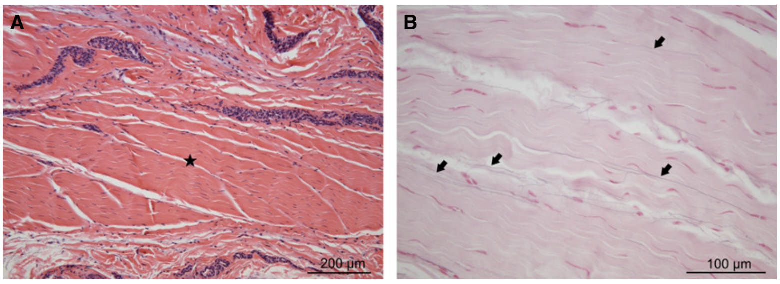

Fig. 5 The carpal extensor retinaculum. (A) In the dense, middle layer (asterisk), compact bundles of collagen fibers are separated by thin sheaths of the areolar connective tissue. The non-dense fibrous tissue is visible externally and internally. (B) Elastic fibers are visible between the collagen fibers in the dense fibrous component of the retinaculum (arrows). (A) H&E staining. (B) Weigert's resorcin fuchsin staining.

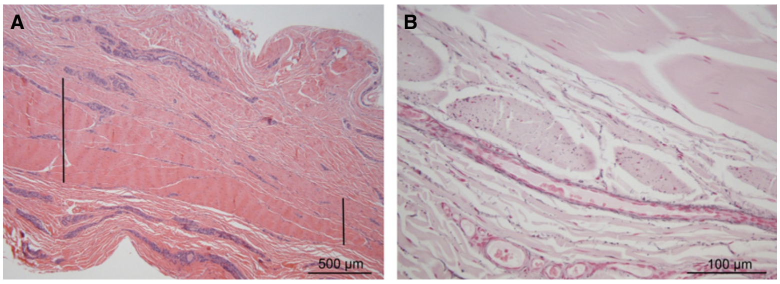

Fig. 6 The metacarpal flexor retinaculum. (A) In longitudinal sections the decrease in the retinaculum thickness that occurs in the distal direction is clearly visible (markers). (B) Elastic fibers are distributed in the areolar connective tissue and inbetween the collagen bundles at the periphery of the dense tissue. (A) H&E staining. (B) Weigert's resorcin fuchsin staining.

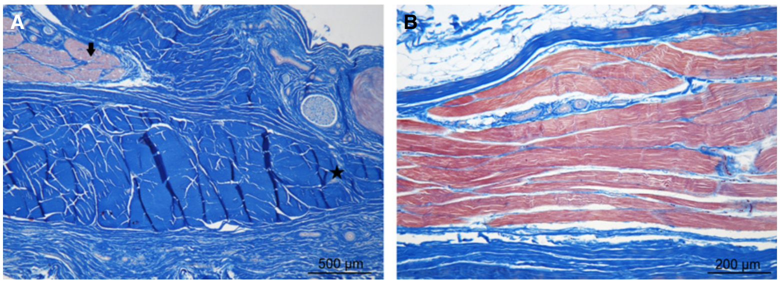

Fig. 7 The lateral branch of the metacarpal flexor retinaculum with skeletal muscle fibers. (A) Transverse section – axially the main compact component of the retinaculum ends smoothly (asterisk) surrounded by joint external and internal layers of areolar connective tissue. Muscular tissue is visible at the internal side of the retinaculum (arrow). (B) Longitudinal cut through the skeletal muscle reveals the additional layer of collagen fibers located on the internal side. (A,B) Heidenhain's Azan trichrome staining.

with more transverse oriented fibers were present internally as well as externally. Sometimes these fibers were more oblique or longitudinal, but always at some angle to the fibers of the main component (Fig. 2B). Thin bundles of fibers with different orientations could also be observed between collagen bundles of the main layer. In two horses, skeletal muscle fibers were present underneath the lateral branch of the metacarpal flexor retinaculum (Fig. 7). These fibers were adjacent to the internal side of the retinaculum, surrounded internally by the additional layer of collagen bundles (Fig. 7). Between the muscular fibers, areolar connective tissue with blood vessels and nerves was present. Few elastic fibers were detected accompanying the vascular network and in the space between collagen bundles of the metacarpal flexor retinaculum. Only single elastic fibers were observed between collagen fibers in the middle, main component of the metacarpal flexor retinaculum. Few were visible at the peripheries, between the collagen fibers of the thin external and internal layer (Fig. 6B).

