

TDK THESIS

Ines Likon

2021

Department and Clinic of Equine Medicine
University of Veterinary Medicine Budapest

Magnetic resonance imaging of the proximal metacarpal region in endurance horses:
investigation of the effect of training

By Ines Likon

Supervisor:

Annamaria Nagy DrMedVet DipACVSMR DipECVSMR PhD FRCVS

Budapest, Hungary

2021

Contents

List of Abbreviations.....	1
1. Introduction.....	2
2. Literature review.....	2
2.1 Lameness and proximal metacarpal pain in endurance horses.....	2
2.1.1 Anatomy of the proximal metacarpal region.....	3
2.1.2 The use of MRI in diagnosing proximal metacarpal pain.....	5
2.1.3 Exercise and age-related orthopaedic adaptations in horses.....	10
2.2 Study objectives.....	13
2.3 Hypotheses.....	13
3. Materials and Methods.....	13
3.1 Image Acquisition.....	14
3.2 Image analysis.....	15
3.3 Statistical analysis.....	16
4. Results.....	17
4.1 Horses.....	17
4.2 Measurements.....	18
4.2.1 Thickness of the palmar cortex of Mc III.....	18
4.2.2 Suspensory ligament measurements.....	22
5. Discussion.....	23
5.1 Conclusions.....	27
Abstract.....	28
Absztrakt.....	30
Reference List.....	32
Acknowledgements.....	34

List of Abbreviations

AL-DDFT – accessory ligament of the deep digital flexor tendon

C2 – second carpal bone

C3 – third carpal bone

C4 – fourth carpal bone

DDFT – deep digital flexor tendon

DP – dorsopalmar

LM – lateromedial

Mc II – second metacarpal bone

Mc III – third metacarpal bone

Mc IV – fourth metacarpal bone

MR – magnetic resonance

MRI – magnetic resonance imaging

SDFT – superficial digital flexor tendon

SL – suspensory ligament

1. Introduction

Lameness is the most common veterinary problem in endurance horses. It is often caused by proximal metacarpal pain. Magnetic resonance imaging (MRI) has been used to diagnose injuries in these structures that are not detectable using radiography or ultrasonography. Magnetic resonance imaging anatomy and normal anatomical variations in the proximal metacarpal regions have been described but possible changes due to training have not been documented. Recognition of adaptive changes and their differentiation from pathological lesions is paramount for accurate diagnosis. The main objective of this study was to investigate endurance training induced changes in the proximal palmar cortex of McIII and the proximal SL, the two structures that are most commonly injured in the proximal metacarpal region of endurance horses.

2. Literature review

2.1 Lameness and proximal metacarpal pain in endurance horses

Endurance riding is a competition to test the stamina and fitness of the horse over an endurance course against the clock, without compromising the welfare of the horse (Federation Equestre Internationale, 2021). Lameness is the most common veterinary problem in endurance horses (Misheff, 2010; Nagy et al., 2017). In a recent study 80% of endurance horses in England and Wales had suffered from lameness at some point during their career, and 53.2% of these horses had experienced at least one episode of lameness in the last 12 months (Nagy et al., 2017). Furthermore, 40.0% of horses had been eliminated from a race due to lameness at least once during their career. Orthopaedic injury was identified as one of the major reasons for career end or career break in Dutch endurance horses (Sloet van Ooldruitenborg-Oosterbaan et al., 2010). Evidence-based data on the prevalence of specific orthopaedic injuries in endurance horses is lacking. In a lameness textbook, proximal metacarpal pain is described as one of the most common causes of lameness in endurance horses (Misheff, 2010).

Proximal suspensory desmitis can lead to mild to severe acute lameness or can present as a chronic problem. It is hypothesised that in endurance horses this injury occurs when horses fatigue in the later stages of the race or a strenuous training session, with unfit novice horses or horses with a previous SL injury being at higher risk. Proximal suspensory desmitis is typically diagnosed with ultrasonography after the pain has been localised to the proximal metacarpal region using diagnostic anaesthesia. Magnetic resonance imaging (MRI) examination is indicated if the ultrasonographic appearance of the suspensory ligament (SL) is normal or if ultrasonographic abnormalities do not explain the severity of lameness. The precise knowledge of the degree of damage to SL and the involvement of other surrounding structures is of vital importance for implementing the best treatment plan and prevention of re-injury (Misheff, 2010).

Stress pathology of the proximal palmar aspect of the third metacarpal bone (Mc III) includes avulsion and stress fractures, as well as more subtle bone and ligamentous change. These abnormalities are normally diagnosed with radiography and ultrasonography, but occasionally the definitive diagnosis can only be reached with MRI and/or scintigraphy (Misheff, 2010).

Abnormalities of second metacarpal bone (Mc II) and fourth metacarpal bone (Mc IV) alone are seldom the cause of lameness localised to the proximal metacarpal region. Associated interosseous ligament pathology and/or reactive synostoses between the Mc II or the Mc IV and the Mc III are much more likely to be clinically significant. These reactive synostoses are often seen in association with injuries of the palmar cortex of the McIII and/or the SL, and they can often only be diagnosed with MRI (Misheff, 2010).

2.1.1 Anatomy of the proximal metacarpal region

The osseous structures of this anatomical region include the Mc II, Mc III and Mc IV. The Mc II and Mc IV are joined to the Mc III with the interosseus ligaments. The second carpal bone (C2) and a variable portion of the third carpal bone (C3) usually articulate with Mc II, whereas the fourth carpal bone (C4) articulates with Mc IV (Dyce et al., 2010; Thrall, 2017). The superficial digital flexor tendon (SDFT) runs deep to the skin and subcutaneous fascia. The deep digital flexor tendon (DDFT) lies between the SDFT and the palmar surface of the SL and is enclosed within the carpal sheath together with the SDFT. The DDFT is joined by its accessory ligament, which originates from the palmar carpal ligament as well as from the palmar aspect of the C3 with accessory fibres from C4 (Dyce et al., 2010; Thrall, 2017).

The SL runs palmar to Mc III between the axial surfaces of the Mc II and Mc IV. The SL originates mostly from the proximopalmar aspect of the Mc III, but also has an accessory head that originates from the palmar distal aspect of the C3, and some additional fibres from the axial aspect of Mc IV. Proximally there are two lobes of the SL, which contain variable amounts of muscle and adipose tissue. In the distal third of the metacarpal region, the SL divides into lateral and medial branches that insert on the abaxial aspects of the proximal sesamoid bones (Dyce et al., 2010; Thrall, 2017).

On the dorsal aspect of the metacarpus run the tendons of the m. extensor carpi radialis, m. extensor carpi obliquus, m. extensor digitorum communis, m. extensor digitorum lateralis and m. ulnaris lateralis. The extensor carpi radialis tendon inserts on the proximal tubercle on the dorsal aspect of the Mc III and the extensor carpi obliquus tendon inserts on the dorsal aspect of the base of the Mc II. The tendon of the ulnaris lateralis muscle inserts on the accessory carpal bone and the proximal aspect of Mc IV. The tendon of the lateral digital extensor muscle runs deep to the lateral collateral ligament of the carpus, before continuing distally to its insertion site on the metacarpal tuberosity of the Mc III. The extensor retinaculum is a dorsal reinforcement of the antebrachial fascia that holds the tendons of the extensor muscles in place (Dyce et al., 2010).

The medial and the lateral collateral ligaments of the carpus insert on the abaxial aspect of the base of the Mc II and Mc IV, respectively (Nagy and Dyson, 2009). There are two additional small ligaments connecting the carpus to the metacarpal bones. The medial ligament originates from in-between the C2 and C3 and inserts between the Mc III and the base of the Mc II, while the lateral ligament originates from in-between C3 and C4 and inserts between Mc III and the base of Mc IV (Nagy and Dyson, 2009).

The flexor retinaculum is a tight fascia extending from the accessory carpal bone to the medial collateral ligament of the carpus and the palmar aspect of Mc II and Mc IV, surrounding the palmar aspect of the carpus and the proximal metacarpal region (Dyce et al., 2010).

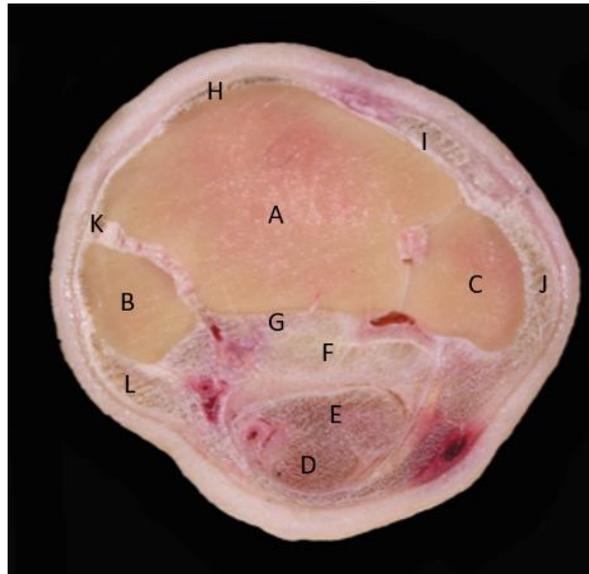


Figure 1. Cross sectional anatomical specimen of the proximal metacarpal region. (A) third metacarpal bone, (B) second metacarpal bone, (C) fourth metacarpal bone, (D) superficial digital flexor tendon, (E) deep digital flexor tendon, (F) accessory ligament of the deep digital flexor tendon, (G) suspensory ligament, (H) m. extensor digitorum communis tendon, (I) m. extensor carpi radialis tendon, (J) m. extensor carpi obliquus tendon, (K) m. extensor digitorum lateralis tendon, (L) m. ulnaris lateralis tendon.

