

**Functional morphometric analysis of tarsal bones,
ligaments including histological examination of fascia
with respect to tarsal joint internal alignment and
loading biomechanics**

by

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ABSTRACT

The tarsus or hock is a complex joint of the hind limb consisting of seven different bones and having various articulations. The tarsal bones are held together in intimate contact through the attachments of soft tissue such as ligaments, tendons and fascia. The tarsal joint is commonly involved in equine hindlimb lameness and other pathological problems. Despite suggestions that tarsal conformation is a contributory factor to performance, lameness and orthopedic health, limited studies are available in the literature regarding tarsal bone morphometry, objective radiographic conformation or alignment changes in response to loading of the tarsus in horses, and the role of soft tissue in maintaining the joint stability and range of motion under loading is not known possibly due to insufficient measurable tarsal parameters.

In addition to this, faulty conformation and uneven loading of the tarsal bones is believed to result in tarsal pathology, ligamentous ruptures and joint instability of the equine hind limb. The aims of this thesis were:

1. To investigate the morphometric and radiographic anatomy of the equine tarsus and develop reliable measurable parameters that can be used to consistently and objectively measure tarsal conformation in horses.
2. To provide baseline data for the developed parameters from tarsal radiographs including the effects of rotations, and inter-rater repeatability.
3. To use developed parameters to assess the change of alignment of the tarsal joint under loading.
4. To investigate the potential role of the tarsal ligaments in the load redistribution within the tarsus, joint stability and range of motion of the tarsal joint.

5. To deduce the potential role of fascia in load redistribution through mechanical connectivity, internal composition (histological) and thickness variation proximal and distal to the tarsus.

A pilot study on 10 cadaveric equine hind limbs from 7 adult horses (8.15 ± 5.28 years), was used to specify the characteristics and identify reliable landmarks of the bones of the equine tarsal joint to be used for further investigation through radiology to assess the tarsal conformation. “Zero degree Lateromedial” (ZLM) and “Zero degree Dorsoplantar” (ZDP) views were established and 88 measurable parameters with validated anatomical landmarks were developed, including 39 parameters from the ZLM view and 49 parameters from the ZDP views, consisting of individual, as well as correlated angular and ratios parameters to avoid any effects of different tarsal size or magnification effects associated with the radiography technique. LM and DP views were used as the minimum required to gain a 3D appreciation of tarsal bone movements, while a complete investigation would require the inclusion of oblique views as well.

Then subsequent studies established: i.) baseline data of these parameters from tarsal radiographs; ii.) effects of rotations, vertical and horizontal rotations of projection angles of the primary X-ray beam (radiography machine) on the developed tarsal measurements to established acceptable range of rotations; iii.) assessment of the inter-rater reliability of the selected parameters; iv.) changes in alignment of the tarsal bones under ex vivo loading; v.) Role of soft tissue like ligaments and fascia in loading and joint range of motion (during flexion, extension and incremental load.

Increased load on the tarsal joint produced measurable radiographic changes in the alignment and direction of movement of the tarsal bones ex vivo. Six reliable parameters showed the potential to detect changes. Soft tissues such as ligaments were shown to play a significant role

in maintaining joint stability and normal range of joint motion. Severing all major tarsal ligaments caused significant changes in tarsal flexion ($P < 0.001$). Cutting the plantar aspect of the long plantar ligament caused the largest significant change in tarsal extension of $2.3^\circ \pm 0.5$ ($P < 0.000$).

Both transverse and longitudinal arrangements of intertarsal ligaments within the tarsus and the arrangement of major tarsal ligaments external to the bones suggest a normal tightly packed alignment of the tarsus allows slight transverse movements of the tarsal bones during loading and prevents over extension and flexion of the tarsal joint, suggesting the existence of more complex mechanisms involving other soft tissue such as fascia that help in maintaining the loading stability and influence the joint range of motion. In consideration of this, this research was then directed towards the broader field of fascial anatomy.

Fifteen hind limbs were obtained from adult horses of mixed breeds and an anatomical exploration of fascia by dissection was completed with the purpose being to lay the groundwork for understanding how tension is distributed from the crural region to the distal hindlimb. Characteristics such as relative fascial thickness, attachment points, connectivity and collagen fiber's orientation, internal composition by histological examination were recorded and described to deduce their potential functional significance.

In conclusion, the tarsal bone geomorphometric and loading data, along with information generated on the ligaments and fascia in this study, will allow reliable quantitative assessment of tarsal conformation and eliminate judgmental errors or variation between observers using subjective visual assessment for the tarsus. Locomotory control and the postural stability of the hind limb is largely dependent on the functional integration of different structures which is enabled via the fascial architecture providing mechanical connectivity. Overall, this data will

improve our understanding of tarsal loading biomechanics and help in the understanding of the pathogenesis of injury and locomotor dysfunctions. The measurement protocols will require further investigations on large groups of different horse breeds for wider potential usefulness, adaptability and validation. The use of the developed parameters to differentiate between normal conformation and any faulty or pathological conformation will potentially allow investigators to identify possible relationships between morphological measurements and the occurrence of pathology.

DEDICATION

“And indeed We created man (Adam) out of an extract of clay (water and earth). Thereafter We made him (the offspring of Adam) as a Nutfah (mixed drops of the male and female sexual discharge and lodged it) in a safe lodging (womb of the woman). Then We made the Nutfah into a clot (a piece of thick coagulated blood), then We made the clot into a little lump of flesh, then We made out of that little lump of flesh bones, then We clothed the bones with flesh, and then We brought it forth as another creation. So Blessed is Allah, the Best of creators.” (Holy Quran)

To my parents, the guiding star in the maze of this hard life,

To my wife, my helpmate in life's journey,

Without them, none of this would have been possible.

To my brothers and sisters,

my supporters I dedicate

this simple work.

DECLARATION

This is to certify that:

- i. The thesis comprises only my original work towards the PhD.
- ii. Due acknowledgement has been made in the text to the specific contributions of other persons.
- iii. The thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Zeeshan Akbar

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- Zeeshan Akbar*^{1,2}, Oday Alawi Al-Juhaishi¹ and Helen M.S. Davies¹, 2019. Radiographic morphometric parameters on dorsoplantar radiographs for evaluation of the equine tarsus at Banff Center for the Arts in Banff, Alberta

from July 26-29, 2019 of American Association of Veterinary Anatomists (AAVA-2019), University of Calgary, Canada.

- Akbar, Z.*^{1,2}, Oday A. Al-Juhaishi¹ and H.M.S. Davies¹, 2019. Effect of possible rotations during radiography on the developed radiographic tarsal measurements of dorsoplantar view of the equine tarsus at the International Federation of Associations of Anatomists (IFAA 2019) from the 9th to the 11th August, London, United Kingdom.

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Chapter 1: Literature Review

1.1 INTRODUCTION:

1.2 Summary

The occurrence of tarsal joint pathologies is believed to be dependent on the mechanical function of the joint. In addition, it is also significantly influenced by several anatomical factors, two of which will be the primary focus of this thesis. The first of these relates to the tarsus conformation. The conformation is the shape or physical appearance or outline due to the arrangements of muscles, bones, and other tissues of the body in an animal (Beeman 1973; Stashak 1987). The conformation is closely related to its function (Gnagey, Clayton, & Lanovaz, 2006). Thus, good conformation is believed to have many advantages such as balancing of uneven forces, thus helping in handling overload and ultimately lead to less pathology (Gnagey et al., 2006; Rooney, 1969; Vanderperren, Raes, Van Bree, & Saunders, 2009).

Therefore, poor conformation has been proposed as an associated factor in decreasing the performance, affecting orthopedic soundness or health and lameness in equines (Rooney 1969; Stashak 1987; McIlwraith et al., 2003; Anderson et al., 2004). Lameness is the most important and common cause of losing performance in horses of all categories regardless of their age, breed, and sex (Rossdale et al., 1985; Olivier et al., 1997; Nahms 1998; Kane et al., 2000). It has great economic importance to the equine industry because of being a major contributor to costs related with both veterinary treatment and wastage.

Thus, lameness can cause huge economic losses in the racing industry which can be considered as one of the biggest industries in Australia (Bailey et al. 1997). Faulty conformations such as angular limb deformities (such as varus and valgus deformities) are

commonly reported in young foals and even observed in adult horses (Bramlage & Auer, 2006; Caron, 1988; García-López & Parente, 2011; Witte & Hunt, 2009).

In addition to this, there could be many contributory factors both internal and external which can impact on musculoskeletal disorders or injuries in racing and other professional horses such as genetics, age, number of race starts, surfaces of race track, pre-existence of pathology, training load intensity, any trauma and conformation and biomechanics (Magnusson 1985; McIlwraith et al., 1987; Kobluk et al., 1990; Dolvik and Klemetsdal 1996; Anderson et al., 2004).

Quantitative measurements of different traits of conformation have been attempted using various methods such as radiography, photogrammetry, use of high-speed motion cameras recordings and three dimensional (3D) computerized motion analysis systems in horses. These conformational traits included the lengths of segment, circumferences and angles of joints (Anderson et al., 2004; Weller et al., 2006b; Adair III et al., 2016). However, there are certain factors impacting the accuracy such as placement of markers over skeletal landmarks, change in positioning of the camera in relation to the horse body or limb, complex joint configurations and segment lengths and reduction of a 3D structure into 2D are potential sources of errors and are thus major limitations for these techniques.

Furthermore, these methods only mention one or two tarsal parameters to measure its general conformational alignment. In addition, no specific details for the assessment of morphological or other features of individual tarsal bones within the complex tarsal structure have been provided. However, despite frequent suggestions that faulty tarsal conformation could affect the function and predispose the tarsus to damage, objective measurements methods or protocols of tarsus conformation are not commonly used. Although radiography has some limitations, such as superimposition of bones images on each other and capturing

a complex three-dimensional structures in two dimensional images, radiography is still a simple and easily accessible, widely used in field and clinics, more cost effective and non-invasive diagnostic technique with immense potential for use for morphometrical studies (Pharr and Fretz 1981; Auer et al., 1983; Barr 1994; Abdunnabi 2011). Furthermore, radiology is also an integral part of pre-sale or purchase examinations in horses. Therefore, radiography was extensively used in the subsequent studies of this thesis.

More recent injury management in humans and studies on human locomotion has embraced a new thinking of functionality of connective tissue in terms of its architecture and mechanical connectivity in consideration of its relationship with other systems of body in contrast to the classical strategy of isolating muscles, bones and joints.

This idea acknowledges the importance of fascia as an integrative, communicative matrix of the body. This idea has proven to provide insight into the understanding of locomotory patterns and injuries in the human body. The mapping of the fascial system has helped to visualise the progress of injury to various other areas of the body through interconnected lines of fascia. This mapping was helpful in demonstration of pain progression and dysfunction via these fascial lines and the impact on the overall locomotory function (Myers, 1997; Stecco, 2015). Such mapping of the equine fascial system has been attempted but is not yet fully described or comprehensive (Schultz & Elbroend, 2013).

The second anatomical factor investigated in relation to loading biomechanics of the tarsal joint, is the overall stability (range of joint motion) of the tarsal joint through tarsal ligaments and their mechanical connectivity with the rest of the hind limb via the fascial system. (Further details are described in the relevant chapters).

Although this approach appears quite general, it can be argued that a better understanding of the anatomical and loading mechanisms is a further fundamental step. This could help in understanding the optimum management of and factors associated with the occurrence of frequent pathologies not only in the tarsal joint, but also in other connected structures of the hind limb.

Thus, it can be concluded that conformation and functional biomechanics of the equine tarsus have potential relationships between tarsal conformation and the loading biomechanics for joint stability and tarsal damage. The current literature review provides a basic brief description of the tarsal joint anatomy, syndesmology, frequent pathologies of tarsus along with assessment of conformation mainly through radiology and various other methods.

1.2.1 Anatomical description:

The body is supported by skeleton (Figure 1.1) which is a frame work of hard structures which support and protect the soft tissues of body, provide the system of levers used in locomotion (Dyce, Sack, & Wensing, 2010b). The skeleton includes bones and cartilages, along with ligaments which bind these together might be included as well. The skeleton can be divided into (1) axial (2) appendicular, and (3) splanchnic or visceral skeleton. The number of bones of the skeleton of an animal varies with age, because of the fusion during growth of skeletal elements, and even in adults of same species numerical variations occur.

The following review discusses the current state of knowledge of the structures and functions of the equine tarsus, pathogenesis of tarsal damage, assessment of morphometrical conformation and loading mechanics of the tarsal joint in the horse.