The analyzed region of the carpal flexor retinaculum comprised two layers of dense, compact collagen bundles separated by loose connective tissue surrounding the radial artery and vein in a figure-of-eight shape. In the outermost compact layer the collagen fibers were organized in bundles oriented in transverse, longitudinal as well as oblique direction. In the deep layer, adjacent to the synovial membrane of the flexor carpi radialis tendon sheath, the collagen bundles were interwoven in a crisscross manner with two layers of bundles coursing from the medial and lateral sides (Fig. 8A). The collagen fibers around the radial artery and vein were loosely organized, coursing in different directions, and also formed concentric layers around the blood vessels. Between the artery and the vein we consistently found bundles of nerve fibers. The areolar connective tissue was richly vascularized with blood vessels of different calibers coursing in different directions. Elastic fibers were present in high numbers in the vascular walls and in the figure-of-eight-shaped areolar connective tissue component, except the epineural connective tissue. Less elastic fibers were observed in both compact layers, visible only around vessels and between collagen bundles (Fig. 8B).

With the exception of the muscular tissue underneath the lateral branch of the metacarpal flexor retinaculum, we did not find any differences in anatomical or histological structures between horses of different breeds, ages or sexes.

Discussion

Interest in the anatomical and histological structures of the deep fasciae has increased recently as researchers try to fully understand the roles of these structures in movement coordination and the pathogenesis of several orthopedic pain syndromes. Two different structural types of deep fasciae have been described in humans in a study aiming at clarifying macroscopic and microscopic differences between the pectoral and femoral fascia, which are morphologically and functionally different (Stecco et al. 2009). According to our results the general structure of the equine antebrachial fascia corresponds to the type described for the human deep fasciae of the free parts of the thoracic (upper) and pelvic (lower) limbs (Stecco et al. 2008, 2009). The equine antebrachial fascia – similar to the deep fascia of the human thigh – is a thick, whitish layer of connective tissue, separable from the underlying muscles due to the presence of the epimysium, which permits the muscles to slide independently from the fascia (Stecco et al. 2009). Our study also revealed the presence of a thin layer of areolar connective tissue between the epimysium and the antebrachial fascia, which further facilitates the sliding between the fascia and muscular plane (Stecco et al. 2009). The presence of a few layers of parallel collagen fiber bundles showing different orientations in adjacent layers corresponds with the structure of the human fasciae of the upper and lower limbs (Stecco et al. 2008). We have also identified the external and internal layers of areolar tissue rich in elastic fibers (Stecco et al. 2008). The main difference reported between the fasciae of the upper and lower limbs in humans is the presence and distribution of elastic fibers. In the deep

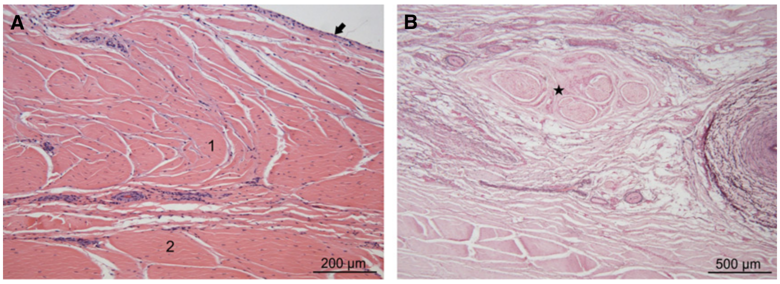

Fig. 8 The carpal flexor retinaculum. (A) In the dense fibrous tissue adjacent to the synovial membrane of the flexor carpi radialis tendon sheath (arrow) two layers of compact collagen bundles form a crisscross network (1, 2). (B) In the areolar connective tissue located between the radial artery and vein numerous elastic fibers are present, except in the epineural connective tissue of the nerve trunk (asterisk). (A) H&E staining. (B) Weigert's resorcin fuchsin staining.