2.1.2 The use of MRI in diagnosing proximal metacarpal pain

Magnetic resonance imaging is an imaging method that allows examination of both osseous and soft tissues structures in three dimensions. The use of MRI is generally indicated when pain causing lameness has been localized to a region and a definitive diagnosis could not be achieved using radiography and ultrasonography (Murray and Dyson, 2010)

Magnetic resonance imaging can be performed in horses using high-field closed, or low-field open systems. The closed systems require the horse to be under general anaesthesia, while some open systems allow for acquisition of images in the standing horse under sedation. Open systems have lower magnetic field strength, resulting in lower signal to noise ratio, and consequently lower image resolution, compared to higher strength magnets. Furthermore, during standing imaging, motion artefacts are a greater problem than when the horse is under general anaesthesia. Nevertheless, avoiding general anaesthesia makes standing MRI safer and more cost effective (Mair et al., 2005; Murray and Dyson, 2010). In a low-field MRI system for the standing horse, the patient is sedated and positioned before fitting the receiver radiofrequency coil around the region of interest and moving the U-shaped magnet into position. Such systems allow imaging of the forelimb up to the carpus, and the hindlimb up to the tarsus (Mair et al., 2005).

Magnetic resonance imaging has been shown to have considerable effect on case management in horses presenting with proximal metacarpal pain, especially when primary diagnostic findings had been equivocal and initial treatment response had been poor (Labens et al., 2020). Magnetic resonance imaging was particularly useful for imaging the region of the origin of the SL and it was valuable for detection of primary bone pathology in the proximal metacarpal/metatarsal region.

In a study of 50 lame horses with carpal and proximal metacarpal pain, the most common MRI abnormalities in the proximal metacarpal region included abnormal mineralisation, loss of fibre structure and/or increased signal intensity within the medial interosseous ligament and pathologic changes on the adjacent aspects of McII and McIII (Nagy and Dyson, 2012). A more recent and larger scale study analysed low-field MRI findings in 359 lame horses with pain localized to proximal metacarpal region. The most common findings included palmar metacarpal bone injury associated with suspensory desmopathy, bone abnormalities at the medial palmar aspect of Mc III, periosteal and/or endosteal abnormalities at the medial palmar aspect of Mc III and abnormalities in the medial intermetacarpal articulation. Pathology of C3 and abnormalities in the interosseus articulation between Mc II and Mc III were also recognised. Damage to the SL was observed mostly in medial lobe or in both lobes, with the highest prevalence of abnormalities observed on the dorsal margin. Collagenous tissue damage was reported at a similar frequency to muscle tissue damage. Poorly defined interface between collagenous and muscle and/fat tissue or loss of normal architecture correlated well with the presence of lameness. Enlargement of the ligament or adhesions to other structures was also reported. The most frequently detected pathology in the Mc III was thickening and irregularity of the medial palmar cortex as well as periosteal and endosteal abnormalities. In this study endurance horses were overrepresented for palmar trabecular bone pathology, SL injury and damage to the second to third metacarpal articulation, when compared to other disciplines (Murray et al., 2020).

Both studies identified Mc III and SL as the most commonly affected structures in the proximal metacarpal region, and the medial aspect as the predilection site for the described pathologies. High-field MRI studies also reached similar conclusions (Barrett et al., 2018; Brokken et al., 2007). In cutting Quarter horses, Mc III sclerosis, Mc III resorption and Mc III bone contusion at the proximal SL origin, as well as the SL dorsal margin fibre irregularity, were the most common abnormalities causing proximal metacarpal lameness. Brokken et al. (2007) also reported abnormalities of SL and AL-DDFT, as the major contributing factors of lameness localised to the proximal metacarpal region of sport horses.

Along the statistical limitations of these two studies, it is important to note that they did not include endurance horses and may therefore be of limited relevance for this study.

2.1.2.1 Principles of MRI

All atomic nuclei consist of protons and neutrons and have a net positive charge. Certain atomic nuclei, such as the hydrogen or the phosphorus nucleus possess a property known as spin, which is dependent on the number of protons. The spinning nucleus induces a magnetic field, which is behaving like a bar magnet, with its magnetic poles aligning along its axis of rotation. Only hydrogen nuclei are considered in clinical settings because they are abundant in the body and are mobile in fat and water (Grover et al., 2015).

Application of a strong, external magnetic field aligns individual hydrogen nuclei parallel with, or perpendicular to the external field. These nuclei are thereafter excited by the application of a second radiofrequency magnetic field, which is applied perpendicular to static magnetic field. The absorption of this energy facilitates the transition of the nuclei to a higher energy state, which in turn enables the subsequent release of energy upon relaxation of the nuclei. The energy absorbed and subsequently emitted by the nuclei induces a voltage change, that can thereafter be detected, amplified, and displayed (Grover et al., 2015; Murray and Dyson, 2010).

There are two basic types of pulse sequences capable of producing an MRI image: gradient echo and spin echo (Murray and Werpy, 2011).

There are two types of relaxation: longitudinal and transverse relaxation, and they are described by the time T1 and T2, respectively. T1 describes the energy lost to the environment, while the T2 describes the energy lost to the surrounding nuclei. Based on the latter T1 and T2 weighted images are produced. T1-weighted images highlight the structural characteristics of bone and soft tissues, whereas T2-weighted images emphasize the fluid characteristics of tissues and are sensitive for detecting synovial effusions, cysts, and oedema. Fat suppressed images are also usually obtained during routine scanning protocols, as they enable better detection of fluid-based pathology in bone marrow or cancellous bone (Bolas, 2011).

It is standard practice to acquire images in sagittal, dorsal, and transverse orientation. Information is presented in the form of tomographic slices, the orientation and thickness of which can also be set during image acquisition (Murray and Dyson, 2010).

Causes of artefacts seen on MRI images can be classified into motion, magnetic field inhomogeneity and digital imaging artefacts. Motion artefacts are caused by movement during the acquisition of MRI data and present as ghosting or repeated picturing of a structure throughout the entire image. Movement can originate from the voluntary motion of the horse or may be physiological in nature (e.g., breathing). Motion artefacts can be minimized by immobilising and/or padding the area of interest or by utilizing specialized image acquisition techniques. The latter however usually prolongs the image acquisition time. Inhomogeneity artefacts lead to image distortion or alterations in signal intensity and can be the result of temperature fluctuations, presence of metal in the magnetic field or magnet inhomogeneity. Furthermore, several other artefacts related to data acquisition and manipulation such as chemical shift artefact, phase cancellation artefact and magic angle effect, may be of importance on a case-to-case basis. Generally speaking, if an abnormality is only seen in one pulse sequence in one orientation and cannot be reproduced, then there is a high likelihood that the finding is an artefact (Murray and Werpy, 2011).

2.1.2.2 MRI anatomy of the proximal metacarpal region

Normal cortical and subchondral bone have low signal intensity (appear black) and have clearly defined endosteal, periosteal or osteochondral margins. Cancellous bone has normally a more heterogeneous appearance in T1- and T2-weighted images, because the bone marrow fat and connective tissues distributed throughout are generally of high signal intensity. On fat suppressed images medulla also appears of low signal intensity (Murray and Werpy, 2011).

The cortex of Mc III has homogeneous low signal intensity and its thickness on the dorsal, palmar, medial and lateral aspect is progressively increasing from proximal to distal. A study by Dyson and Nagy (2011), found that the proximal palmar cortex of Mc III is thicker medially than laterally. The medulla of Mc III has heterogeneous intermediate signal intensity that is increasing from proximal to distal. The medulla of Mc II and Mc IV is only present in the proximal aspect of the proximal metacarpal region, extending up to 5 cm distal to the base of the bones. The cortices of these bones are thicker on the abaxial aspect, while axially at the attachment sites of interosseus ligaments, they are relatively thinner and can be slightly irregular. A smooth bony prominence is often present on the axial aspect of the

base of the Mc II, at the insertion site of the flexor carpi radialis tendon (Nagy and Dyson, 2009).

Normal collagen tissue of tendons emits little to no signal and therefore appears mostly black on all image sequences. The connective tissue between tendon fibrils and at the boundaries of the tendon emits more signal, which may give the tendons a mesh like appearance when imaged with higher resolution sequence parameters. The margins of normal tendons are generally clearly defined, smooth and uniform in appearance (Murray and Werpy, 2011).

The SDFT is oval-shaped in cross section at the level of the carpometacarpal joint. More distally it becomes teardrop shaped with its apex pointing laterally. The DDFT is triangular, or teardrop shaped in cross section with its apex pointing medially at the level of the carpometacarpal joint and becomes more rounded distally. The digital extensor tendons, the lateral digital extensor, the extensor carpi radialis and the extensor carpi obliquus tendons have an elongated to an elongated-oval shape in cross section in this region. These structures are of uniform low signal intensity with smooth and well-defined margins (Nagy and Dyson, 2009).