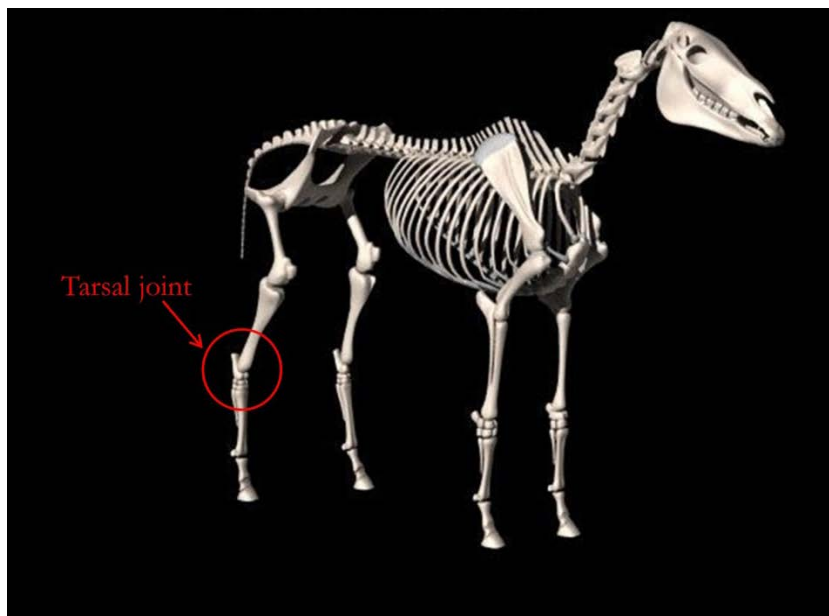


Figure 1. 1: Horse skeleton showing tarsal joint location in relation to the body.

The figure also illustrates location of tarsal joint in relation to hind limb of horse adopted (<https://flippednormals.com/downloads/horse-skeleton-sculpture/>).

The bones have been commonly divided into four different types according to their shape and function by different authors like: long, short, flat and irregular bones. The bones can be divided into: flat, short, vertebral and sesamoid bones (Michell & Watkins, 1989) or long, short, flat and sesamoid bones (Liebich, König, & Maierl, 2004; Nickel et al., 1986b). The long bones are typically elongated, cylindrical in shape having enlarged extremities, act as supporting columns and levers. Morphometrically, the short bones such as the tarsus present somewhat similar dimensions in length, breadth, and thickness. Their chief function appears to be that of diffusing concussion (Getty, 1975b; Sisson, 1917).

The tarsus (or “hock”) is defined as a region in the hind limb (Figure 1.1) composed of different bones, the ossa tarsi usually numbering six but exceptionally seven in the horse and forming a number of articulations arranged in three rows. The proximal row consists of relatively large tarsal bones: the talus medially, and the calcaneus laterally. The middle row consists of only a single central tarsal but the distal row comprises up to four tarsal bones (Dyce et al., 2010b; Sisson, 1917, 1975).

The tarsus also includes the distal tibia, tarsal bones, and proximal second, third, and fourth metatarsus (MTII, III, and IV) maintained in intimate contact by the help of their soft-tissue attachments. Proximally, the cochlear surface of distal tibia articulate with the trochlea of talus in the tarsocrural joint; distally the distal row of tarsal bones articulate with three metatarsal bones in the tarsometatarsal joint (Stashak, 2002).

1.2.1.1 Bone ossification in the tarsal joint:

The ossification in the tarsus is continuous after birth, and can be recognized by radiography where the joint space appeared wider because of the presence of more cartilage compared to an adult horse (Ruohoniemi, Hildén, Salo, & Tulamo, 1995; Shelley & Dyson, 1984). The tarsus reveals relatively rounded centers of ossification. The ossification proceeds rapidly and the individual bones became recognizable in reasonable approximation to their adult shape by two weeks of age but may still be more rounded (Shelley & Dyson, 1984).

The tibia has three centers of ossification along with a supplementary one for the lateral malleolus which is really the distal end of the fibula and is a separate piece at birth (Getty, 1975a). It has a well-circumscribed oval body which fuses to the tibia by three months (Brown & MacCallum, 1975). The fibula in young foals is not completely mineralized at birth and may not be visible by radiography (Jeffcott, 1984). The incomplete ossification of the tibial malleoli or the trochlear ridges of the talus (tibiotarsal) bone at birth may be obvious as an irregular rough contour of the bone. Adult features, such as the medial proximal and distal tubercles of the talus (tibiotarsal) bone and other prominences of the talus, appear undeveloped (Shelley & Dyson, 1984).

The calcaneus tarsal bone, the largest bone of the tarsus, develops from two centers of ossification, one for the main body (approximate time of appearance of ossification centers is 135-155 days of gestation) of the bone while other includes the tuber calcaneus which develops as a separate center of ossification (approximate time of appearance of ossification centers is 325 days of gestation to soon after birth) (Getty, 1975c). The ossification of the tuber calcaneus proceeds in a proximodorsal direction and it fuses to the main part of

calcaneus around the age of 16 and 24 months (Banks, 1972; Shively & Smallwood, 1980; Smallwood et al., 1984).

The first and second tarsal bones are often unfused in young foals and have two separate centers of ossification, but are usually fused together before birth (Getty, 1975a; Shelley & Dyson, 1984). To evaluate the extent of ossification or fusion of ossification centers in 23 foals, a grading system was devised by sectioning and macroscopic examination to record information about the ossification centers and growth plates. In 40 percent of foals aged between 52 and 104 days, fusion of the first and second tarsal bones were found, while in the remainder the bones were separated, or fused incompletely or unilaterally (Brown & MacCallum, 1975). All other tarsal bones developed from one center of ossification and approximate time of appearance of ossification centers is 290-335 days of gestation (Getty, 1975a). The ossification and epiphyseal closure of the proximal third metatarsal proceeds before birth (Getty, 1975c).

1.2.1.2 Morphology of the tarsal joint bony components:

The tarsus is composed of different small tarsal bones, forming a number of articulations arranged in three rows.

The tarsus also includes the distal tibia, tarsal bones, and proximal second, third, and fourth metatarsus (MTII, III, and IV) maintained in intimate contact by the help of their soft-tissue attachments (Auer, 2012). Proximally, the cochlear surface of distal tibia articulate with the trochlea of talus in the tarsocrural joint; distally the distal row of tarsal bones articulate with three metatarsal bones in the tarsometatarsal joint (Stashak, 2002). The detailed morphometry of each constitutive bone of the tarsal joint is described in the next Chapter.

1.2.1.3 Tarsal bone arrangement and articulations:

The tarsus consists of seven bones having numerous articulations including the talus and calcaneus in the proximal row, the central tarsal in the middle row, and the first tarsal, second tarsal, third tarsal, and fourth tarsal in the distal row forming a complex arrangement of articulation of the tarsal joint. The tarsus of horses usually comprises six bones as the first and second tarsal bones are fused together, forming a single irregular bone, but occasionally there are seven bones as the first and second tarsal bones may remain separated (Getty, 1975a; Nickel et al., 1986a; S. Sisson, 1917).

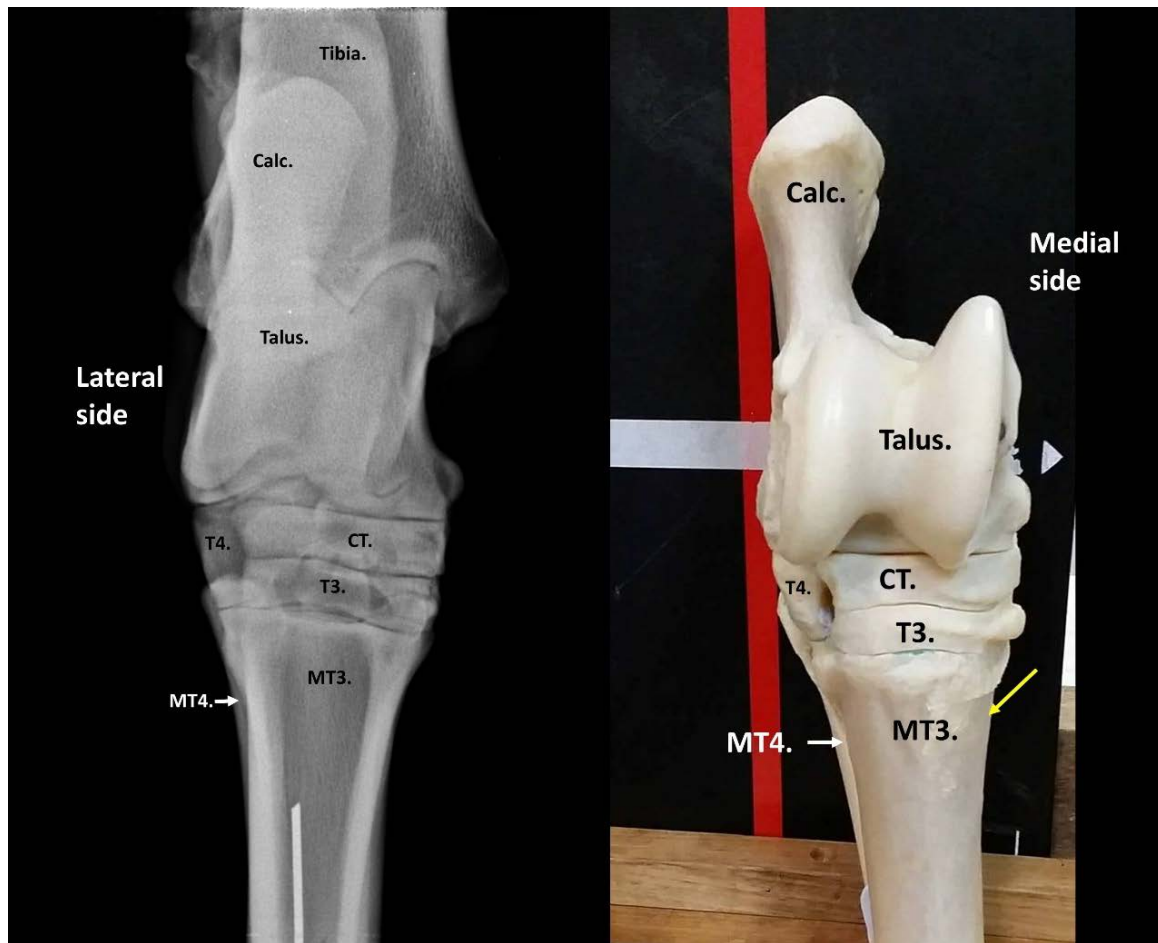


Figure 1. 2: Position of the tarsal bones in right tarsal joint with radiographic and bones reconstruction dorsal views.

Arrangements of constituent bones and joints of the tarsus in the horse, Abbreviations, Calc. calcaneus bone, CT. central tarsal bone, T3, third tarsal bone. T4, fourth tarsal. MT3, third metatarsal. MT4. Fourth metatarsal, Arrow. Showing the position of second metatarsal.

The distal end of the tibia and the proximal aspect of the talus articulate together to form the tibio-tarsal joint (TT). The talus and calcaneus articulate with each other to form the talocalcaneal joint (TC). The central tarsal and talus bone articulate to form the proximal inter-tarsal joint (PIT), and the joint formed by the central tarsal bone and the distal row of tarsal bones is called the distal inter-tarsal joint (DIT). The distal row of tarsal bones

articulates with the proximal aspect of the metatarsal bones to form the tarso-metatarsal joint (TMT) (Dyce, Sack, & Wensing, 2010a; Kraus-Hansen, Jann, Kerr, & Fackelman, 1992).

1.2.1.3.1 Tarsocrural joint:

The tarsocrural or tibio-tarsal joint is a ginglymus joint based on the deeply grooved shape on the cochlear articular surface of the distal end of tibia with the extensive trochlear surface of the talus. The distal end of the tibia is adapted to fit the trochlea of talus. These articulations are at an angle (12 to 15 degrees) dorsolateral to the sagittal plane (Dyson & Ross, 2011; Getty, 1975a). Most of the joint movement occurs in the tarsocrural joint and the tibia articulates almost in a circular path along the ridges of the talus. However, some rotations and translations might occur at other sites (Auer, 2012; Lanovaz, Khumsap, Clayton, Stick, & Brown, 2002).

1.2.1.3.2 The talocalcaneal-centroquartal (proximal intertarsal):

In the proximal intertarsal joint, the talus and calcaneus articulate with the central and fourth tarsals. The articular surfaces of the proximal intertarsal joint are flat and form an amphiarthrosis (Nickel et al., 1986c). This is a plane joint and is capable of only small amounts of gliding (shear) movement (Dyson & Ross, 2011). This joint is considered to be almost immobile but micromovements do occur during ambulation and weight bearing.