fasciae of the thoracic limb, many elastic fibers are reported, mixed with the collagen fiber bundles forming an irregular mesh, whereas in the pelvic limb they are present only in the areolar connective tissue between the different fibrous layers and at the periphery of the fasciae (Stecco et al. 2008). Our samples, however, collected from the thoracic limbs, are more similar in structure to the fasciae of the human lower, rather than upper, limb. This might be explained by the hypothesis that the difference in quantity of elastic fibers between upper and lower limbs in humans is due to their different function (Stecco et al. 2008). The thoracic limbs in humans are not engaged in habitual locomotion aimed at moving the body forward and in keeping the trunk in an upright position – this task is realized by the pelvic limbs. These are engaged in postural control, their muscles act against gravitational forces, similar to the thoracic and pelvic limbs in horses; in principle, they facilitate simple movements of locomotion. The elastic fibers are thought to ease the precise and fine movements of the human upper limbs (Stecco et al. 2008), which are not necessary in the equine thoracic limb. Those diversities arise from the obvious differences between obligate bipedal humans and quadruped horses, which are evolutionarily adapted to strenuous high-speed runs under the great load of their body mass, employing all four limbs. Therefore, in the authors' opinion, our results support the hypothesis stated by Stecco et al. (2008) that the dense, stronger layers of the deep fasciae might be implicated in tension transmission generated by muscles and participate in peripheral motor coordination. These fascial properties might be used in equine training programs, analogously to the way they are employed by human athletes (Rivero, 2009). Plyometric training for humans is composed of exercises that include stretching–shortening cycles (SSCs), i.e. rapid, powerful eccentric contraction of muscles followed by a stretch reflex and explosive release of kinetic energy stored in myofascial tissues (like an elastic recoil in tendons), which is added to that produced in isometric and concentric contraction of muscles. SSCs have been shown to provide more force than concentric contractions alone (Heiduk, 2015). The great locomotor capacity of horses depends in general on elastic-strain energy (from stretching muscle-tendon complexes and myofascial tissues), which contributes to the force required to elevate the center of mass during movement (Alexander & Bennet-Clark, 1977; Alexander, 2002; Rivero, 2009). Most equine natural gaits consist of SSC movements (Bobbert & Santamaría, 2005; Butcher et al. 2009), and most equine training methods use natural gaits at different intensities and durations. It is assumed that such approaches represent a natural stimulus to plyometric training in horses (Rivero, 2009). Furthermore, Stecco et al. (2008) hypothesize that the multilayer structure of the deep fasciae of the limbs probably ensures resistance to pressure without consequent damage and adaptation to volume changes during muscle contraction and movement (Stecco et al. 2008). Additionally, the regular architecture of collagen fibers crisscrossing in adjacent layers allows the mechanism of elastic recoil due to superior capabilities to transform and store energy (Klinger & Schleip, 2015). This leads to another important aspect, i.e. immobilization during convalescence from musculoskeletal injuries and its consequences for fascial tissues. It was shown by Järvinen et al. (2002) in an experimental model that immobilization causes loss of the regular collagen fiber arrangement and an increase in the amount of the endomysial and perimysial connective tissue with multidirectional, irregular mesh-like fiber arrangement, leading to the loss of elastic properties. It results in lower capabilities to transform and store elastic energy, and impedes movement quality and effectiveness; this should be taken into consideration when designing rehabilitation protocols for horses.