Many ligaments have a more heterogeneous signal intensity and more variable appearance and structure than tendons, due to higher variation in connective tissue composition and ligament fibre orientation. Normal ligaments have smooth and well-defined margins, and the cortical bone at the origin and insertion is smooth on both the endosteal and periosteal surfaces. Normal variations in ligament size and appearance may occur, and as rule of thumb symmetry between structures in contralateral limbs or between structures within the same limb is an indication of normality (Murray and Werpy, 2011).

The proximal SL has clearly distinct medial and lateral lobes that merge approximately 5-5.5 cm distal to the carpometacarpal joint (Nagy and Dyson, 2009). The dorsal margins of the lobes can be slightly irregular proximally and become more clearly defined approximately 3-4 cm distal to the carpometacarpal joint (Nagy and Dyson, 2009). The medial lobe is normally rectangular in shape and has greater lateromedial (LM) width than dorsopalmar (DP) depth, while the lateral lobe is more square-shaped, has greater DP depth than the medial lobe and is slightly bigger on the abaxial aspect. The amount and distribution of the muscle and adipose tissue within each lobe is variable and can be seen as an area of intermediate to high signal intensity or intermediate to low signal intensity on T1-weighted or T2-weighted images and fat suppressed images, respectively. Close to the origin, the muscle and adipose tissue can be distinctly visualized in both lobes individually, while more distally these tissues become progressively more diffusely distributed. The ligament is

surrounded by loose connective tissue of intermediate to high signal intensity and the palmar metacarpal vessels and nerves running abaxially to the ligament (Nagy and Dyson, 2009). The AL-DDFT has heterogeneous intermediate signal intensity on T1-weighted and T2-weighted images and low signal intensity on fat suppressed images. At the level of the carpometacarpal joint, it has ill-defined margins, while more distally the margins are better defined and the signal intensity is more homogeneous. The signal intensity of the AL-DDFT is always greater than that of the SDFT and DDFT. The lateral and medial collateral ligaments of the carpus have elongated shape in cross section and are of low signal intensity, while the medial and lateral carpometacarpal ligaments are normally characterised by intermediate signal intensity. The interosseus ligaments are seen as an area of heterogeneous intermediate signal intensity between the metacarpal bones (Nagy and Dyson, 2009).

Normal synovial fluid has high signal intensity on T2-weighted and fat suppressed images and low signal intensity on T1-weighted images. Capsular tissue clearly defines the margins of the synovial fluid and should be smooth and of uniform thickness (Murray and Werpy, 2011).

Synovial structures that can be visualised in the proximal metacarpal region include the carpometacarpal joint and the carpal sheath. The carpal sheath can only be clearly seen if there is some distension in it. The palmar recess of the carpometacarpal joint may extend medially and laterally to the proximal aspect of the SL and/or the AL-DDFT, several cm distal to the carpometacarpal joint (Nagy and Dyson, 2009).

The articular cartilage has intermediate to high signal intensity on T1-weighted images and intermediate to low signal intensity on T2-weighted images. It should be clearly defined from the adjacent subchondral bone, be of uniform thickness and the articular surface and chondro-osseous margins should be smooth. Evaluation of the articular cartilage is limited in low-field images, and subtle findings should be interpreted with caution (Murray and Werpy, 2011)

Lastly, the flexor retinaculum, which is a tight fascia surrounding the palmar aspect of the carpus and the proximal metacarpal region, is of homogeneous low signal intensity and its lateral aspect is slightly thicker than the medial (Nagy and Dyson, 2009).

2.1.3 Exercise and age-related orthopaedic adaptations in horses

Structural changes occur in response to mechanical stimulation that is capable of inducing bone deformation. Cyclic dynamic strain facilitates osteogenesis and thus an increase in bone

mass, while the absence of mechanical stimulation will result in bone resorption and decreased bone mass. This process appears to be highly age and site specific. The immature skeleton can adapt to a greater degree than bones of mature horses. Increase in bone mass in response to exercise of the C3 and Mc III, was shown to be most marked at the dorsal locations (Firth, 2006; Smith and Goodship, 2008).

Firth *et al.* (2012) demonstrated that conditioning exercise from a young age, particularly when including gallop exercise, produced horses that had stronger Mc III and proximal phalangeal bone (P1). The authors of this study propose that the observed persistent increased resistance of bone to strain, was mainly due to bone enlargement and not as a result of increased bone density. Density increased during training and decreased during paddock rest, but bone strength continued to increase throughout the study due to the slow growth that was still occurring during rest periods. Nunamaker and colleagues (1990), demonstrated an almost 40% reduction in strain of the Mc III in mature horses, compared with young horses.

Several authors have documented a reduced incidence of bone injury associated with exercise regimes that rely on short bursts of high-speed training. The latter induces high loading rates, which in turn elicits an osteogenic response that ultimately contributes to an increase in the bone mass and thus increased resistance to strain (Smith and Goodship, 2008). An increase in bone mass was observed by Rajão *et al.*, (2019), in response long-term exercise of moderate intensity in adult endurance racehorses. The most significant changes in this study included an increase in cortical bone thickness of the radius and Mc III and an increase in bone density of Mc III, the calcaneus and the accessory carpal bone.

In general, the functional adaptation of tendons appears to be much less pronounced than that of the bone, especially in mature animals. Nevertheless, exercise appears to have important influence on tendon matrix in equine athletes, but these effects have been found to vary with age as well as with the type and functionality of the tendon (Smith and Goodship, 2008).

The histological structure of equine tendons and ligaments stabilizes at around two years of age. Pasture turn-out and controlled low-level exercise programmes were shown to promote healthy development of tendons and ligaments in young foals. Addition of high intensity exercise proved detrimental to these tissues (Firth, 2006; Smith and Goodship, 2008).

The response of the adult tendon to exercise remains rather controversial. Exercise appears to advance age-related changes within tendons of the mature horse, such as reduction of the angle and length of the crimp (wave) pattern of the fascicle, disruption of large collagen

fibrils and changes in the extracellular matrix composition. These findings suggest that after skeletal maturity, the synergistic effect of ageing and exercise causes an inevitable accumulation of microdamage, which predisposes to clinical injury (Firth, 2006; Smith and Goodship, 2008).

Tendons and ligaments with a high incidence of exercise-related injury in the horse tend to be those that act as elastic springs to store energy and contribute to energetic efficiency of locomotion, such as the SDFT and SL. Exercise induced hypertrophy, reflected in the increased cross sectional area, is postulated to alter the mechanical properties of these tendons, thus making them less efficient at storing energy and more susceptible to injury (Smith and Goodship, 2008).

Joints can also be influenced by mechanical stimulation. The remodelling of the subchondral bone was shown to reduce the absorption of high impact loads and inflict damage upon the overlying articular cartilage. Long term loading was associated with stiffening of the subchondral bone, that proposedly lead to occurrence of cartilage fibrillation and breakdown, rather than adaptation (Smith and Goodship, 2008). Mild to moderate exercise produces no long-term effect and that short bouts of heavy exercise superimposed on a confinement regimen will likely have an adverse effect on long-term viability of the articular cartilage (Firth, 2006; Smith and Goodship, 2008).

Increased exercise in the adult horse results in a thickening of the trabeculae of the subchondral bone, thickening of the subchondral plate, and a thickening of both the calcified and hyaline layers of the overlying articular cartilage. As expected, the effect of the forces acting on cartilage also affects the subchondral bone (Firth, 2006; Smith and Goodship, 2008). In a study by Kawcak *et al.* (2000), examining the effect of controlled treadmill exercise on carpal and metacarpophalangeal joint, there was a significant increase in the subchondral bone density of the metacarpal condyles but not in the carpus in response to exercise.

Even though proximal palmar metacarpal pain associated with the proximal SL desmitis and/or stress pathology of the proximal palmar aspect of the Mc III, is one of the main causes for lameness in endurance horses, current scientific literature offers very little information on the response of these structures to exercise. The understanding of the latter is of vital importance for differentiating exercise-induced adaptive changes from injuries and for creating evidence-based training recommendations, with the aim of facilitating maximal performance and preventing injury and lameness.

2.2 Study objectives

The overall aim of this study was to objectively describe training-related adaptive changes of the proximal SL and proximal palmar cortex of Mc III of novice and experienced endurance horses, before and after six months of endurance training and competition.

Specific objectives:

- To compare objective training-related adaptive changes of the SL and proximal palmar cortex of Mc III between novice and experienced horses.
- To compare objective training-related adaptive changes of the SL and proximal palmar cortex of Mc III between images obtained before and after six months of endurance training and competition.

2.3 Hypotheses

1. The proximal palmar cortex of Mc III will be thicker medially than laterally, and its thickness will be increasing from proximal to distal.
2. The palmar cortex of Mc III will be thicker in experienced than in novice horses and will also increase with age.
4. The thickness of the palmar cortex of Mc III will increase following 6 months of endurance training and competition.
4. There will be an increase in SL measurements between the pre- and post-season measurements in some horses.