1.2.1.3.3 The distal intertarsal joint:

The distal intertarsal joint is narrower than the width of the tarsus as the fourth tarsal extends dorsally to the proximal row. In this joint all the tarsal bones of the distal row except the fourth tarsal are in contact with the central tarsal. The joint is an amphiarthrosis (Nickel

et al., 1986c) or plane joint considered to be rigid and almost immobile having small gliding (shear) movements (Dyson & Ross, 2011).

1.2.1.3.4 The tarsometatarsal joints:

This joint is characterized by flat articular surfaces between the distal row of tarsals and on the proximal aspects of metatarsal bones (Liebich et al., 2004; Nickel et al., 1986c).

1.2.1.3.5 The talocalcaneal or intertarsal joints:

The talocalcaneal joint includes the articulation between the talus and calcaneal tarsal bones. It also includes intertarsal (amphiarthroses) joints which are situated between the bones of each row of tarsals (Nickel et al., 1986c). These are vertical joints between the bones of the same row. They are closely opposed and allow very little movement (Liebich et al., 2004).

1.2.1.4 SOFT TISSUES SURROUNDING THE TARSUS:

The bones of the tarsus are held together with bony attachments of ligaments and maintained in their normal configuration or alignment by remaining in contact through the soft tissues like joint capsule and fascia (Auer, 2012). The tarsus is also secured by a combined joint capsule which attaches from the distal part of the tibia proximally and extends distally to the tarsal bones of the distal row blending with collateral ligaments as well as attaching to the second, third and fourth metatarsal bones (MtII,III,IV) thus covering all the synovial compartments (Lanovaz et al., 2002).

1.2.1.4.1 Ligaments surround the tarsus:

There are multiple ligaments surrounding the hock and these can be further divided according to their location and length (Liebich et al., 2004). The long collateral ligament connects the distal end of the tibia to the proximal end of the metatarsal bones, but it also attaches to the small tarsal bones as well, and these ligaments are taut during extension. The other short collateral ligaments originate cranial and deeper to the long collateral ligaments from the medial and lateral malleolus of the tibia and attach to the talus and calcaneus bones of the tarsus respectively. These ligaments are tense during flexion and relaxed during extension of the hock joint (Updike, 1984; Wissdorf, Gerhards, & Huskamp, 2010).

1.2.1.4.2 Lateral collateral ligaments (ligamentum collaterale tarsi laterale):

The medial and lateral collateral ligaments each have long and short parts (Updike, 1984). The long lateral collateral ligament origin is superficial and it arises on the lateroplantar aspect of the lateral malleolus of the distal tibia, is directed almost straight distally, and is

attached at the distolateral aspect of the calcaneus and fourth tarsal, third metatarsal and fourth metatarsal bones. It also forms a canal for the passage of the tendon of the lateral digital extensor muscle (Figure 1.3).

The short lateral collateral ligaments lie deeper to the long lateral collateral ligament and their origin is the craniolateral aspect of the lateral tibial malleolus and they attach to the lateral surface of calcaneus and the planter proximolateral aspect of talus (Auer, 2012).

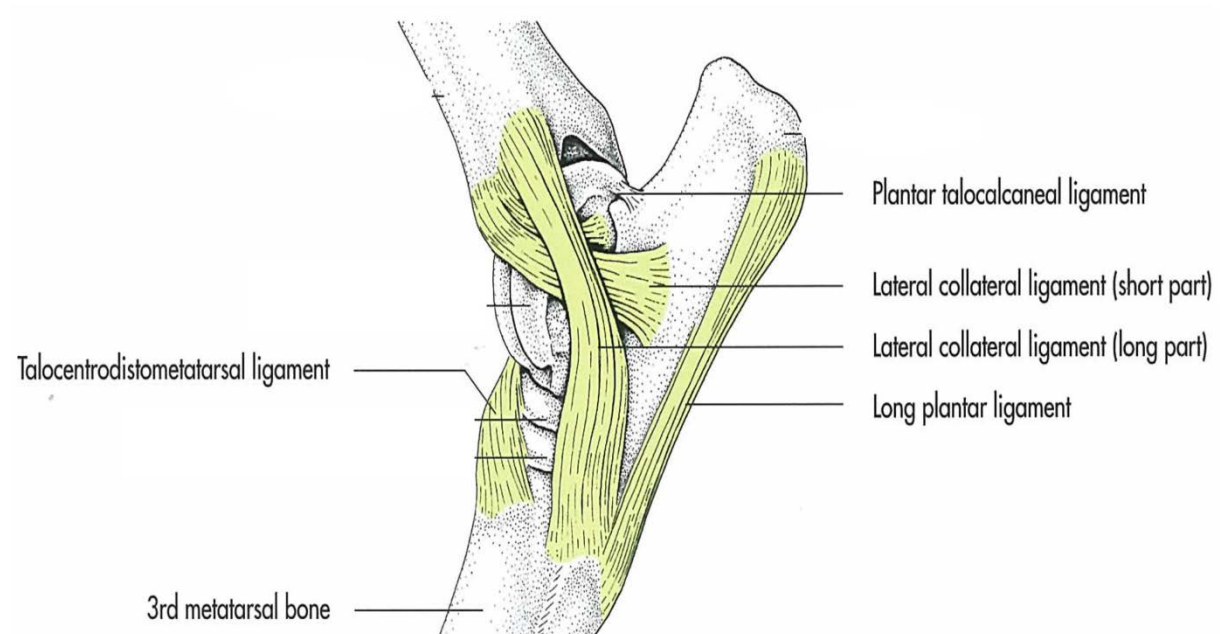


Figure 1. 3: Adopted from Cervený, 1980. Ligaments of the left tarsus of the horse (lateral aspect).

1.2.1.4.3 Medial collateral ligaments (ligamentum collaterale tarsi mediale):

The long medial collateral ligament (as indicated in figure 1.4) originates from the caudo-medial aspect of the medial malleolus of the tibia and extends to the second metatarsal,

third metatarsal and also the medial aspects of the distal tarsal bones. The short medial collateral ligament lies deeper to the medial aspects of the calcaneus and sustentaculum tali (Dyson & Ross, 2011).

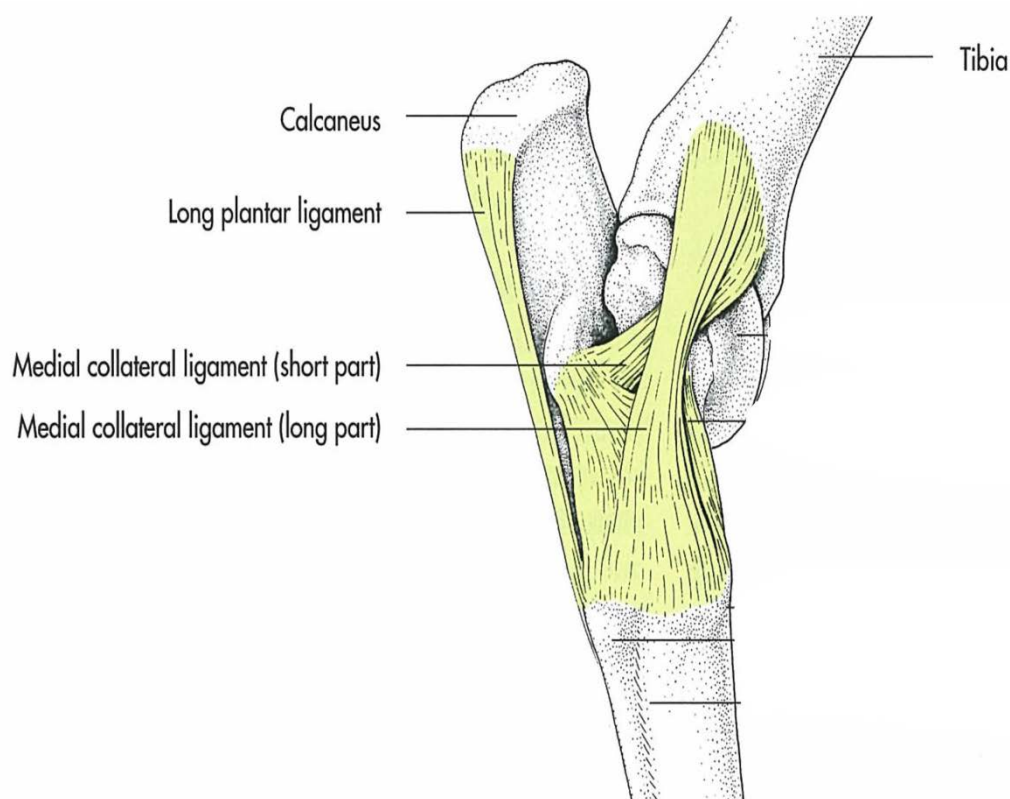


Figure 1. 4: Adopted from Cervený, 1980. Ligaments of the left tarsus of the horse (medial aspect).

1.2.1.4.4 Long plantar ligament:

The long plantar ligament is just like a flat, strong band which originates at the proximal planter surface of the calcaneus, extends distally, and ultimately attaches to the fourth tarsal and the fourth metatarsal bone.

1.2.1.4.5 Dorsal tarsal ligament:

The dorsal lateral ligament expands distally from the distal tuberosity of the talus and covers the central tarsal, third tarsal and proximal extremity of the third and fourth metatarsal bones (Dyson & Ross, 2011; Liebich et al., 2004).

1.2.1.4.6 The interosseous ligament (IL):

This ligament originates from the plantar aspect of the fourth tarsal and the calcaneus and extends proximally (Bell, Baker, Foreman, & Abbott, 1993). This anatomical relationship of the interosseous ligament and the tarsus may explain the coexistence of distal hock joint pain and suspensory injury, a positive response to tarsal flexion in some horses with primary interosseous ligament pain, and confusion of diagnostic analgesic techniques used to differentiate distal hock joint pain from proximal interosseous desmitis (Dyson & Ross, 2011).

1.2.1.4.7 Various short ligaments:

There are many short intertarsal ligaments connecting the adjacent bones of the tarsus and metatarsus and also that bridge the joint spaces among the adjacent bones of the same level or the neighbouring level (ligamenta tarsi interossea) (S. J. Dyson & M. W. Ross, 2011; Liebich et al., 2004).

1.2.1.4.8 Tarsal Sheath:

The tarsal sheath is a long pouch of about 16-20 cm at the musculotendinous junction of the lateral digital flexor tendon in the caudo-distal aspect of the crus. The tarsal sheath forms a larger pouch in between the common calcanean tendon and lateral digital flexor muscle. The distention of the tarsal sheath is termed thoroughpin or true thoroughpin, but the condition may have distinct causes (Stashak, 1987). The sheath is compressed by retinacular bands, so if the sheath is distended it gives the sheath a loculated appearance. Such longitudinal swelling is quite a common finding in lame and sound horses together either unilateral or bilateral and sometimes associated with pain and lameness (Ross and Dyson, 2011).

1.2.1.4.9 Tendons surround the tarsus:

In horses, the long digital extensor tendon covers the dorsal region of the tarsus and its position is maintained by the retinaculi. The lateral digital extensor tendon unites with the long digital extensor tendon distal to the tarsal region. The fibularis tertius (peroneus tertius) tendon along with the tibialis cranialis muscle tendon runs parallel to the tendon of the long digital extensor. The peroneus tertius tendon is penetrated by the tibialis cranialis muscle tendon and which then divides into three branches, the medial, middle, and lateral branches, before inserting on the central, third, and fourth tarsal bones as well as the proximal metatarsal region (Figure 1.5). The medial branch is better known as the cunean tendon, which at one time was thought to be responsible for the development of spavin.

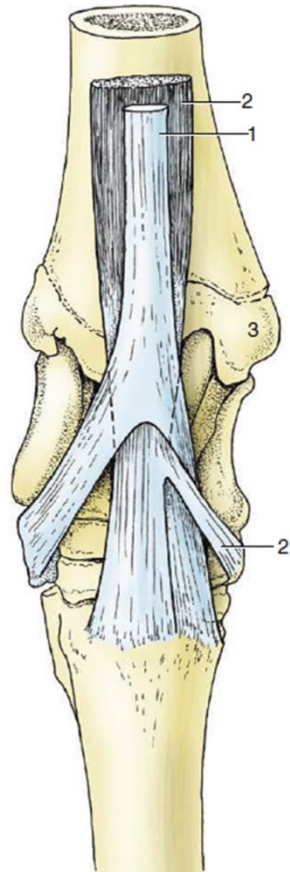


Figure 1. 5: Adapted from Dyce et al. 2010. The insertion of flexors of right tarsus (dorsal aspect).

1, peroneus tertius, dividing into dorsal and lateral branches; 2, tibialis cranialis, splitting into dorsal and medial (cunean, 2') branches; 3, the medial malleolus of tibial distal extremity.