In the light of our results based on anatomical dissection of equine thoracic limbs, structures commonly referred to as retinacula, i.e. carpal extensor retinaculum, carpal and metacarpal flexor, are continuous with the antebrachial fascia and not separable or independent. They appear as the reinforcements of the fascia presenting local specialization according to their function and forces acting in the specific regions of the limb. For example, the transverse alignment of the collagen fibers in the carpal extensor retinaculum secures the extensor carpi radialis tendon from displacement and probably prevents the retinaculum fibers from tearing during flexion of the carpal joint. Similarly, the flexor retinaculum at the level of the carpal canal and proximal metacarpal region assure correct positioning of the digital flexor tendons during movement and muscle contraction. This seems to be especially important in the context of the extreme hyperextension of the fetlock joint during loading, which can be observed in a high-speed gallop or when the thoracic limb contacts the ground after a jump. Consequently, the thick layers of collagen fiber bundles in the metacarpal flexor retinaculum are oriented in the direction of the acting force. The high resistance of this fascial structure to the tensile forces during the high-speed run is evidenced by the occurrence of splint bone fractures caused by increased tension from the fascial attachments during racing (Bertone, 2011). Our observations that the lateral aspect of the metacarpal flexor retinaculum is clearly thicker than the medial are in agreement with results presented in a study of the proximal metacarpal region of the horse using magnetic resonance anatomy (Nagy & Dyson, 2009). At the level of the carpal canal we performed histological examination of the superficial compartments of the flexor retinaculum containing the radial artery and vein. In the compact layers of collagen bundles, the orientation of fibers is multidirectional and individual layers seem to crisscross each other. This is probably an adaptation of the retinaculum structure to forces acting in different directions at the mediopalmar side of the carpus during locomotion and specific movements as well as to the flexion of the carpal joint. Additionally, this kind of fiber organization might ease blood flow in the radial vein. According to Probst et al. (2008) the term ‘carpal flexor retinaculum’ should be replaced by ‘palmar anular carpal ligament’ as the structure covers not only flexor tendons but also blood vessels and nerves. It is important to mention that semantics in the field of collagenous fibrous tissues is something of a vogue area, as the fascia research is multidisciplinary and occupies researchers from different professional backgrounds. Classically, the skeletal ligaments are defined as dense bands of collagenous tissue (fibers) that span a joint and then become anchored to the bone at either end (Frank, 2004). Retinacula are described as strap-like thickenings of dense connective tissue that bind down structures near joints. They prevent tendons from bowstringing and are typically regional specializations of the deep fascia (Benjamin, 2009). However, the same author points out that structures with retinacular function are known by a variety of names, i.e. fibrous pulleys, annular ligaments or fibrous sheaths (Benjamin et al. 2008; Benjamin, 2009). As ligaments and retinacula are also described as parts of the fascial body-wide tensional network consisting entirely of fibrous collagenous soft connective tissue (Findley & Schleip, 2007), it is worth recallin the etymology of these terms: ligament derives from Latin ‘ligare’ (to bind) and retinaculum from ‘retinare’ (to retain) (Stecco et al. 2010a). In humans, the flexor retinaculum of the wrist was also described as a reinforcement of the antebrachial fascia (Stecco et al. 2008, 2010a; Won et al. 2012), and it was suggested that the term ‘flexor retinaculum’ should be abandoned because it does not correspond to any specific autonomous structure (Stecco et al. 2010a). Analogous to our results, collagen bundles of the antebrachial fascia became thicker and were arranged in multiple layers in mediolateral and lateromedial directions, from proximal to distal, creating a structure that is continuous and not separable from the fascia, appearing as a thickening (Stecco et al. 2010a). Besides the linguistic considerations, the results of our dissection support the statement of Schleip & Müller (2013) that many anatomical areas consist of gradual transitions between different tissue architectures in which a clear distinction often appears as arbitrary and misleading. In a study focusing on the ankle retinacula in humans (Stecco et al. 2010b), the authors describe several histological differences between the crural fascia and the extensor retinacula. An analogous comparison in our study brought slightly different results and supports the statement of local specialization of fascial structures according to their specific location. Similarly to the results of Stecco et al. (2010b) the equine antebrachial fascia and the extensor and flexor retinacula are composed of multiple layers of collagen bundles. However, only in the metacarpal flexor retinaculum are the collagen bundles more densely packed than in the antebrachial fascia; the carpal extensor retinaculum seems to be thinner and the collagen bundles are separated by thicker areolar connective tissue. In the area of the carpal flexor retinaculum, dense layers show clear multidirectional organization and are separated by the areolar connective tissue surrounding the blood vessels. Stecco et al. (2010b) report that they did not observe any elastic fibers in the retinacula. In contrast, we observed elastic fibers in the carpal extensor retinaculum, probably preventing the retinaculum from tearing during flexion of the carpal joint when it is exposed to horizontal tensile forces. In the metacarpal flexor retinaculum, some elastic fibers were also present, and numerous elastic fibers were present in the