3. Materials and Methods

A total of 12 endurance horses were selected for the study, including six novice and six experienced horses. Participation was voluntary and by invitation. Owners gave their written consent for their horses' participation. Horses were eligible for the study if they were thought to be sound by their riders and their owner intended to train and compete (if appropriate) them in the 2021 season by their riders and intended to compete in the 2021 season. The first 12 applicants were selected. Novice horses were defined as horses that were undergoing endurance training but have never competed at international level or at a distance

> 80 km at a national level. Horses were considered experienced if they have completed at least two ≥ 120 km international endurance rides. All horses were examined twice, in January and February 2021 ('pre-season') and approximately six months later ('post-season'), July through September 2021. Clinical, training and competition history was obtained prior to admission to the Equine Clinic of the University of Veterinary Medicine Budapest using a questionnaire. The horses first underwent clinical examination and gait evaluation, performed by an experienced clinician (Diplomate of the American and European Colleges of Veterinary Sports Medicine and Rehabilitation), and were included in the study if they did not exhibit lameness higher than 1/8 in either of the forelimbs (Dyson and Nagy, 2011). Data on the number and distance of competitions and any time off training >2 weeks during the study period were collected at the time of the second examination.

3.1 Image Acquisition

All measurements were carried out by myself, after having received training from my supervisor. Standing magnetic resonance imaging of the proximal metacarpal region of both forelimbs was performed under sedation with a combination of acepromazine (0.01–0.05 mg/kg IV), romifidine, detomidine (0.012 mg/kg IV) and butorphanol (0.02 mg/kg IV). Top-up sedation was administered as required during the scanning procedure. The horse was appropriately positioned, and the receiver radiofrequency coil fitted around the leg. The magnet was then moved into position, so that the proximal metacarpal region was centrally located (Figure 2). Pilot sequences were run to check the positioning, correct for any lateral axis deviation, and set the position and angle of subsequent scans. The sequences used and their parameters are described in Table 1.



Figure 2. A horse undergoing magnetic resonance image acquisition of the proximal metacarpal region.

Table 1. The magnetic resonance imaging sequences used and their parameters. T1 - T1-weighted, T2* - T2*-weighted, GRE - gradient echo, T2 FSE - T2-weighted fast spin echo, STIR - short tau inversion recovery, in transverse (TRA), sagittal (SAG) in frontal (FRO) planes. TR - repetition time, TE - echo time.

Sequence	Slice thickness [mm]	TR [ms]	TE [ms]
T1 GRE TRA FAST	5	52	8
T2* GRE TRA FAST	5	68	13
T2 FSE TRA FAST	5	1544	88
STIR FSE TRA FAST	5	5072	22
T1 GRE SAG FAST	5	52	8
T2* GRE SAG FAST	5	68	13
STIR FSE SAG FAST	5	2536	22
T1 GRE FRO FAST	5	52	8
T2* GRE FRO FAST	5	68	13
STIR FSE SAG FAST	5	2536	22

3.2 Image analysis

The LM width of the entire SL and the DP depth and lateromedial width of each lobe of the SL were measured on T1-weighted transverse MR images, 2, 3, 5 and 7 cm distal to the level of the carpometacarpal joint. The DP depth was measured as the greatest distance between the dorsal and palmar margins of the ligament, obtained perpendicular to its palmar margin. The lateromedial width was measured as the greatest distance between the medial and lateral margins of the ligament.

The thickness of the palmar cortex of Mc III was also measured on T1-weighted transverse MR images, at the same levels as described above. At each level three measurements were made, at 25%, 50% and 75% of the distance between the most medial and most lateral aspects of the palmar cortex of Mc III. All measurements were performed in Horos (Horos Project, 2021; <https://horosproject.org>). Repeatability study was performed on a sample of 10 limbs of 5 horses prior to obtaining the results. Three measurements were obtained for each parameter and relative standard deviation was calculated (relative standard deviation = [standard deviation x 100]/sample mean), using Microsoft Excel (Microsoft Corp., 2019; Redmond, Washington 98052-8300, United States). Subjective MR image analysis was also

performed by experienced assessors, but these results are a part of a larger study and are beyond the scope of this thesis.

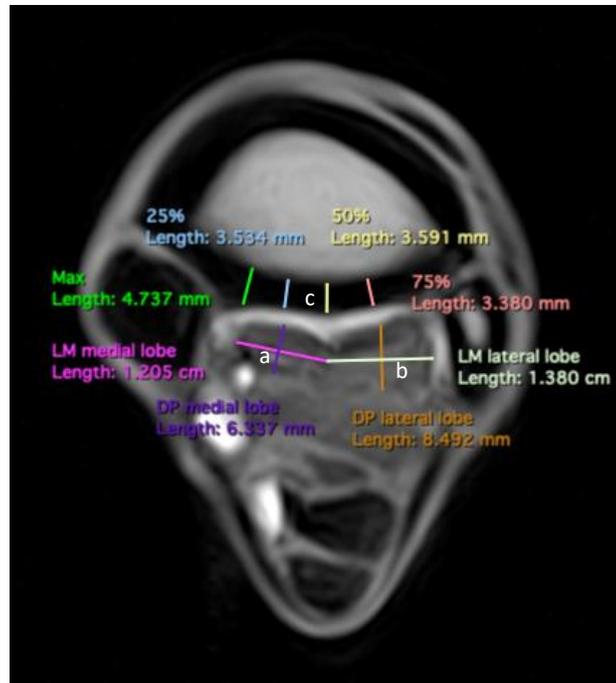


Figure 3. Transverse low-field T1-weighted gradient echo magnetic resonance image obtained 3 cm distal to the carpometacarpal joint. Medial is to the left and dorsal to the top. (a) medial lobe of the suspensory ligament, (b) lateral lobe of the suspensory ligament, (c) palmar cortex of Mc III, (LM) lateromedial width measurement, (DP) dorsopalmar depth measurement, (Max, 25%, 50%, 75%) palmar cortex thickness measurements from medial to lateral.

3.3 Statistical analysis

Paired samples t-test and independent samples t-test or their non-parametric equivalents (i.e., Wilcoxon signed-rank test and Mann–Whitney U test), were used test for the difference in the thickness of the palmar cortex of Mc III as well as the LM width and DP depth of the SL and its lobes, between pre- and post-season measurements, novice and experienced horses. The thickness of the palmar cortex of the Mc III medially at 25% and laterally at 75%, the overall palmar cortex thickness of the Mc III from proximal to distal, the LM width and DP depth of each lobe, and the DP depth of the lateral and medial lobes of SL, were also compared with these tests. Statistical analyses were performed in SPSS (IBM Corp., 2019; New Orchard Road Armonk, New York 10504-1722, United States). Statistical significance was set at $p < 0.05$.

Additionally, Cohen's d and point-biserial correlation were computed for the results of the independent samples t-tests, as a measure of effect size, using Microsoft Excel (Grissom and Kim, 2012; Lakens, 2013),

4. Results

4.1 Horses

Clinical examination and MRI of the proximal metacarpal region were performed in 12 horses pre-season and in 11 horses post-season. One horse got sold during the study period. The horses' signalment, experience, competition and off-training data during the study period are summarized in Table 2. The mean time between the examinations was 203 days (range: 184-226 days).

No horses showed forelimb lameness or clinical signs of proximal metacarpal pain on the initial examination. Six horses developed lameness between examinations, three horses showed forelimb lameness and three horses hindlimb lameness. The forelimb lameness was localized mostly to the fetlock region in two horses; one of them also had a small component of proximal metacarpal pain. One horse had a transient lameness associated with mild SDF tendinopathy in the mid-metacarpal region. All horses were in training at the time of the second examination. One horse showed consistent forelimb lameness (unknown to the owner), which was localized to the foot.

In total, 324 images of 46 limbs were analyzed. The images of all limbs were overall of acceptable quality for clinical diagnosis. At 2 cm distal to the carpometacarpal joint the margins of the SL were poorly defined in the majority of limbs and measurements could not be obtained reliably. This level was therefore excluded from analysis of SL measurements. Repeatability of measurements was confirmed (coefficient of variance $\leq 2\%$), for both the palmar cortical and SL measurements.

Table 2. Signalment and experience of participating horses and their competitions and days off training during the study periods. Novice – horses that have never competed at international level or at a distance longer than 80 km at a national level, experienced – horse that have completed at least two ≥ 120 km international endurance rides, CEN – national-level competitions, CEI – FEI-approved international competition.