The deep digital tendon is actually formed by the union of the proximal metatarsal region of the thin medial digital flexor tendon with the lateral digital flexor tendon (Cauvin, 2011; Dik, 1993). The distal tendon of the long digital extensor muscle passes through the tarsus from the dorsolateral aspect, and it is enclosed in a synovial sheath as well. Focal lesions of the long digital extensor tendon can be diagnosed ultrasonographically but are mostly not associated with lameness (Barone, 2010; Ross and Dyson, 2011).

1.2.1.4.10 Tarsal joint capsule:

The tarsal joint is composed of four synovial compartments, the tarsocrural compartment lubricates the tarsocrural joint along with the dorsal aspect of the proximal intertarsal joint and this is the largest component having four pouches: dorsomedial, dorsolateral, plantaromedial and plantarolateral. The proximal intertarsal pouch communicates with the tarsocrural joint dorsally and also lines the talus and calcaneus proximally and the plantar aspect of the central and third tarsal bones distally. Any loose osteochondral fragments can move freely between the tarsocrural and proximal intertarsal joint as there is a large fenestration present in between these two joints, so any problem or disease of one compartment would necessarily involve the other compartment as well (Ross and Dyson, 2011).

The PIT joint communicates with the tibio-tarsal synovial pouch, and such communications are also found in some other joints to some extent but not necessarily bilaterally in the same horse. These communications are of great importance regarding the spread of medication or disease. The involvement of more joints requires more attention as disease of one joint may affect other compartments as they may function like one. As the location of the joint capsule is superficial so they are more prone to injury, trauma, infection and penetration of the tarsus (Kainer, 2002; Serena et al., 2005).

There could be different methods of spread of substances like diffusion in the absence of frank communication as well (Martin Gough & Munroe, 1998; Serena et al., 2005). It was demonstrated in 3 % of live horses that the communication between the tarsocrural joint and distal tarsal joint can be evaluated through the injection of radiodense contrast medium (Dyson & Romero, 1993). However, in cadaver studies injection of mepivacaine in the

centrodistal joint or tarsometatarsal joint could spread to the tarsocrural joint in 88-92% of limbs within 15 minutes (MR Gough, Munroe, & Mayhew, 2002).

1.2.2 Assessing frequent pathologies in the equine tarsal joint

In considering the potential adaptive changes of the tarsal joint, it is first necessary to recognize the bone types comprising this joint. The adaptations of bone driven by a dynamic loading, and only a short duration of loading is necessary to induce an adaptive response (Turner, 1998). As per 'Wolff's law', the bone in a healthy animal adapts remodelling according to loading in response (Wolff, 1892).

Ducher et al. (2005) found an increase in trabecular bone density in response to exercise in the forearms of tennis players (Ducher et al., 2005). The bone volume fraction, trabecular number and trabecular thickness were found to increase with no changes in overall external dimensions of the distal tibia of sheep (Barak, Lieberman, & Hublin, 2011).

In normal horses, the radiopharmaceutical uptake was found to be greater on dorsal and lateral aspects in the distal tarsus, suggesting enhanced adaptive bone modelling in response to a high loading pattern (Rachel C Murray, Dyson, Weekes, Branch, & Hladick, 2004). While incomplete ossification of cuboidal tarsal bones contribute to musculoskeletal abnormalities like angular limb deformities in foals (Caron, 1988).

The axial deviation of a limb (varus or valgus) is referred to as angular deformity in the frontal plane distal to some point of origin (Figure 1.6). This angular deformity can result from incomplete ossification of the third tarsal bone, laxity of the supporting soft tissues or hypoplasia of the central tarsal bone or as abnormalities of epiphyseal and metaphyseal configuration in neonatal foals (Auer et al., 1982; Leitch, 1985; Caron, 1988).

The type of exercise or training, intensity of exercise or training and the loading pattern modulate the subchondral bone and cartilage thickness in the central and third tarsal bones. The thickness differences from medio-laterally reflect the loading (Branch, Murray, Dyson, & Goodship, 2005; Rachel Clare Murray, Branch, Dyson, Parkin, & Goodship, 2007). The distal tarsal joints absorb energy in the early phases of stance which is used to contribute to propulsion in the later stance phases (Dyson & Ross, 2011). In horses having some structural problems or feeling pain in any of the limbs, the affected limb became unloaded and contralateral limbs had to bear varied amounts of vertical forces to support the body weight (Ishihara, Bertone, & Rajala-Schultz, 2005; Starke, May, & Pfau, 2015; Weishaupt, 2008). As a result, the body moves asymmetrically between the two steps of one stride during hind limb lameness which presents a more challenging scenario for the pelvic movement pattern as well (Buchner, Savelberg, Schamhardt, & Barneveld, 1996; Faber et al., 2001).



Figure 1. 6: Angular limb deformity (valgus) in 2-month-old foal with LM and Plantarodorsal (PID) projections.

The incomplete ossification at time of birth leads a deformation and wedging of third tarsal bone, not defined boundaries of central tarsal and a triangular shaped fragment displaced at dorsal side Adapted from Vanderperren et al., 2009.

Tarsal valgus deformation was observed in some rare cases because of complete fibula formation in miniature ponies as indicated in figure 1.7 (Tyson et al., 2004).



Figure 1. 7: Plantaro-dorsal (PID) view adapted from Vanderperren et al., 2009 of a Shetland foal.

A persistent fibula (arrows) is present along with of the tibia and tarsus resulting in a valgus deformation and malformation of the tarsocrural joint.

1.2.3 Clinical relevance of the tarsal joint and its conformation:

A thorough understanding of the equine hock joint conformation and its variability is fundamental to distinguishing between normal and pathological conditions of this joint. Furthermore, it is of equal importance in monitoring and assessing pathological changes that may occur.

Tarsal conformation:

Better understanding of the relationship of conformation, movement and pathology is essential for decision making of future breeding, sound management and different training regimes (Stashak, 2002). The conformation refers to physical appearance and outline as dictated primarily by bone and muscular structures.

1.2.3.1 Importance of tarsus conformation:

The conformation of the tarsus is very important as it is closely related with its function (Gnagey et al. 2006). In hind limb lameness, the tarsus is one of the most commonly affected joints in horses (Brokken 1978). In horses, a small tarsal angle ($<155^\circ$) 'sickle hocks' or larger tarsal angle ($<165^\circ$) 'straight hocks' have been implicated in the aetiology of tarsal diseases (Stashak 1987; Eksell *et al.* 1998; Marks 2000). A small tarsal angle has been identified as a risk factor for plantar ligament desmitis (Stashak 1987) and for lameness of the hind limb (Axelsson et al., 2001).

The conformation of the tarsus may also have an impact on power generation by the equine hind limb, and ultimately affect performance of a horse in racing (Marks 2000), dressage (Holmström *et al.* 1995), showjumping (Holmström 2000) and working western stock horse

events (Black, 1992; Gnagey et al., 2006). There could be different factors which are significantly associated with hind limb lameness like sire, age and tarsal angle. There could be a number of environmental factors claimed to be injurious to the distal tarsus, such as the training and promotion of 4- and 5-year-old horses for stud shows (Axelsson et al., 2001).

1.2.3.1.1 Ideal Conformation

- Smooth and without obvious swellings
- Tarsus should be higher than the carpus
- Left and right tarsal joints = symmetrical
- Tarsal bones should be substantial; too light a structure increases the likelihood of injury.
- When observing from behind, the whole limb should appear straight and the tarsus should lie in this straight line. There should be no major angulation inward and outwards.
- Metatarsal bone should be near perpendicular to the ground when observing from the side.
- Angle of the right tarsal joint (Sagittal plane standing angle) should not be too wide (post-legged/greater than 165 degrees) or too angled (sickle hocked/less than 155 degrees).
- The range of hock angles is quite wide – a variety of angles can be present without causing lameness, it is the extreme ends of these angles that can pose a threat to soundness. Slight adaptations to angle can also benefit the horse, ie. increased tarsal angulation in dressage horses is beneficial to allow for collection potential.

- The positioning of the pelvis, femur and tibia all require analysing as they can affect the location of the tarsus.
- Deviation from the ideal conformation will place additional stress on tendons and ligaments.

(Eksell *et al.* 1998; Gnagey *et al.*, 2006; Getty, 1953; Marks 2000; Rooney, 1969; Stashak 1987; Vanderperren *et al.*, 2009)

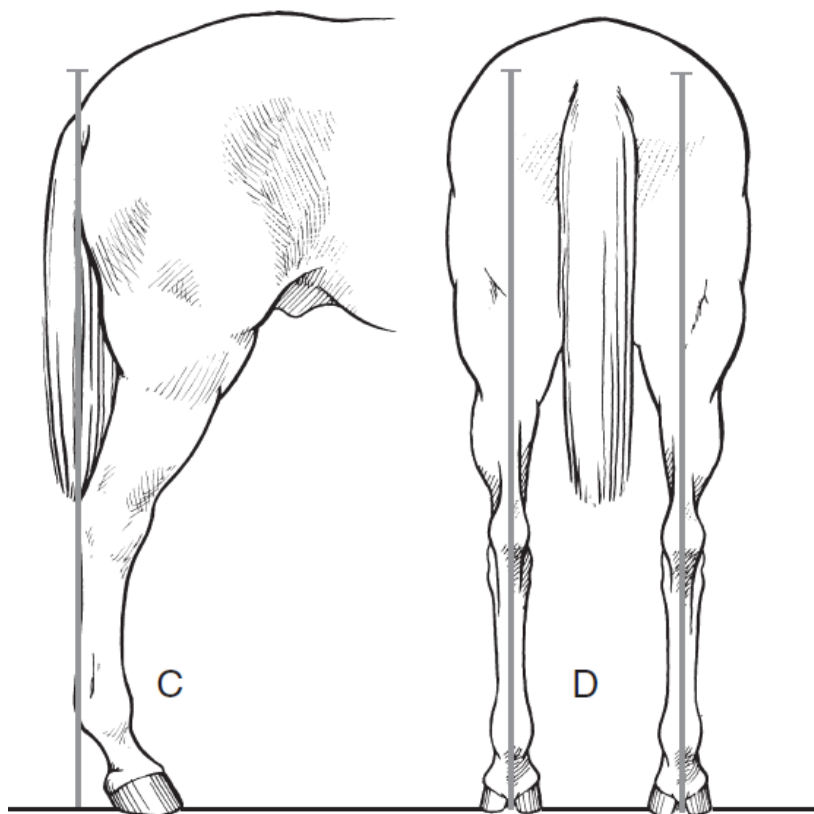


Figure 1. 8: Lateral (C) view of in right hind limb and plantar (D) views of “Ideal conformation” of hind limbs.

Line dropped from point of buttock (tuber ischii) should follow the cannon bone, meaning with a normal hock angle of 155°-165° (D) the line should bisect the limbs. Source:(Stashak & Hill, 2002)

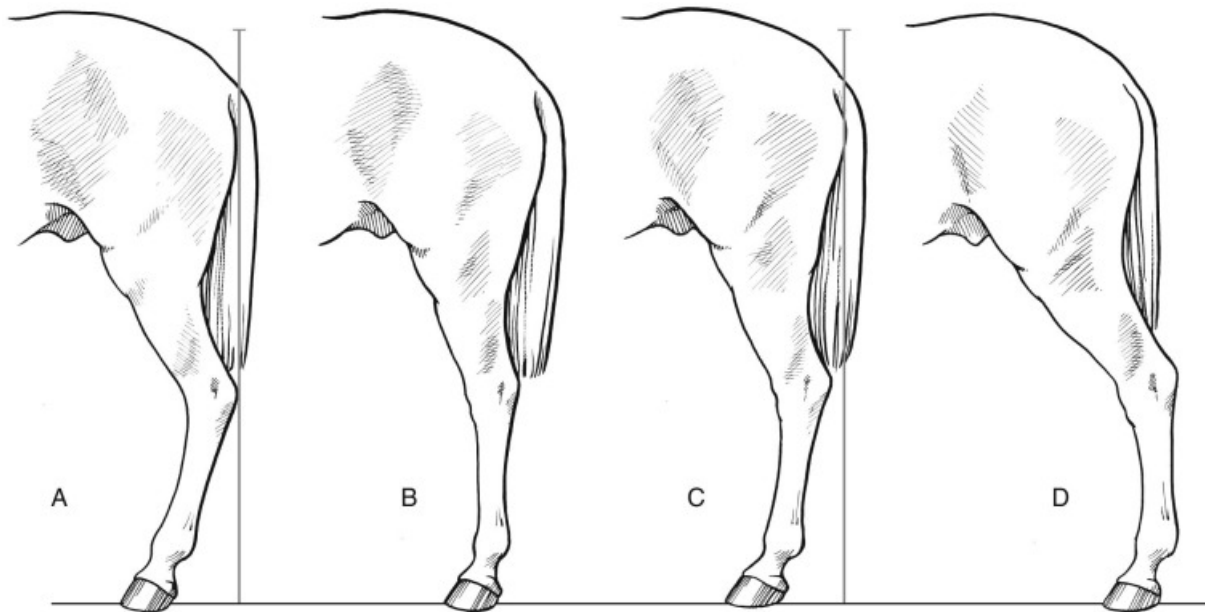


Figure 1. 9: Lateral (A-D) views of left hind limbs. Line dropped from point of buttock (tuber ischii) is not following cannon bone.