figure-of-eight-shaped areolar connective tissue of the carpal flexor retinaculum. It has been reported in the literature that the ankle retinacula as well as flexor retinaculum in humans are rich in nerve fibers and proprioceptors and therefore should be considered not only passive elements of musculoskeletal apparatus but also a proprioceptive organ (Stecco et al. 2007, 2010b). Although we did find nerve fibers and free nerve endings in the antebrachial fascia and retinacula we analyzed, we did not observe the Pacini, Ruffini or the Golgi-Mazzoni corpuscles. In their recent study, Mense & Hoheisel (2016) also did not find any encapsulated receptors in the medial thoracolumbar fascia of adult rats, and the same group of researchers (Tesarz et al. 2011) reported the lack of such receptors in the human thoracolumbar fascia. In these studies all nerve endings had the appearance of free nerve endings (unmyelinated terminal axons). The authors state the hypothesis that probably encapsulated proprioceptors do not exist in all types of fascia or are not equally distributed over all parts of a given fascia, which could explain their absence in the evaluated samples (Mense & Hoheisel, 2016). This might also be the case in the equine antebrachial fascia, although we do not have other reports on its innervation to compare the results. However, lack of receptive corpuscles does not necessarily negate the proprioceptive role of the antebrachial fascia and its derivatives in horses. Mense & Hoheisel (2016) noted that, according to their previous study, unmyelinated afferents may have a low mechanical threshold and could serve as proprioceptors (Hoheisel et al. 2005). We also did not observe any nerve fibers oriented perpendicular to the collagen fibers, which could support the hypothesis stated by other authors that they are stimulated by the stretching of the collagen fibers (Stecco et al. 2007, 2010b). In our study, nerve fibers were present in a large number in the areolar connective tissues and only a few between compact bundles of collagen fibers. Conclusions of other authors (Benjamin, 2009; Mense & Hoheisel, 2016) suggest that the expanded presence of nerves and receptors in close contact with densely packed collagen fibers, which probably have to transmit the mechanical forces, would constantly excite the receptive endings during movement, and this would be undesirable in normal conditions. Additionally, our results show that the presence of nerve fibers was associated with richly vascular loose connective tissue or vascular network permeating the fascia, similar to the case for tendons and ligaments (Hagert et al. 2005, 2007). However, some of these nerve fibers might be adrenergic and control local blood flow (Benjamin, 2009). In our opinion, further research focused on innervation of equine fascia is necessary to clarify those issues. On the other hand, our results concerning vascular supply of the equine antebrachial fascia and retinacula are in agreement with those described in human deep fasciae of the upper and lower limbs (Stecco et al. 2008; Bhattacharya et al. 2010). Our study also evidenced the rich vascular arcade comprising arterioles, venules and capillaries penetrating throughout the fascia which might mirror the dynamic metabolism of the fascial tissue. Further studies are needed to fully investigate all equine fascial derivatives, especially those present in the digit as well as the connection between the trunk and limbs. We also consider evaluation of the deep fasciae of the equine hindlimb as an area for our future study. Insights from such studies may make valuable contributions to the concept of the relationship between the structure and function of deep fasciae (Stecco et al. 2008), as the forelimbs and hindlimbs in cursorial quadrupeds play different roles in locomotion (Payne et al. 2005a; Biancardi & Minetti, 2012). Thoracic limbs in horses carry ca. 60% of static bodyweight (Biancardi & Minetti, 2012); they are optimized for support and body mass absorption during the stance phase of the stride (Payne et al. 2005b). Altogether, 57% of the vertical impulses during locomotion are applied through the forelimbs, whereas 43% pass through the hindlimbs (Payne et al. 2005b), which are considered primary propulsors of the horse (Payne et al. 2005a). Another interesting point is the possible variables in the fascial structure associated with locomotor muscles of the shoulder and hip girdle in horses due to different type of connection of the thoracic and pelvic limbs to the trunk. The scapula is attached to the trunk only by a *synsarcosis,* whereas the hindlimb connects with the sacrum via the sacroiliac joint (Payne et al. 2005a; Kainer & Fails, 2011).

In conclusion, fascial structures in the equine thoracic limb create a continuous network presenting local specializations according to specific location and in relation to the acting forces. Their structure is in general analogous to those evidenced in the human lower rather than upper limb. The substantial difference between human and equine antebrachial fascia is the small amount of elastic fibers in the latter. Additionally, equine retinacula in the thoracic limb represent a less compact structure, with the exception of the metacarpal flexor retinaculum. They also contain elastic fibers, in contrast to human retinacula. We also did not observe any encapsulated receptors; however, this does not negate the proprioceptive role of equine fascial tissues. In our opinion, any far-reaching conclusions concerning function of the equine fascial system require further morphological research and interpretation with a holistic approach based on the biomechanics of equine movement.

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Authors' contributions

A. Skalec contributed to study concept and design, acquisition and interpretation of data, and drafting of the manuscript. M. Egerbacher contributed to study design, data analysis, and provided critical contributions to the manuscript. Both authors approved the final manuscript.

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