Horse	Age (years)	Breed	Gender	Experience level (highest level of competition)	Competitions	>2 weeks of training
1	5	Arabian	mare	Novice (no competition)	2 x 40 km, 1 x 80 km, 16 days rest	16 days
2	7	Anglo-Arabian	mare	Novice (40 km CEN)	1 x 40 km, 2 x 80	18 days
3	9	Arabian	gelding	Experienced (CEI ** 120 km)	1 x 100 km	none
4	6	Arabian	mare	Novice (80 km CEN)	1 x 80 km, 1 x 100 km	14 days
5	8	Arabian	mare	Experienced (CEI ** 120 km)	2 x 20 km	None
6	16	Shagya Arabian	mare	Experienced (CEI*** 160 km)	1 x 40 km	21 days
7	11	Shagya Arabian	mare	Experienced (CEI*** 160 km)	1 x 160 km	16 days
8	15	Shagya Arabian	gelding	Experienced (CEI*** 160 km)	100 km	35 days
9	17	Shagya Arabian	mare	Experienced (CEI*** 160 km)	60 km	56 days
10	3	Shagya Arabian	gelding	Novice (no competition)	none	none
11	7	Shagya Arabian	mare	Novice (no competition)	sold	sold
12	4	Arabian	gelding	Novice (no competition)	none	none

4.2 Measurements

4.2.1 Thickness of the palmar cortex of Mc III

There was no significant average difference between pre- and post-season measurements. At 2 and 3 cm distal to the carpometacarpal joint the measurements for experienced horses were significantly greater on the medial aspect of the palmar cortex than for novice horses ($p < 0.05$, $-0.61 < |r| < 0.23$), (Table 3), (figure 4). At 5 and 7 cm distal to the carpometacarpal joint there was no significant average difference between novice and experienced horses.

The measurements at 25% of the lateromedial width of the palmar cortex of McIII were significantly larger than measurement at 75% ($p < 0.05$, $0.64 < |r| < 0.92$) for all levels, except at 7cm post-season (table 4). The overall palmar cortex thickness was progressively thicker from proximal to distal, and this difference was statistically significant (p value < 0.01 , $0.53 < |r| < 0.75$) (Table 5).

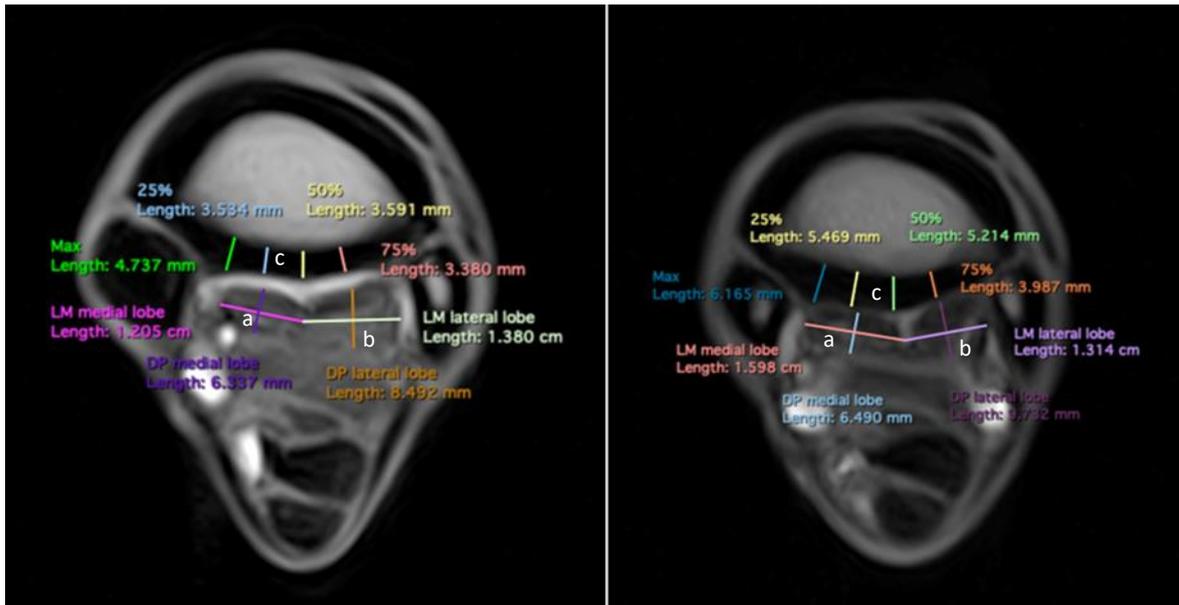


Figure 4 Transverse low-field T1-weighted gradient echo magnetic resonance images from of a novice (left) and from an experienced horse (right), obtained 3 cm distal to the carpometacarpal joint. Medial is to the left and dorsal to the top. (a) medial lobe of the suspensory ligament, (b) lateral lobe of the suspensory ligament, (c) palmar cortex of Mc III, (LM) lateromedial width measurement, (DP) dorsopalmar depth measurement, (25%, 50%, 75%, Max) palmar cortex thickness measurements from medial to lateral.

Table 3. Results of the independent samples t-test comparing the thickness of the palmar cortex of the third metacarpal bone (Mc III) between novice and experienced horses at 2, 3 5 and 7 cm distal to the carpometacarpal joint., 25 – 25%, 50 – 50%, 75 – 75% of the lateromedial width of the palmar cortex, pre – pre-season, post – post-season

Variable	Mean novice horses [mm]	Mean experienced horses [mm]	Mean difference [mm]	p value	95% confidence interval [mm]		r value
2cm25pre	2.74	3.99	-1.25	<0.01	-2.04	-0.47	-0.56
2cm50pre	2.53	3.67	-1.15	0.01	-1.98	-0.32	-0.50
2cm75pre	2.48	2.73	-0.25	0.48	-0.99	0.48	-0.15
2cm25post	3.66	4.97	-0.94	0.01	-1.63	-0.26	-0.53
2cm50post	2.97	3.55	-0.58	0.18	-1.46	0.29	-0.28
2cm75post	2.51	3.00	-0.49	0.14	-1.16	0.18	-0.31
2cmMaxpost	3.54	4.86	-1.32	0.01	-2.34	-0.31	-0.50
3cm25pre	3.92	5.10	-1.17	<0.01	-1.93	-0.41	-0.55
3cm50pre	3.83	4.81	-0.97	0.02	-1.80	-0.15	-0.45
3cm75pre	3.61	3.50	0.12	0.68	-0.47	0.70	0.08
3cmMaxpre	4.97	6.89	-1.92	0.01	-3.22	-0.62	-0.53
3cm25post	3.46	4.83	-1.37	<0.01	-2.17	-0.58	-0.61
3cm50post	3.48	4.64	-1.16	<0.01	-1.90	-0.41	-0.57
3cm75post	3.15	3.44	-0.29	0.43	-1.07	0.48	-0.17
3cmMaxpost	5.16	6.54	-1.38	0.03	-2.64	-0.12	-0.45
5cm25pre	4.49	5.16	-0.66	0.14	-1.57	0.25	-0.29
5cm50pre	4.29	4.61	-0.32	0.42	-1.14	0.50	-0.16
5cm75pre	4.28	4.43	-0.15	0.70	-0.96	0.66	-0.08
5cmMaxpre	6.22	7.20	-0.98	0.07	-2.04	0.08	-0.36
5cm25post	4.54	4.71	-0.17	0.67	-0.99	0.65	-0.09
5cm50post	4.24	4.37	-0.13	0.75	-1.00	0.73	-0.07
5cm75post	4.37	4.34	0.03	0.93	-0.64	0.70	0.02
5cmMaxpost	6.52	7.13	-0.63	0.23	-1.65	0.42	-0.26
7cm25pre	5.66	5.69	-0.04	0.94	-1.13	1.05	-0.01
7cm50pre	5.34	5.37	-0.03	0.95	-1.03	0.97	-0.01
7cm75pre	5.52	5.36	0.17	0.73	-0.84	1.18	0.07
7cmMaxpre	7.04	7.56	-0.52	0.36	-1.69	0.64	-0.19
7cm25post	5.35	5.13	0.22	0.71	-1.04	1.49	0.08
7cm50post	5.19	4.85	0.34	0.55	-0.81	1.49	0.13
7cm75post	5.31	4.75	0.56	0.30	-0.55	1.67	0.23
7cmMaxpost	7.11	6.82	0.29	0.69	-1.20	1.80	0.09

Table 4. Results of the paired samples t-test comparing the thickness of the palmar cortex of the third metacarpal bone (Mc III) medially at 25% (25) and laterally at 75% (75) of the lateromedial width of the palmar cortex Mc III, pre-season, and at 2 cm distal to the carpometacarpal joint at 75% of the palmar cortex of Mc III, pre-season.

Variables	Mean	Mean	Mean difference [mm]	p value	95% confidence interval [mm]		r value
	25%	75%					
2cm25pre - 2cm75pre	3.36	2.60	0.76	<0.01	0.46	1.06	0.77
2cm25post - 2cm75post	3.51	2.73	0.78	<0.01	0.56	1.00	0.84
3cm25pre - 3cm75pre	4.51	3.55	0.95	<0.01	0.61	1.29	0.64
3cm25post - 3cm75post	4.09	3.28	0.80	<0.01	0.44	1.17	0.68
5cm25pre - 5cm75pre	4.82	4.36	0.47	<0.01	0.16	0.77	0.76
5cm25post - 5cm75post	4.62	4.36	0.26	0.01	0.06	0.46	0.87
7cm25pre - 7cm75pre	5.67	5.44	0.23	0.03	0.02	0.45	0.92
7cm25post - 7cm75post	5.25	5.06	0.19	0.13	-0.06	0.45	0.91

Table 5. Results of the paired samples t-test comparing the overall palmar cortex thickness of the third metacarpal bone (Mc III), from proximal to distal at 2,3,5 and 7 cm distal to the carpometacarpal joint, pre – pre-season, post – post-season.