(A) Sickie hocked conformation with mean normal hock angle 150° - 153° , (B) concave dorsal surface, (C) under camped (D) camped out conformation. Source:(Stashak & Hill, 2002)

1.2.3.2 Factors that can disturb the tarsus conformation:

The factors which can contribute to enhance compression and rotation of the distal tarsals can predispose the horse to distal tarsal osteoarthritis (Baxter et al. 2003). These factors could be performance activities, such as jumping, dressage, and western performance; shoeing practices like shoes with outside trailers or calks; conformational defects like a straight or more angled tarsal joint; or pathology such as crushed or fractured tarsal bones or poorly developed tarsal cuboidal bones.

Any abnormality in tarsal conformation can lead to decreased performance and soundness and ultimately could result in lameness. The radiographic lesions in Thoroughbred yearlings about 2-3 years old have been correlated with their subsequent racing performance (Cohen, Carter, Watkins, & O'Connor, 2006; Kane et al., 2003). Horses with smaller hock joint angles often develop osteoarthritis of the distal hock joints and soft tissues injury and may be prone to the development of fractures of the central and third tarsal bones. Horses with larger tarsal angles may be less prone to tarsal pain but could be more predisposed to the development of suspensory desmitis. A decreased dorsal tarsal angle was associated with a high incidence of osteoarthritis and involved distal intertarsal and tarsometatarsal joints in Icelandic horses (Axelsson et al. 2001).

1.2.3.3 Conformational variations and deformities:

The alignment of the skeletal components provides a framework for the attachment of muscles. The hind limb bone structure and muscling should be healthy for the intended use. The relationship of bones lengths, angles of joint, and the overall height dictate the amount of power produced and type of action.

1.2.3.3.1 Lateral deviations:

A horse with a small hock angle means that, although the horse can step under its body easily, but it would not be able to support itself properly due to decreased resistance in the hock. Therefore, a normal hock angle is advantageous for the function of the hind quarters (Schmidt, 1928).

Hind limbs with more angulation have longer limb length and produce more vertical, folding action required for a high level dressage horse collection. The hock angle $<53^\circ$ are considered as sickle hock when viewed from the sides (Holmström et al. 1993). If the overall length of the hind limb is too long, it is similar to sickle-hocked conformation or camped out and it can be associated with these conditions (Stashak & Hill, 2002).

Angulated (sickle) hocks with an angle $<150^\circ$ are considered to be more prone to disease in the distal joints, and more likely to get a curb, most probably if there is a longer tuber calcis, but many can be effectively managed and will be sound. Extremely angulated racehorses may bruise their ergot regions and may be termed “run down” (Marks, 2000).

In a study by Holmström, The absence of sickle-hocked horses in the elite horse groups might be due to two factors, the small hock angles increased the risk of injury, or it may impair the ability to attain the level of collection required for good performance of a horse in advanced classes (Holmström, Magnusson, & Philipsson, 1990).

It is generally regarded as a handicap for a horse to have a large hock angle, whereas a somewhat smaller hock angle $<155^\circ$ is considered by Bengtsson (1983) to be not particularly detrimental to a horse's soundness and performance (Bengtsson, 1983). But in a study by Holmström, both groups of elite horses had larger hock angles than the other groups (Holmström et al., 1990).

A positive relationship was found between large hock angles and soundness in Standardbred trotters (Dalin, Magnusson, & Thafvelin, 1985), and various performance traits in show jumpers. The large hock angle might lead to rigid and incorrect hind limb action as well as an increased strain on the joints of the hind limb (Bengtsson, 1983; Forsell, 1927; Wrangel, 1911).

1.2.3.3.2 Plantar deviations:

Straight hocks were related to increased range of motion of the tarsus such as longer strides and swing duration. The gait quality was improved by maximising protraction and retraction (Stashak & Hill, 2002).

1.2.3.3.3 Medial (Cow hocks) deviations:

In this conformation, the horse's hind limbs are base narrow to the hock and base wide from hock to digits. This conformation is a common conformational deviation. In this condition the hocks became too close, pointing each other and the feet widely separated to one another. Cow hocked is believed to be a worse conformational deviation because of excessive stress on the hock joint, which might lead to bone spavin. In western performance horses a combination of sickle hock and cow hock was observed commonly (Stashak & Hill, 2002).

1.2.3.3.4 Movements of the tarsus:

The abduction of the distal part of the hind limb caused hock flexion in an oblique axis of motion as per oblique lateral orientation of the trochlear ridges of the talus combined with the major musculature all being lateral (Gnagey et al. 2006). In the swing phase outward rotation of the third metatarsal bone is followed by a small inward rotation during the stance phase when the hock is compressed. Thus, during the gallop both hind limbs move outside of the forelimbs and the tarsus flexes and moves backwards in the midline and extends before landing. There is a mechanism of locking in between the talus, central tarsal, third tarsal and third metatarsal (MtIII) by different processes and ridges on the different aspects of bones that fit into the corresponding fossae on the opposite articulating surfaces (Badoux, 1987). The lack of motion was observed by Schamhardt et al. (1984) in the small tarsal bones

during static loading. The oblique dorsal ligament originates from a small origin on the talus and extends distally; spreads across the central and third tarsal bones and is thought to play an important role in limiting the motion(Dyson & Ross, 2011).

Some studies of the tarsus have shown that greater compression on the disto-medial aspect of the tibia and the proximo- lateral aspect of the third metatarsal bone (MtIII), suggesting the transfer of compressive load from medial to lateral passing through the tarsus (Roony et al. 1969;Lindsay et al. 1982). In the progression of osteoarthritis of the centrodistal joint the subchondral bone sclerosis did not appear to be a primary factor but was considered to reflect an uneven distribution of biomechanical forces within the joint (Björnsdottir, Ekman, Eksell, & Lord, 2004).

1.2.4 Current commonly used methods for assessing joint pathological changes:

Tarsal joint injury is a major cause of serious problems and early retirement in performance horses. As such, disorders of tarsal joint should be detected as early as possible for their appropriate correction timely and to avoid any irreparable damage. The equine tarsal joint is one of the most affected regions of the hind limb associated with lameness, and diagnostic imaging is routinely applied (Vanderperren et al., 2009). Radiography is an important diagnostic aid, along with other more advanced diagnostic techniques such as ultrasonography, computed tomography, magnetic resonance imaging, nuclear scintigraphy or diagnostic arthroscopy. These should be used in combination with accurate history, physical exam or where radiography is insufficient and a more detailed diagnosis is necessary (Park, Wrigley, & Steyn, 2002; Akbar et al. 2019).

These methods can most often provide an accurate diagnosis either independently or in combination. However, early detection of any conformational deformity and other pathological changes which can lead to a clinical issue of tarsal joint is often limited by some factors such as costs, equipment and other factors associated with each of these methods. Hence, it remains that a relatively cheap and simple method of evaluation is needed to determine such changes at early stages to avoid further anomalies.

1.2.4.1 Geometrical Examination:

Geometry is a branch of mathematics for the studies of relationships and measurements of surfaces, lines and angles. The geometrical data of a human or an animal can represent their shapes and conformational variations for the quantitative assessment or analysis. Therefore, the

body shape or a particular organ or any body structure conformational studies have been an area of interest for many researchers in order to understand the real morphology.

1.2.4.1.1 Importance of geometrical studies:

The importance of geometrical studies can be assessed by its involvement in many studies of different organs and relations between organs in different species, within and between individual species. Therefore, there are a number of reasons for performing this type of study like:

1. Geometrical data can be used to identify effects of bone shapes on loading and force transmission through joints to assess the mechanical significance of the shape. This is an important utility for evaluating the capability of individuals to achieve great performances.
2. Geometrical studies can be used to assess the similarities and variations between and within individuals.
3. It could be necessary to determine the required surgical interference before starting an operation.
4. Geometrical studies can be conducted for prediction and prevention purposes for some clinical cases.

1.2.4.2 GEOMETRICAL STUDIES OF EQUINE TARSAL CONFORMATION:

Conformation is a major factor given serious attention during the evaluation of the purchase value of Thoroughbred racehorses, because horses with poor conformation may detract

from its value considerably and they are assumed to be more susceptible to injuries (Love et al., 2006).

However, the available parameters were insufficient to assess tarsal conformation in mature Thoroughbreds (Love et al. 2006). This limitation was attributed to problems or difficulties in using a quantitative and repeatable measurement techniques (Weller et al. 2006). Little objective information is available about the effects of tarsal conformation on joint movements or joint forces (Gnagey et al., 2006). Although the tarsus has different joints, intertarsal articulations and seven bones, most tarsal geometrical studies have been conducted to determine the angular hock deformity and some dimensions and their different ratios of different tarsal bones.

1.2.4.2.1 Tarsal angle:

Gnagey, Clayton and Lanovaz (2006) compared tarsal kinematics and kinetics in horses with different angles and classified them as large, intermediate and small tarsal angles in 16 horses as they stood squarely with the hind hooves vertically beneath the hip joint, when the sagittal plane standing angle of the right tarsal joint was measured.

The different tarsal angles were classified and determined by using reflective markers, on the lateral aspect of the right hind limb. The markers were overlying palpable bony landmarks to represent the segment angles and centers of rotation for the limb joints. The conformational measurements were taken from a standing file in which the horse was positioned in a standardized pose. The tarsal joint angles were classified as small, intermediate and large; the intermediate category was defined as the angles that were within one standard deviation (SD) of the group mean (Holmström et al., 1990).

The horses were trotted at a constant velocity (standardised for the size of the horse) over a force plate (Khumsap, Clayton, & Lanovaz, 2001). They classified tarsal angles as small ($<155.5^\circ$), intermediate ($155.5\text{--}165.5^\circ$) or large ($>165.5^\circ$). The calculation of net joint moments and net joint powers across the tarsus was done using ground reaction forces combining with morphometric data using inverse dynamics of joint angles. The tarsus was observed to flex during the impact phase and extended in the late stance of all horses. The angles of the tarsus were stratified related to the tarsal angle in a standing position throughout stance. The horses with large standing angles demonstrated less flexion and less energy absorption during the impact phase at the tarsus than those with intermediate or small angles. They also generated less vertical impulse than horses with small standing angles. The net extensor moment at the tarsus during the stance phase was lower for horses with large standing angles (Gnagney et al., 2006).

In a complex study Weller et al. (2006) investigated the relationship between conformation with occurrence of injury and performance in racehorses which were used for racing over fences. They evaluated 108 racehorses (over 2 years) and their medical and performance data was recorded. The conformational measurements were measured in 3D with a computerized motion analysis system. To evaluate the effect of conformational measures on the race performance, linear multiple regression models were used and for the assessment of risk of injury, logistic regression models were used. An increased intermandibular width, flexor angle of the shoulder joint and coxal angle (this angle was between the ilium and ischium) were demonstrated to have a positive effect on the performance measures. Performance was decreased with increasing girth, valgus conformation of the metacarpophalangeal (MCP) joint and the length of the hind digit. It was noted that increased

MCP joint angle and with carpus valgus conformation, the risk of superficial digital flexor tendon injury was increased (Figure 1.10). More importantly the valgus conformation of the tarsus increased the risk of pelvic fracture which decreased with an increasing coxal angle. This valgus conformational deformity was not only demonstrated to be detrimental to performance of the racehorses but also increased risk of injury, perhaps resulting in higher loads on musculoskeletal structures. The coxal angle was found to have an effect on both risk of injury and performance measures. A better alignment of the muscles along the axis of the pelvis might have better force transmission and decrease the bending moment of the muscles on the bones. This study provided preliminary data necessary for the planning of further larger scale studies on the effect of conformation on performance and risk of injury as it may aid in identifying good racehorses likely to perform well and avoid risks of injury. The lateral deviations of the tarsal joint as viewed from both the cranial and caudal aspects were associated with a high risk of tendonitis. This might have happened due to the fact that unevenly distributed forces acting on the musculoskeletal system during locomotion were extended across the limb in the lateromedial direction. The lateral deviation of the tarsus was also associated with an effusion in the digital tendon sheath and may again be related to loading asymmetry of the tendon sheath.

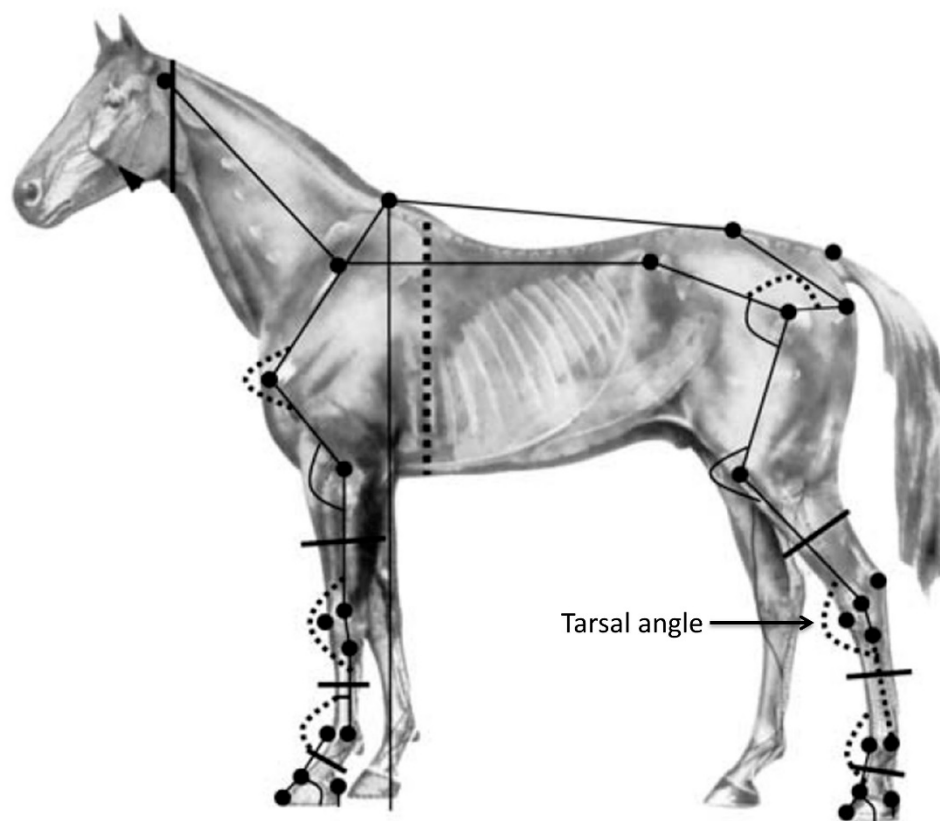


Figure 1. 10: Schematic diagrammatic representation of a horse, positions for markers, joint angles, segment lengths and circumference parameters (bold lines) are indicated used in this study, adopted from Weller et al. 2006.

The conformational parameters which contributed significantly to the regression models for musculoskeletal injuries or performance are indicated by dotted lines including the tarsal joint as indicated above.

1.2.5 Radiological assessment of tarsal conformation:

Radiography is still a readily available non-invasive and important diagnostic tool which is widely used both inside clinics and in the field. There exist a wide range of morphological variations between tarsus radiographs in clinic archives because of conformational variations among animals even within the species and in between species. Radiography was considered among the best diagnostic imaging techniques in the evaluation of bone and mineralized lesions in horses (Desmaizières & Cauvin, 2005). However, radiology has some limitations like in diagnosing some minor abnormalities such as damage in subchondral bone (Kawcak, McIlwraith, Norrдин, Park, & Steyn, 2000), articular cartilage (Moore & Schneider, 1995) and detection of some subclinical lesions or radiographic lesions that could have remained even after the recovery (Dyson 1988).

1.2.5.1 Standard views of radiology:

The tarsus radiographic anatomy is quite complicated but the clinically most important areas are localised in the dorsal half of the joint which is anatomically the least complicated region (Verschooten & Schramme, 1994). There could be different radiological projections to determine the radiological status of an area of interest but in some situations, time, and money or some other factors might limit the number of projections required. Sometimes only one projection is made. The degree of misinterpretation caused by using only one projection has not been evaluated (Eksell et al. 1998).

In equine radiography, a complete radiography of the tarsus includes usually four standard projections: dorsoplantar, lateromedial, dorsolateral-plantaromedial oblique, and

dorsomedial-plantarolateral oblique views. In order to enhance the visualization of a particular area of interest, some additional views may be required (Baird, 2002). In selected cases, flexed lateromedial and flexed dorsoplantar (also known as skyline) views provide valuable information or conformation detail (Butler, C. M. Colles, S. J. Dyson, S. E. Kold, & P. W. Poulos, 2008).

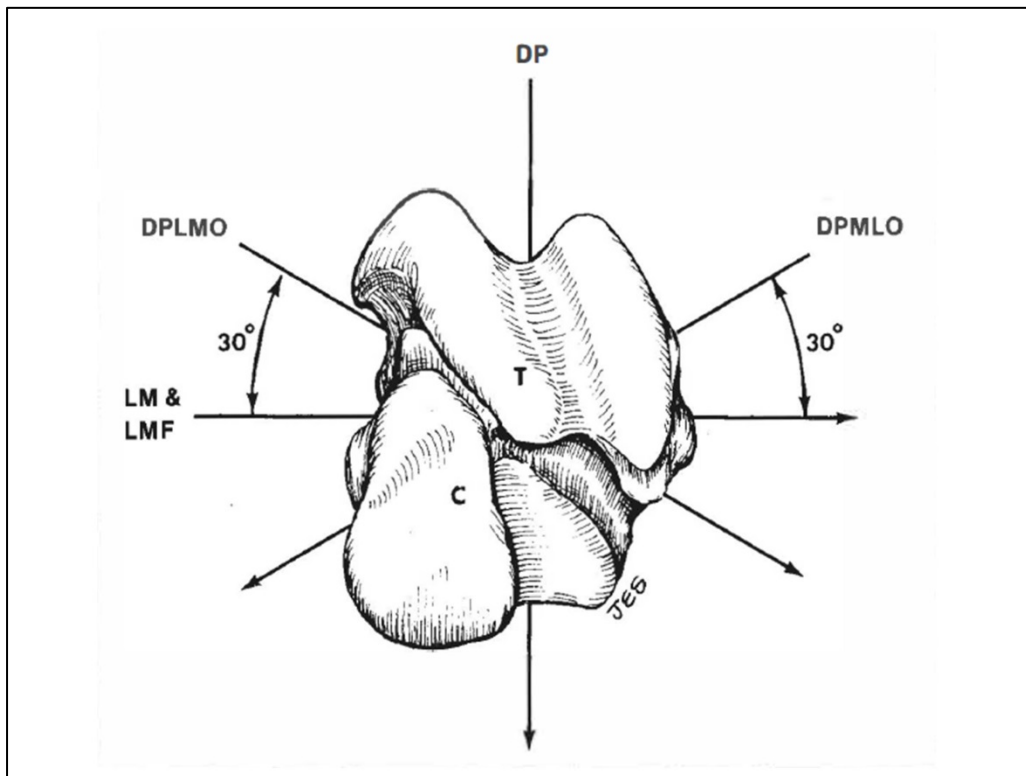


Figure 1. 11: Diagrammatic representation of the four standard views in radiographing, adopted from Shively and Smallwood, 1980. Drawing of proximal left tarsal bones of the horse (proximal aspect) to demonstrate the orientation of central x-ray in the four standard radiographic views.

The talus (T) and calcaneus (C) are indicated respectively. The dorsoplantar (DP) lateromedial (LM), dorsoplantar lateromedial oblique (DPLMO) and dorso-plantar mediolateral oblique (DPMLO) views are illustrated in respect of a central ray. The lateromedial view of the flexed tarsus (LMF view) orientation is also shown. The cassette is positioned perpendicular to the central ray for each radiographic view.

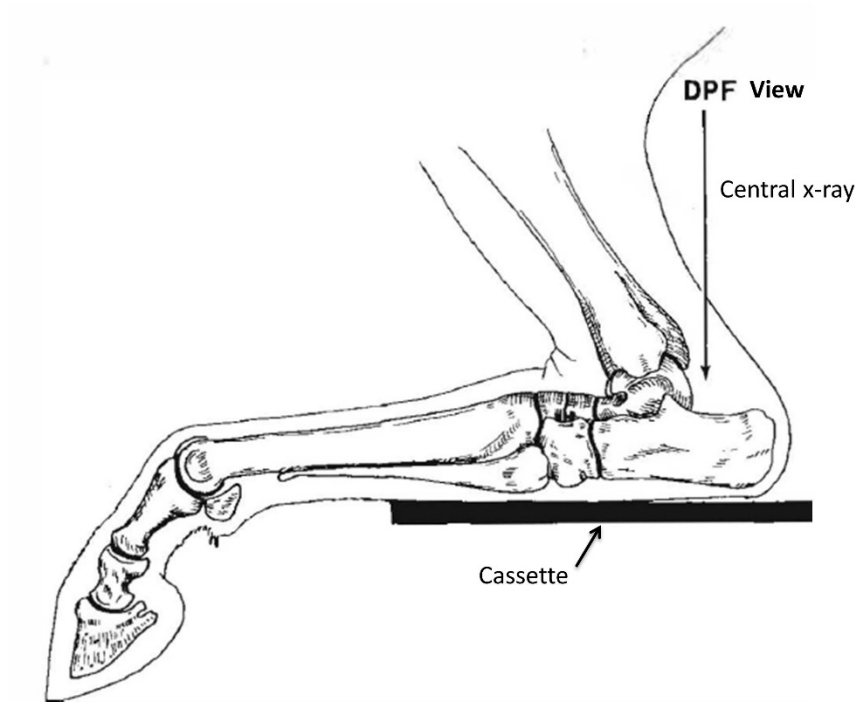


Figure 1. 12: Adopted from Shively and Smallwood, 1980. Drawing for the dorsoplantar flexed view of the tarsal region having calcaneus and talus with the flexed tarsus (DPF view) illustrating the limb, cassette and central ray orientation.

1.2.5.2 Tarsal bone dimensions:

Dahn and Ueltschi, (1989) in their study evaluated three normal radiographic views of the tarsus from 270 horses from different breeds (like Irish (23.3%), the Swiss half-breed (19.3%), the Selle-Français (14.8%), the Germans (12.5%) and the Dutch (10.0%)) including dorsoplantar (DP), lateromedial (LM) and oblique dorsolateral-plantaromedial (30° Obl) of each tarsus or “hock”. The study included mainly young and sound horses to determine the radiological evaluation of normal equine hocks. The mean dimensions of certain tarsal bones were measured; the geldings had greater bones dimensions as compared to mares. There was an increase of reactions at the origin of the M. interosseus with relation to the age. In their study they also determined that medichrome films increased the number of discrete abnormalities determined. This is of particular importance for the purchase evaluations of young horses. The central beam (angle) direction is also an important consideration in order to get symmetrical radiographs. They found a minimal change in this angle can expose or hide many discrete abnormalities. They also described the normal radio-graphical anatomy of the equine tarsus and its principal variations.

There was a slight difference between bone dimensions of right and left (larger) which was not statistically significant. They evaluated different ratios from these dimensions. The width of the talus was 73% of its height. The width of the calcaneus was slightly less than half its height. The widths of the talus and calcaneus were quite similar. The mares had a smaller talus and calcaneus than the geldings, although the differences were small but considering the large number of animals, they were statistically significant. The heights of the central tarsal bones and the third tarsal bone were slightly lower in the mares as compared to the geldings respectively. On the other hand, the average thickness of the subchondral bone

plates of these bones were identical for the two genders. Anomalies were rare in tarsal bones and their occurrence was symmetrical.

The shape of the calcaneus was evaluated on the lateral radiographs; a clear majority (84%) of the tuber calcanei were double, single (16%), flattened at top. There was a single horse with convex top (unilaterally). A clear majority (87.2%) of the cases present had fused first and second tarsal bones but some bones (3.7%) were perfectly separated. The comparison of radiographs of the same horse, taken very slightly displaced by the angle (5 °) or the height (5 cm) of the alignment of the central ray showed good reproducibility.