Variables	Mean	Mean	Mean difference [mm]	p value	95% confidence interval [mm]		r value
	upper level [mm]	lower level [mm]					
2cmpre - 3cmpre	3.34	4.58	-5.78	<0.01	-1.67	-0.79	0.53
3cmpre - 5cmpre	4.58	5.09	-3.49	<0.01	-0.81	-0.21	0.73
5cmpre - 7cmpre	5.09	5.94	-4.77	<0.01	-1.23	-0.48	0.64
2cmpost - 3cmpost	3.40	4.29	-5.16	<0.01	-1.24	-0.53	0.62
3cmpost - 5cmpost	4.29	5.02	-4.63	<0.01	-1.06	-0.40	0.67
5cmpost - 7cmpost	5.02	5.58	-3.01	0.01	-0.95	-0.17	0.75

4.2.2 Suspensory ligament measurements

There was no significant average difference between pre- and post-season measurements for all but one measurement. The lateromedial width of the medial lobe at 3 cm appeared to be shorter post-season, compared to pre-season ($p=0.026$, $r=0.47$).

There was no significant average difference between most SL measurements for novice and experienced horses. The DP depth of the lateral lobe of the SL was larger post-season in experienced than in novice horses at 3 and 5 cm distal to the carpometacarpal joint ($p<0.05$, $-0.62 < |r| < -0.46$). The DP depth was also larger in experienced than in novice horses at 7 cm distal to the carpometacarpal joint ($p=0.02$, $r=-0.42$).

Table 6. Results of the paired samples t- comparing dorsopalmar (DP) depth of the lateral and medial lobes of the suspensory ligament at 3 and 5 cm distal to the carpometacarpal joint, medlobe – medial lobe, latlobe – lateral lobe, pre – pre-season, post – post season

Variables	Mean DP depth medial lobe [mm]	Mean DP depth lateral lobe [mm]	Mean difference [mm]	P value	95% confidence interval [mm]		r value
3cmDPmedlobepre - 3cmDPlatlobepre	7.27	9.45	-2.17	<0.01	-2.95	-1.40	0.13
3cmDPmedlobepost - 3cmDPlatlobepost	7.22	8.95	-1.73	<0.01	-2.45	-1.01	0.29
5cmDPmedlobepre - 5cmDPlatlobepre	7.68	8.15	-0.47	0.17	-1.17	0.23	0.48
5cmDPmedlobepost - 5cmDPlatlobepost	7.11	7.82	-0.71	0.04	-1.38	-0.04	0.25

Measurements of the SL lobes could be performed reliably at 3 cm distal to the carpometacarpal joint. By 5 cm the lobes were fused in 8/12 limbs (66.7%). The LM width of both the medial and lateral lobes was significantly greater than their DP depth ($p\text{-value} < 0.001$, $-0.18 < |r| < 0.41$) (table 7). The DP depth of the lateral lobe was significantly greater than DP depth of the medial lobe where the two lobes could be measured separately ($p\text{-value} < 0.05$, $0.13 < |r| < 0.48$) (table 6).

Table 7. Results of the paired samples t-test comparing the lateromedial (LM) width and dorsopalmar (DP) depth of the suspensory ligament lobes at 3 and 5 cm distal to the carpometacarpal joint, medlobe – medial lobe, latlobe – lateral lobe, pre – pre-season, post – post season

Variables	Mean	Mean	Mean	P value	95% confidence		r value
	LM width [mm]	DP depth [mm]	difference [mm]		interval [mm]		
3cmLMmedlobepre - 3cmDPmedlobepre	14.83	7.27	7.55	<0.01	6.79	8.32	0.37
3cmLMlatlobepre - 3cmDPlatlobepre	12.97	9.45	3.51	<0.01	2.89	4.14	0.30
3cmLMmedlobepost - 3cmDPmedlobepost	13.80	7.22	6.58	<0.01	5.67	7.49	0.04
3cmLMlatlobepost - 3cmDPlatlobepost	13.03	8.95	4.08	<0.01	3.48	4.67	0.41
5cmLMmedlobepre - 5cmDPmedlobepre	12.62	7.68	4.94	<0.01	4.20	5.68	0.41
5cmLMlatlobepre - 5cmDPlatlobepre	11.55	8.15	3.40	<0.01	2.52	4.28	0.31
5cmLMmedlobepost - 5cmDPmedlobepost	12.02	7.11	4.90	<0.01	3.92	5.89	0.22
5cmLMlatlobepost - 5cmDPlatlobepost	11.58	7.82	3.76	<0.01	3.01	4.51	-0.18

5. Discussion

This is the first study to describe MRI findings in the proximal metacarpal region of non-lame endurance horses in full training and competition. To our knowledge this is also the first study to perform sequential MRI examinations in performance horses with the aim of documenting MRI changes as a response to exercise.

In agreement with our hypothesis, the medial aspect of the palmar cortex of the Mc III was thicker in experienced than in novice horses. This indicates that long-term exercise induces thickening of the palmar cortex in the proximal metacarpal region. This process appears to be site specific, significant increase in medial aspect of the palmar cortical thickness between novice and experienced horses was only observed at 2 and 3cm distal to the carpometacarpal joint. This can be explained by results of previous studies. This is the region of the origin of the SL and where most osseous abnormalities of the proximal palmar Mc III occur, with

or without associated SL lesions (Murray et al., 2020; Nagy and Dyson, 2012). The greater thickness in experienced horses suggest that the medial palmar cortex of Mc III responds to long term exercise with thickening. Thickening of the dorsal-cortex of Mc III in response to exercise is well documented (Firth, 2006; Smith and Goodship, 2008), however according to our knowledge there is no information on the adaptation ability of the palmar-cortex of Mc III. Age may also contribute to the increase in cortical thickness; all horses in the experienced group were > 8 years old. Our sample size was too small to allow statistical assessment of the effect of age as a continuous variable on palmar cortical thickness (or other objective measurements).

The palmar cortex was significantly thicker on the medial aspect of the limb, which agrees with previous studies (Dyson and Nagy 2011). The greater cortical thickness in the medial aspect is thought to reflect greater loading of the medial aspect of the limb, which is also supported by other diagnostic imaging observations. On radiographs the medial aspect of the proximal palmar cortex of Mc III has greater opacity than the lateral (Nagy and Dyson, 2012). On scintigraphic images of normal horses, there is greater radiopharmaceutical uptake in the medial than in the lateral aspect of the palmar cortex. (Weekes et al., 2006). In our study significant difference between novice and experienced horses was only seen in the medial aspect of the palmar cortex, which gives further evidence that this region is affected by exercise the most in the proximal palmar cortex of Mc III. Several authors have suggested that variation in limb structure and gait efficiency may play an important role in the adaptation process of cortical bone (Davies and Watson, 2005; Firth et al., 2012). These variables are rather difficult to describe objectively to allow meaningful statistical analysis and were therefore not included in the study.

The mean difference between the measurements in the medial and the lateral aspect was progressively smaller from proximal to distal, suggesting that uneven mediolateral loading is mostly present in the most proximal aspect of the Mc III. The overall thickness of the palmar cortex itself was progressively thicker from proximal to distal, which agrees with previous observations (Nagy and Dyson, 2009).

The minimum speed of international endurance competition is 13 or 14 km/h, which equals to 3.6 or 3.9 m/s. Most endurance horses are subjected to interval training, which includes galloping (approximately 20 km/hr or 5.6 m/s) for short distances. To our knowledge there is no scientific data to provide evidence for the minimum speed that results in long-term bone remodelling.

Another explanation for the lack of detectable changes in the proximal Mc III thickness post-season can be that low-field MRI is unable to detect subtle changes that may have developed and that using more sensitive imaging methods with higher resolution (e.g. high-field MRI or high-resolution computed tomography) these changes could be detectable.

The LM width of the medial lobe of the SL was significantly greater than its DP depth, the same trend could also be observed for the lateral lobe. This has been previously described in the medial lobe (Nagy and Dyson, 2009), but not in the lateral lobe. The DP depth of the lateral lobe was significantly greater than DP depth of the medial lobe, except for pre-season measurements at 5 cm distal to the carpometacarpal joint. This is in agreement with previous observations of (Bischofberger et al., 2006; Nagy and Dyson, 2009).

The histological structure of equine tendons and ligaments stabilizes at around two years of age, and it is proposed that these tissues are most susceptible to adaptations to exercise during this time of maturation. Pasture turn-out and controlled low-level exercise programs were shown to promote healthy development of tendons and ligaments in young foals, exercise is postulated to advance age-related changes within tendons of the mature horse. Exercise induced hypertrophy is hypothesised to alter the mechanical properties of these tendons, thus making them less efficient at storing energy and more susceptible to injury (Firth, 2006; Smith and Goodship, 2008).

There was no significant average difference between pre- and post-season measurements of SL for all but one measurement. The latter is likely an incidental finding. Even the effect of long-term endurance exercise appears to be rather insignificant on the SL, as only an increase of DP depth of the lateral lobe of the SL post-season at 3 and 5 cm distal to the carpometacarpal joint was observed. Most lesions in the proximal SL are detected in the medial lobe (Murray et al., 2020), therefore these small increases in size are also likely to be incidental findings. However, only a study on a larger number of horses could confirm this. As previously mentioned, the effect of age was not possible to analyze due to the small sample size.

Our work provides reference values of Mc III palmar cortical thickness and proximal SL size in endurance horses in full training and competition. This will help veterinarians when interpreting scans of clinical cases and will also serve as reference in future studies.

During the study period 6/12 horses developed lameness, which is in agreement with a previous study, where 53% of endurance horses developed lameness in the 12 months preceding data collection (Nagy et al., 2017). One horse got sold five months after the first examination and had not showed lameness until that time. Of the three horses that had

developed forelimb lameness, in only one horse was lameness associated with proximal metacarpal pain and only partly. This is not necessarily contrary to textbooks that state that proximal metacarpal pain is one of the most common reasons of proximal metacarpal pain. Our sample size was too small to establish prevalence of causes of lameness and this was not the scope of this thesis.

This study had some limitations. The low-field standing system has lower signal-to-noise ratio than the high-field systems, and this in turn results in lower resolution or longer imaging times. The latter increases the occurrence of movement artefacts that can have a great negative impact on image quality. It is not always possible to position the limb straight in the magnetic field, which may also affect the quality images. The inherent limitations of low-field MR imaging of the proximal metacarpal lesion might have resulted in decreased ability to detect more subtle changes and may have also affected the results of statistical analysis. Despite the limitations, the huge advantage of the low-field system must not be forgotten; it allows MRI examination in the standing horse, which makes the examination accessible for horses in training and competition.

Our sample size was relatively small, which could have adversely affected the statistical power of our study. Sample sizes in studies using live, client-owned animals are limited by financial constraints. Welfare and ethical considerations also support using the smallest possible sample size that can be used to detect meaningful results. This was an absolute pilot study in this field of research, therefore sample size or power calculation could not be reliably performed as no estimated effect was available. Using a low-field MRI system inherently introduced some limitations. Other than image quality and resolution, the relatively thick (3-5 mm) slice thickness must also be mentioned. The exact location of the reference images is likely to have been influenced by positioning of the limb and the slice thickness. A small difference in the location of reference images in relation to the carpometacarpal joint might have influenced our results. Horses belonged to three different trainers and had individually tailored training programs, which may have also influenced our results. Further studies on a larger number of horses and using a more sensitive diagnostic imaging method are indicated to detect more subtle changes induced by exercise over short period of time.

5.1 Conclusions

In experienced endurance horses the medial proximal aspect of the palmar cortex of the Mc III is thicker than in novice horses, which reflects the effect of cumulative long-term exercise and possibly age. Endurance training and competition of 6-7 months was insufficient to induce changes in the palmar cortex that are detectable with low-field MRI. The size of the SL does not seem to change as a result of 6-7 months or even years of exercise.

Abstract

Background: Proximal palmar metacarpal pain, associated with proximal suspensory desmitis and/or stress pathology of the proximal palmar cortex of the third metacarpal bone (McIII), is one of the most common causes of lameness in endurance horses. However, the effect of exercise on these structures remains poorly elucidated.

Objectives: The main aim of this study was to objectively describe training-related adaptive changes in the proximal palmar cortex of McIII and in the proximal aspect of the suspensory ligament (SL) of novice and experienced endurance horses, before and after six months of training.

Materials and Methods: Six novice and six experienced non-lame endurance horses with no history of proximal metacarpal pain were selected for the study. Clinical and magnetic resonance imaging (MRI) examinations were performed twice, approximately six months apart. Between examinations horses received endurance training appropriate to their age and competition level. All measurements were made at 2, 3, 5 and 7 cm distal to the level of the carpometacarpal joint on transverse T1-weighted gradient echo images. The thickness of the palmar cortex of McIII was measured at 25, 50 and 75% of the lateromedial width of the palmar cortex. Measurements of the LM width and DP depth of the SL and its lobes were also obtained.

Paired samples t-test and independent samples t-test or their non-parametric equivalents were used to test for difference in the thickness of the palmar cortex of McIII, the LM width and DP depth of the SL and its lobes between novice and experienced horses, pre- and post season measurements and horses of different age categories.

Results: The medial aspect of the palmar cortex of McIII was significantly thicker in experienced than in novice horses at 2 and 3 cm distal to the carpometacarpal joint ($p < 0.05$). Horses of ≥ 8 years of age also had a thicker medial palmar cortex of McIII than younger horses at these levels ($p < 0.05$). There was no statistically significant difference between pre- and post-season measurements of the thickness of the palmar cortex of McIII in any location. No significant changes were observed in LM width and DP depth of the SL or its lobes, between pre and post season measurements, novice and experienced horses or between different age categories.

Conclusions: Low-field MRI was unable to detect objective changes in the proximal palmar cortex of McIII or the proximal SL following six months of endurance training and competition. However, the significant difference in the proximal palmar cortex measurements between novice and experienced horses gives evidence of long-term effect of exercise. Further studies on a larger number of horses and using a more sensitive diagnostic imaging method are indicated to detect more subtle changes induced by exercise over short period of time.

Absztrakt

Háttér: A proximalis palmaris metacarpalis fájdalom, proximalis egyenítőszalag sérüléssel és/vagy a hármás metacarpalis csont (McIII) proximalis palmaris cortexének stressz eredetű elváltozásaival összefüggésben, távlovakban a sántaság egyik leggyakoribb oka. Ennek ellenére ezen struktúráknak az edzéshez és versenyzéshez köthető változásait még nem vizsgálták.

Célkitűzések: A tanulmány fő célja az McIII proximalis palmaris kérge és a proximalis egyenítőszalag edzéshez köthető adaptív változásainak leírása kezdő és tapasztalt távlovakban a versenyszezon kezdetén és hat hónappal később.

Anyag és módszer: Hat kezdő és hat tapasztalt távló került kiválasztásra. A tanulmányban való részvételhez előfeltétel volt, hogy nem szerepel proximalis metacarpalis fájdalom a kórelőzményben, és a ló az első vizsgálat során nem mutat elülső végtag sántaságot. A mozgás- és a mágneses rezonanciás (MRI) vizsgálatokat kétszer végeztük el, a versenyszezon előtt és kb. hat hónappal később. A két vizsgálat közt a lovak edzettségi szintjüknek és koruknak megfelelő távlovás edzésprogramban részesültek. Valamennyi mérést a carpometacarpalis ízületől 2, 3, 5 és 7 cm-re distalisan végeztük tranzverzális T1-súlyozott 'gradient echo' felvételeken. Az McIII palmaris cortexének vastagságát a palmaris cortex lateromediális szélességének 25, 50 és 75%-nál mértük. Szintén megmértük az egyenítőszalag lateromediális szélességét és dorsopalmaris vastagságát. Az McIII palmaris kérge vastagságábeli és az egyenítőszalag méretbeli különbségeit kezdő és tapasztalt lovak között, a versenyszezon előtt és után, valamint különböző korú lovakban, párosított és független t-teszttel, illetve azok nem-parametrikus megfelelőivel vizsgáltuk.

Eredmények: Az McIII palmaris kérgének mediális része jelentősen vastagabb volt tapasztalt lovakban, mint a kezdő lovaknál a carpometacarpalis ízületől 2 és 3 cm-re distalisan ($p < 0.05$). Ezekben a szintekben a ≥ 8 éves lovakban szintén vastagabb volt az McIII mediális palmaris cortex, mint a fiatalabb lovakban ($p < 0.05$). Nem volt statisztikailag szignifikáns különbség a versenyszezon előtt és után készített McIII palmaris cortex mérések között. Ugyancsak nem detektáltuk szignifikáns változást az egyenítőszalag méreteiben a

szezon előtt és után készített mérések alapján, kezdő és tapasztalt, illetve különböző korcsoportú lovak között.

Következtetések: Az álló helyzetű alacsony tesztelés MRI használatával nem detektáltunk objektív változásokat az McIII proximális palmaris cortexében vagy a proximális egyenítőszalagban hat hónap távlovas edzést és versenyzést követően. Azonban a kezdő és a tapasztalt lovak proximális palmaris kérgében mért jelentős különbség az edzés hosszútávú hatását bizonyítja. Nagyobb kísérleti csoporton, érzékenyebb diagnosztikai képalkotó módszerek alkalmazásával végzett további tanulmányok szükségesek ahhoz, hogy az edzés és versenyzés által előidézett enyhébb, rövid idő alatt bekövetkező változásokat dokumentálhassuk.