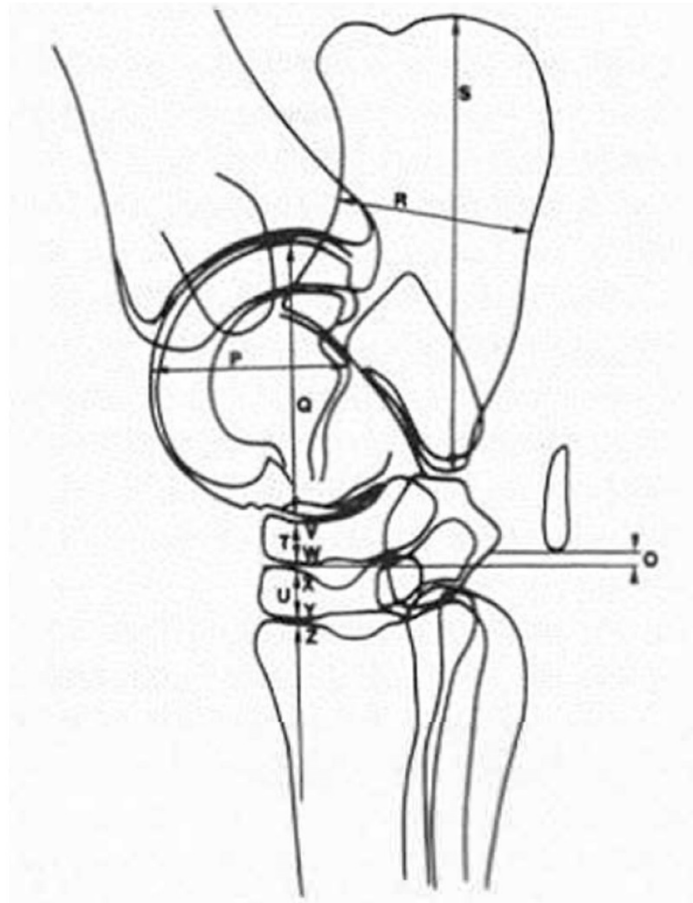


Figure 1. 13: Diagrammatic representation of the radiographic lateral view, for measuring points for bone dimensions adopted from Dahn and Ueltschi, (1989).

The diagram indicates different points like O: Distance distal intertarsal articular distance - chestnut; P: width of the Talus; Q: height of the Talus; R: width of the Calcaneus; S: Calcaneus height; T: height of the central tarsi bone; U: height of the tarsal bone tertium; the metatarsal bone subchondral osseous bone of the central tarsi bone proximal (V); distal (W) proximal (X) and distal (Y) third tarsal bone and third metatarsal (Z).

1.2.5.3 Other geometrical dimensions:

Barr (1994) measured the carpal angle on lateromedial (LM) radiographs by identifying the longitudinal axes of the radius and the third metacarpus. For the longitudinal axis of the radius, the craniocaudal diameter was measured at two points proximal to the antebrachiocarpal joint. The first point was taken at a distance of 6 cm and the second was at a distance of 12 cm proximally and the mid points were established.

For the longitudinal axis of the third metacarpal, two points were again taken on its long axis at distances of 6 and 12 cm distal to the carpometacarpal joint. Then a straight line was drawn passing through the two midpoints of the radius. Similarly, another line was drawn through the two midpoints of the third metacarpus. The angle formed where the two longitudinal lines met was measured from the dorsal aspect. This study was also conducted precisely for the evaluation of the effects of rotation on the radiographic angle. This study included two main components of this angle which were the longitudinal axes of the radius and third metacarpus, but any bony component of the carpal joint was included. This might lead to changes in the carpal angle even if any deformities in either or both of these long bones. In this study, the carpal angle in normal horses was $176^{\circ} (\pm 0.8^{\circ})$ while in horses with chip fractures it was $175^{\circ} (\pm 0.6^{\circ})$. The study concluded that there was no relationship between back at the knees and the prevalence of chip fractures in horses.

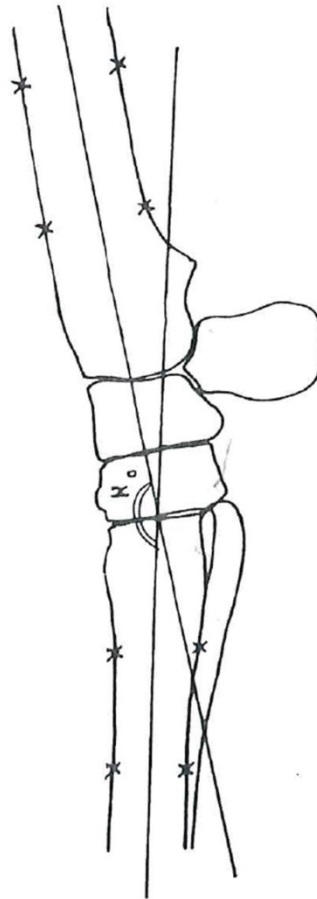


Figure 1. 14: Diagrammatic representation of the radiographic lateral view, for assessment of carpal angle = x degree adopted from Barr, 1994.

Caron (1988) suggested a geometric evaluation of the radiographs by drawing lines on a clear acetate sheet placed over the radiograph looking at deformities in the frontal plane. The angular measurements can be taken at lines bisecting each other from the long bone above and below the involved joint. The use of long radiographic cassettes like 17.5 cm x 42.5 cm in dimensions whenever possible, optimise this evaluation. The point of intersection of two lines can be defined as 'pivot point' of the deformity (Figure 1.14). This point corresponds to the location of the very important site of abnormal development. In general interpretation, a pivot point in the proximal epiphysis of a long bone or metaphyseal growth plate (physis) bears a more favourable prognosis as compared to a pivot point located within a joint. The

severity of the angular limb deviation can be calculated by measuring the angle at the same point of intersection of two lines. The measurements can be affected by kind of study as inconsistency in either position of limb or projections might substantially change the calculated measurements.

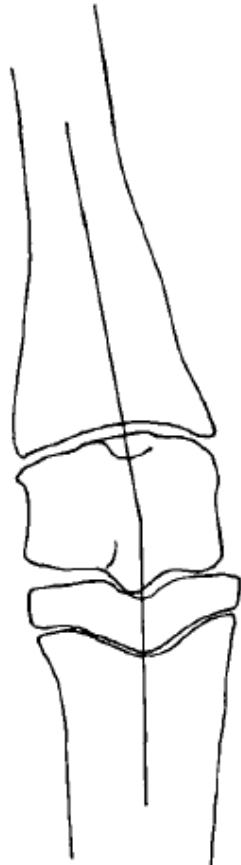


Figure 1. 15: A dorsoplantar radiograph (Schematic representation) adopted form Caron (1988) of the metacarpophalangeal region of a foal, illustration of geometric evaluation of an angular limb deformity. The lines bisecting the long bones above and below the affected area of abnormal development or growth should be noted.

However, the lines have not been standardised in this study and in any protocol of radiographic evaluation because the lateromedial midpoints of these bones cannot be joined by a straight line. The deformities are very difficult to define accurately in radiographic projections as the third metatarsal and the distal tibia are not arranged in the same frontal plane (Clegg, 2003).

Geometric analysis should be followed by morphologic analysis (Bertone, Turner, & Park, 1985; Caron, 1988; Pharr & Fretz, 1981), where abnormalities in the bones or either in soft tissues of joint may be determined as illustrated in figure 1.16 below. If a pivot point is present within the articulation in foals with carpus valgus, a careful examination of the films for abnormalities in the bones comprising the joints might be quite helpful as cuboidal bone abnormalities had a less favourable prognosis. For the evaluation of the cuboidal bones of the tarsus, lateral projection is best utilized.

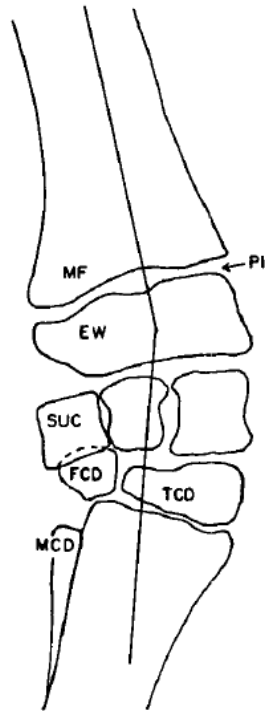


Figure 1. 16: The abnormalities that might be observed on morphological evaluation of carpal radiographs (schematic representation) from foals with angular limb deformities, adopted from Caron (1988).

Many abnormalities may be observed in the same foal; MF-flaring of the metaphysis; PI physeal irregularities; EW wedge shaped epiphysis; SUC displaced (subluxated) ulnar carpal bone; FCD fourth carpal bone dysplasia; MC'D fourth metacarpal bone displacement; TCD third carpal bone dysplasia.

In a study in human tarsus (Vanderwilde, Staheli, Chew, & Malagon, 1988) radiographs were made of the feet of seventy four normal infants and children (6-127 months) and various tarsal angles were measured. For selective eleven clinically useful angles, their means and deviations of the measurements were calculated. Different angular measurements like the talocalcaneal, talus first metatarsal, and calcaneus-fifth metatarsal angles were recorded on the anteroposterior radiographic view.

The angular measurements on the lateral radiograph included the talocalcaneal, tibiocalcaneal, tibiotalar, talus-first metatarsal, and talohorizontal angles. The lateromedial (LM) radiographs were made with the foot in maximum dorsiflexion, and different angular measurements like talocalcaneal and tibiocalcaneal angles were documented and the talocalcaneal index was recorded. The mean values and the ranges of normal measurements changed according with age. There was a decrease in the talocalcaneal index which was dependent on age.

That study provides a standard for assessment of initial and residual deformity in patients especially with some deformities like club foot, vertical talus, metatarsus adductus, or others. In a radiographic study (Simons, 1977) a method was described for making the diagnosis of talonavicular subluxation before ossification of the navicular bone occurs. There are seven basic combinations of deformities thought to occur in deformities like the club foot. The radiographic evaluation before an operation enables the surgeon to understand which of these combinations exists in a particular foot. The radiography during the operation again helps to determine whether or not all the existing deformities have been corrected, and therefore directs decision making for extension or any corrections of the operation.

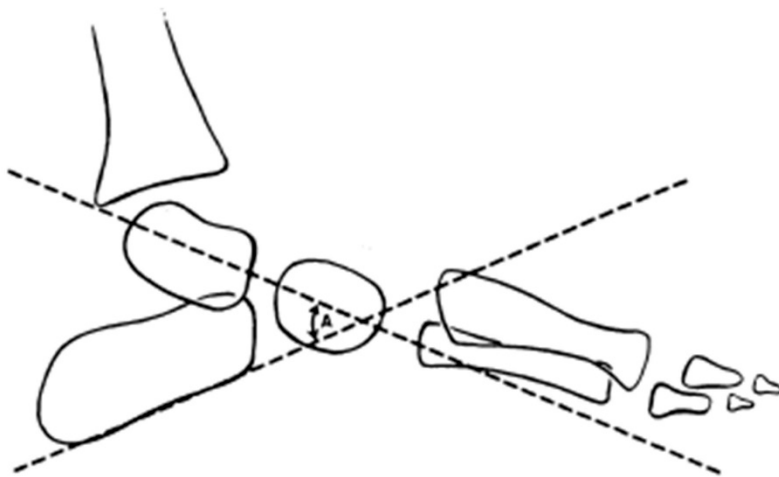


Figure 1. 17: Diagrammatic representation of the radiographic lateral view, for assessment of deformities adopted from Simmon, 1977. A side drawing of the foot, the talocalcaneal angle (A) is measured by drawing lines through the longitudinal axis of the talus and long axis of calcaneus along the plantar surface. The normal range (35 to 55 degrees) was determined for this angle in this study.

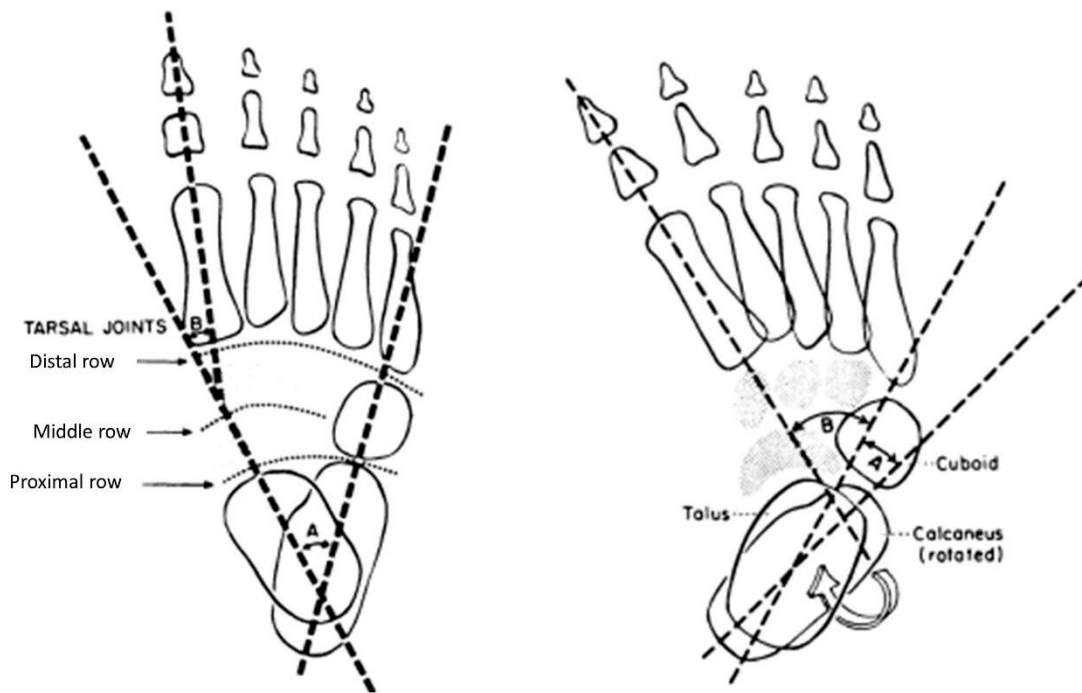


Figure 1. 18: Diagrammatic representation of the radiographic lateral view, for assessment of deformities adopted from Simmon, 1977. Drawing of a normal foot (left side).