Reference List

- Barrett, M.F., Manchon, P.T., Hersman, J., Kawcak, C.E., 2018. Magnetic resonance imaging findings of the proximal metacarpus in Quarter Horses used for cutting: Retrospective analysis of 32 horses 2009–2012. *Equine Vet. J.* 50, 172–178.
- Bischofberger, A., Konar, M., Ohlerth, S., Geyer, H., Lang, J., Ueltschi, G., Lischer, C., 2006. Magnetic resonance imaging, ultrasonography and histology of the suspensory ligament origin: a comparative study of normal anatomy of warmblood horses. *Equine Vet. J.* 38, 508–516.
- Bolas, N., 2011. Basic MRI Principles. In: *Equine MRI*. Blackwell Publishing Ltd, pp. 3–37.
- Brokken, M.T., Schneider, R.K., Sampson, S.N., Tucker, R.L., Gavin, P.R., Ho, C.P., 2007. Magnetic resonance imaging features of proximal metacarpal and metatarsal injuries in the horse. *Vet. Radiol. Ultrasound* 48, 507–517.
- Davies, H., Watson, K., 2005. Third metacarpal bone laterality asymmetry and midshaft dimensions in Thoroughbred racehorses. *Aust. Vet. J.* 83, 224–226.
- Dyce, K.M., Sack, W.O., Wensing, C.J.G., 2010. The Forelimb of the Horse. In: *Textbook of Veterinary Anatomy*. Saunders/Elsevier, St. Louis, pp. 586–623.
- Dyson, S., Nagy, A., 2011. Can lameness be graded reliably? *Equine Vet. J.*
- Federation Equestre Internationale, 2021. Endurance Rules.
- Firth, E.C., 2006. The response of bone, articular cartilage and tendon to exercise in the horse. *J. Anat.*
- Firth, E.C., Rogers, C.W., Rene van Weeren, P., Barneveld, A., Wayne McIlwraith, C., Kawcak, C.E., Goodship, A.E., Smith, R.K.W., 2012. The effect of previous conditioning exercise on diaphyseal and metaphyseal bone to imposition and withdrawal of training in young Thoroughbred horses. *Vet. J.* 192, 34–40.
- Grissom, R.J., Kim, J.J., 2012. Correlational Effect Sizes and Related Topics. In: *Effect Sizes for Research: Univariate and Multivariate Applications, Second Edition*. Taylor & Francis Group, LLC, pp. 97–149.
- Grover, V.P.B., Tognarelli, J.M., Crossey, M.M.E., Cox, I.J., Taylor-Robinson, S.D., McPhail, M.J.W., 2015. *Magnetic Resonance Imaging: Principles and Techniques: Lessons for Clinicians*. J. Clin. Exp. Hepatol.
- Kawcak, C.E., McIlwraith, C.W., Norrdin, R.W., Park, R.D., Steyn, P.S., 2000. Clinical effects of exercise on subchondral bone of carpal and metacarpophalangeal joints in horses. *Am. J. Vet. Res.* 61, 1252–1258.
- Labens, R., Schramme, M.C., Murray, R.C., Bolas, N., 2020. Standing low-field MRI of the equine proximal metacarpal/metatarsal region is considered useful for diagnosing primary bone pathology and makes a positive contribution to case management: A prospective survey study. *Vet. Radiol. Ultrasound* 61, 197–205.
- Lakens, D., 2013. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Front. Psychol.* 0, 863.

- Mair, T.S., Kinns, J., Jones, R.D., Bolas, N.M., 2005. Magnetic resonance imaging of the distal limb of the standing horse.
- Misheff, M.M., 2010. Lameness in Endurance Horses. In: *Diagnosis and Management of Lameness in the Horse: Second Edition*. Elsevier Inc., pp. 1137–1149.
- Murray, R.C., Dyson, S., 2010. Magnetic Resonance Imaging. In: *Diagnosis and Management of Lameness in the Horse: Second Edition*. Elsevier Inc., St. Louis, pp. 239–245.
- Murray, R.C., Tranquille, C.A., Walker, V.A., Milmine, R.C., Bak, L., Tacey, J.B., Bolas, N.M., 2020. Magnetic Resonance Imaging Findings in the Proximal Metacarpal Region of 359 Horses and Proximal Metatarsal Region of 64 Horses Acquired Under Standing Sedation. *J. Equine Vet. Sci.* 94, 103268.
- Murray, R.C., Werpy, N., 2011. Image interpretation and artefacts. In: *Equine MRI*. Blackwell Publishing Ltd, pp. 101–142.
- Nagy, A., Dyson, S., 2009. Magnetic resonance anatomy of the proximal metacarpal region of the horse described from images acquired from low- and high-field magnets. *Vet. Radiol. Ultrasound* 50, 595–605.
- Nagy, A., Dyson, S., 2012. Magnetic resonance imaging findings in the carpus and proximal metacarpal region of 50 lame horses. *Equine Vet. J.* 44, 163–168.
- Nagy, A., Dyson, S.J., Murray, J.K., 2017. Veterinary problems of endurance horses in England and Wales. *Prev. Vet. Med.* 140, 45–52.
- Nunamaker, D.M., Butterweck, D.M., Provost, M.T., 1990. Fatigue fractures in thoroughbred racehorses: Relationships with age, peak bone strain, and training. *J. Orthop. Res.* 8, 604–611.
- Rajão, M.D., Leite, C.S., Nogueira, K., Godoy, R.F., Lima, E.M.M., 2019. The bone response in endurance long distance horse. *Open Vet. J.* 9, 58–64.
- Sloet van Ooldruitenborg-Oosterbaan, M.M., Genzel, W., Van Weeren, P.R., 2010. A pilot study on factors influencing the career of Dutch sport horses. *Equine Vet. J.* 42, 28–32.
- Smith, R.K.W., Goodship, A.E., 2008. The Effect of Early Training and the Adaptation and Conditioning of Skeletal Tissues. *Vet. Clin. North Am. - Equine Pract.*
- Thrall, D., 2017. *Textbook of veterinary diagnostic radiology*, 7th ed. Saunders Ltd.
- Weekes, J., Murray, R., Dyson, S., 2006. Scintigraphic evaluation of the proximal metacarpal and metatarsal regions in clinically sound horses. *Vet. Radiol. Ultrasound* 47, 409–416.

Acknowledgements

The study was supported by the European Union and co-financed by the European Social Fund (grant agreement no. EFOP-3.6.3-VEKOP-16-2017-00005), project title: „Strengthening the scientific replacement by supporting the academic workshops and programs of students, developing a mentoring process). Special thanks go to Hallmarq Veterinary Imaging, which generously enabled us to perform the MRI examinations free of charge.

Furthermore, I would also like to express my sincere gratitude to my supervisor Annamaria Nagy DrMedVet DipACVSMR DipECVSMR PhD FRCVS RCVS, for giving me the opportunity to participate in this research project and for her continued support and encouragement. My completion of this project could not have been accomplished without the help and support of the entire team at the Department and Clinic of Equine Medicine and my family.

HuVetA

ELECTRONIC LICENSE AGREEMENT AND COPYRIGHT DECLARATION*

Name: Ines Likon

Contact information (e-mail): ines.likon@gmail.com

Title of document (to be uploaded): Magnetic resonance imaging of the proximal metacarpal region in endurance horses: investigation of the effect of training

Publication data of document: October 2021

Number of files submitted: 1

By accepting the present agreement the author or copyright owner grants non-exclusive license to HuVetA over the above mentioned document (including its abstract) to be converted to copy protected PDF format without changing its content, in order to archive, reproduce, and make accessible under the conditions specified below.

The author agrees that HuVetA may store more than one copy (accessible only to HuVetA administrators) of the licensed document exclusively for purposes of secure storage and backup, if necessary.

You state that the submission is your original work, and that you have the right to grant the rights contained in this license. You also state that your submission does not, to the best of your knowledge, infringe upon anyone's copyright. If the document has parts which you are not the copyright owner of, you have to indicate that you have obtained unrestricted permission from the copyright owner to grant the rights required by this Agreement, and that any such third-party owned material is clearly identified and acknowledged within the text of the licensed document.

The copyright owner defines the scope of access to the document stored in HuVetA as follows (**mark the appropriate box with an X**):

I grant unlimited online access,

I grant access only through the intranet (IP range) of the University of Veterinary Medicine,

I grant access only on one dedicated computer at the Ferenc Hutýra Library,

I grant unlimited online access only to the bibliographic data and abstract of the document.

Please, define the **in-house accessibility of the document** by marking the below box with an **X**:

I grant in-house access (namely, reading the hard copy version of the document) at the Library.

If the preparation of the document to be uploaded was supported or sponsored by a firm or an organization, you also declare that you are entitled to sign the present Agreement concerning the document.

The operators of HuVetA do not assume any legal liability or responsibility towards the author/copyright holder/organizations in case somebody uses the material legally uploaded to HuVetA in a way that is unlawful.

Date: Budapest, 17.10.2021



Author/copyright owner
signature

—

HuVetA Magyar Állatorvos-tudományi Archívum – Hungarian Veterinary Archive is an online veterinary repository operated by the Ferenc Hutýra Library, Archives and Museum. It is an electronic knowledge base which aims to collect, organize, store documents regarding Hungarian veterinary science and history, and make them searchable and accessible in line with current legal requirements and regulations.

HuVetA relies on the latest technology in order to provide easy searchability (by search engines, as well) and access to the full text document, whenever possible.

Based on the above, HuVetA aims to:

- *increase awareness of Hungarian veterinary science not only in Hungary, but also internationally;*
- *increase citation numbers of publications authored by Hungarian veterinarians, thus improve the impact factor of Hungarian veterinary journals;*
- *present the knowledge base of the University of Veterinary Medicine Budapest and its partners in a focussed way in order to improve the prestige of the Hungarian veterinary profession, and the competitiveness of the organizations in question;*
- *facilitate professional relations and collaboration;*
- *support open access.*