The cuboid blocks medial movement of the foot at the middle row of tarsal joints because of its unique location: it alone occupies a position in both rows of tarsal bones. The talocalcaneal angle (A) is measured by drawing lines through the long axis of both these bones. The normal range for this measurement is 20 to 40 degrees in the young child. The talo-first metatarsal angle (B) is measured by drawing lines through the long axis of the talus and along the long axis of the first metatarsal. The normal range was 0 to -20 degrees. Drawing of a foot with hind foot varus (Right side), as manifested by a decreased talocalcaneal angle (A), and talo-navicular subluxation, as manifested by both hind foot varus of less than 15 degrees and a talo-first metatarsal angle (B) of more than 15 degrees. Talo-navicular subluxation occurs through the medial movement of three bones which move as a unit. The navicular, cuboid and calcaneus move medially through the combined movements of medial translation and supination of the proximal tarsal bones and inversion of the calcaneus beneath the talus in this study.

1.2.5.4 Factors affecting radiographic details used for geometrical measurements:

There could be misalignments of the tarsus, cassette and the x-ray machine during radiography. Therefore, this radiographic variation might need to be investigated.

The lateromedial or dorsopalmar radiographs are two dimensional images so radiographic measurements could be taken from two dimensions and these could be affected by rotations in two directions, these rotations might be around vertical or horizontal directions (Major et al. 1996). The rotation might affect the measurements in both vertical and horizontal axes, as the rotation around the vertical axis during radiography affect the horizontal parameters or measurements as indicated in studies of skull radiography (Ghafari et al. 1995). Based on this fact, evaluating any tarsal lateromedial and dorsopalmar view, should be done by detecting and assessing the presence of any rotation around these axes.

Radiography is highly sensitive to any change in the orientations of the object, rotation of cassette or the radiography machine. In the tarsus, despite the use of various techniques to achieve good LM and DP views, the various radiographs which are available in different clinical archives demonstrates a wide range of apparent variations in morphology/ the alignment or between the different tarsus among various animals of even the same breed. These variations can be seen between breeds, individuals, right and left tarsus of a single individual or even in the same tarsus.

In most of the cases of angular limb deformities, radiographic examination of the affected area is recommended. For good interpretation, it is of prime importance to obtain a true dorsopalmar or dorsoplantar weight bearing projection to minimize rotational effects as unnatural positioning or oblique projections might complicate interpretation. Most of the limbs with angular deformities have sufficient inherent rotational deviation that it might be difficult to obtain a dorsopalmar view of the entire affected limb regions. In this case, for

good interpretation of bones comprising the joint, a dorsopalmar projection at the level of the joints of the involved area should be taken (Caron, 1988).

This wide range of variations in morphology between tarsal joints and even the same tarsus if radiographed twice with some interval seemed to be a hindrance to a clear distinction between normal tarsal morphometry and different tarsal pathological conditions or any defects in conformations of the tarsus. In radiographic technique, the other problem might be the inconsistency that might occur due to misalignment between the object or cassette and the radiography machine, these misalignments have been shown to affect the accuracy of radiographic measurements (Yoon et al. 2001; Han and Hurd 2005a).

Many studies have been conducted to identify the effect of the rotation of any object orientation on its different parameters, which might be a resultant error of misalignment during radiography. The effect of head orientation on cephalometric measurements in postero-anterior radiographs was determined in humans (Major et al. 1996; Yoon et al. 2002). Also, Lonner et al. (1996) and Radtke et al. (2010) investigated the effect of limb rotation on the radiographic alignment of total knee arthroplasties on synthetic femur and tibia (Sawbones) for human study purposes. These kinds of studies ended up with an acceptable range of object rotation through which the parameters did not demonstrate significant errors. Furthermore, some of the studies even offered special ratios to recalculate measurements on the rotated radiographs in order to reduce the requirement to repeat radiographs (Radtke et al. 2010) hence this might be also quite helpful in equine tarsal radiography.

1.2.6 Conclusion:

Frequent problems and pathological disorders have continued to be most frequent causes of lameness of the equine tarsus. There is insufficient information available in current literature on the equine tarsal functional anatomy, the mechanism of loading biomechanics and the pathogenesis of damage or pathological disorders. Such information of tarsus is significant and needs to be addressed. Such problems causing huge economic losses to the equine industry not only in Australia but also worldwide. Thus, require a comprehensive further investigation to attain better wide understanding of the pathogenesis of tarsal damage and best managements to prevent such damage.

1.2.7 Study objectives and research hypotheses

The current experiments reported in this thesis were conducted in order to address some of the knowledge gaps in our current literature of equine tarsal functional anatomy and loading.

The core objective of the study was to design and test different methods for determination of conformation, internal alignment and response to loading (including the role of soft tissues) to maintain better tarsal joint stability.

A number of secondary objectives needed to be reached in order to gain the core aim.

They were to:

- Specify the morphological characteristics of all the constitutive bony components of the tarsal joint.
- Establish some linear measurement and identify reliable landmarks of the bones of the

equine tarsal joint which can provide a clear depiction of orientation and alignment on acquired radiographic images.

- Define consistent equine tarsal lateromedial and dorsoplantar views considering both horizontal and vertical axes.
- Develop tarsal measurable parameters on both lateromedial and dorsoplantar radiographs including a reference structure or line.
- Identify the effect of limb rotation or radiography machine positions around the vertical and the horizontal axes on the developed tarsal parameters.
- Determine an acceptable range of rotation through which the parameters presented the minimum amount of change.
- Evaluate the reliability of the developed tarsal parameters.
- Determine any changes of internal alignment of tarsal bones in response to loading through tarsal parameters.
- Observe the role of the soft tissue (including ligaments and fascia) in the loading biomechanics, load redistribution, stability, and tarsal joint range of motion.
- Assess the specific tarsal morphometry by developing 3D computer modelling.

Chapter 2: Morphometrical study of the bony components of the tarsal joint in horses

2.1 INTRODUCTION:

There is scant morphological data for equine tarsal bones despite the frequent pathology in many sport horses (Gabel, 1980; Lanovaz et al., 2002; Winter, Bruns, Glodek, & Hertsch, 1996). Although some studies gave brief information on bone morphology (Dyce et al., 2010a; Getty, 1975a; Liebich et al., 2004; Sisson, 1917), none looked at morphometric measurements. The other reasons that morphometrical data are scant in the literature may be due to some difficulties associated with meaningful measurements of complex shapes of bones, and also the problem of making such measurements consistent and repeatable.

Different researchers used various methodologies to measure bone dimensions using different materials according to their convenience. For instance, some authors used reliable traditional tools such as callipers and rulers to take their measurements directly from the bone or some parts of specimens (Davis, 1996; Guintard & Lallemand, 2003; Nacambo, Hässig, Lischer, & Nuss, 2007).

Some authors also measured the lengths or widths directly on the bone specimens using standard field-measurements and simple procedures or using other tools like the osteometric board (McFadden & Bracht, 2005).

Many researchers photographed the horse with different skin markers placed on them at different points to measure some of the joint angles and also some bone dimensions (Anderson, McIlwraith, & Douay, 2004; Crevier-denoix et al., 2001). The measurements have

also been taken from digital photographs by cutting surface impressions on paper (paper print) Nacambo et al. (2007), but previous studies of Fuller et al. (1997) and Nielsen et al. (2003), demonstrated that moving of the skin reference points with relation to their landmarks of the bone could result in significant errors and effect the real measurements (Fuller, Liu, Murphy, & Mann, 1997; Nacambo et al., 2007). The other challenge could be changes in the orientation or alignment between the camera and horses, thus rotations like movement of skin markers and alignment of cameras can influence the accuracy of these photographic measurements.

The use of radiographic images to measure bones in human and animal bones has been reported in several studies (Aydinlioglu, Akpinar, & Tosun, 1998; Livshits, Yakovenko, Kletselman, Karasik, & Kobylansky, 1998; Walter & Davies, 2001; Watson, Stitson, & Davies, 2003). At the same time, the possible changes in the focus-film, orientation differences and object to film distances can lead to change in magnifications of the subjects on the acquired images or film (Curry, Dowdey, Murry, & Christensen, 1990; Walter & Davies, 2001).

In addition to this, Bonnel et al. 2011 considered radiographic measurements, as being variable according to the X-ray beam inclination, and hence having no real value on their owns so should be accompanied by biometric analysis (Bonnel, Teissier, Maestro, Ferré, & Toullec, 2011). The identification of reliable landmarks within the tarsal joint on acquired images would potentially help in the understanding of essential conformational details of this complex joint.

Detailed knowledge of these morphometric data could be useful for identification of some reliable landmarks for all essential research on conformation including all the bony structures of the tarsus and thus provide a way to provide a clear depiction of orientation and alignment even on acquired radiographic images.

This morphometric analysis was aimed to

- 1) Specify the characteristics of all the constitutive bony components of the tarsal joint.
- 2) Establish linear measurement and identify reliable landmarks of the bones of the equine tarsal joint, which may then be used to investigate and measure radiological images to assess the tarsal conformation in future studies.

2.2 MATERIALS AND METHODS

2.2.1 Animals

Right and left distal hind limbs were cut at the stifle joint and collected from horses that were euthanized, slaughtered or had died for reasons not related to this study. Bones showing any gross orthopaedic lesion were rejected. The samples used in this study were collected from the Laverton knackery and the postmortem room of the department of pathology, The Faculty of Veterinary Science of the University of Melbourne.

The specimens were collected from 7 Thoroughbreds, 1 Standardbred and 2 Ponies. Ages of these horses varied and ranged between 2 and 13 years old. The hind limbs were collected by transecting them distally at the level of the fetlock joint to collect complete metatarsal bones. This avoided any operator induced damage to the distal extremities of the third metatarsal bones.

2.2.2 Instruments

In the current study the following instruments and materials were used:

1. Steam boiler (Athena Equipment, A.E. Atherton and Sons Pty.Ltd).
2. Laboratory digital thermometer (U-lab Instruments, Eltham, VIC).
3. Dissecting instruments (scalpels, dissecting scissors, butchering knives and surgical hammer).
4. Drying cabinet (Brownbuilt, Deluxe Dryer).
5. Cloth bags.
6. Nylon scrubbing brushes.

7. Vernier calliper (MATUIDOKI Co. Ltd, Japan)
8. Two rulers (18cm and 38cm in length), both graduated in mm.
9. Plastic flexible tape graduated in mm. (IKEA, Melbourne, Australia)
10. A custom-built apparatus.

2.2.2.1 The custom-built apparatus:

This apparatus consisted of six plastic parts: three main and three accessory parts adopted (Alrtib, Philip, Abdunnabi, & Davies, 2013). The main parts included a fixed base and two gliding arms. The base (Figure 2.1) consisted of a flat quadrilateral surface (floor) and two unequal-sized L-shaped walls.

The larger L-shape formed the outer walls on one long side of the apparatus and approximately two thirds of one short side of the apparatus. The smaller L-shape formed the inner walls of the apparatus and was fixed on the floor inside the large L-shape with its sides parallel to it.

This placement of the walls created a short path and a long path and a common space at the intersection of the two paths. The long path represented the space between the two long sides of the two L-shaped walls. The short path lay between the two short sides of the two L-shaped walls. Two stainless steel rulers were fixed one on each upper edge of the internal surfaces of the two sides of the large L-shape. A line perpendicular to the floor was drawn on the inner surface of the short side of the large L-shaped wall to mark the centre of the long path.

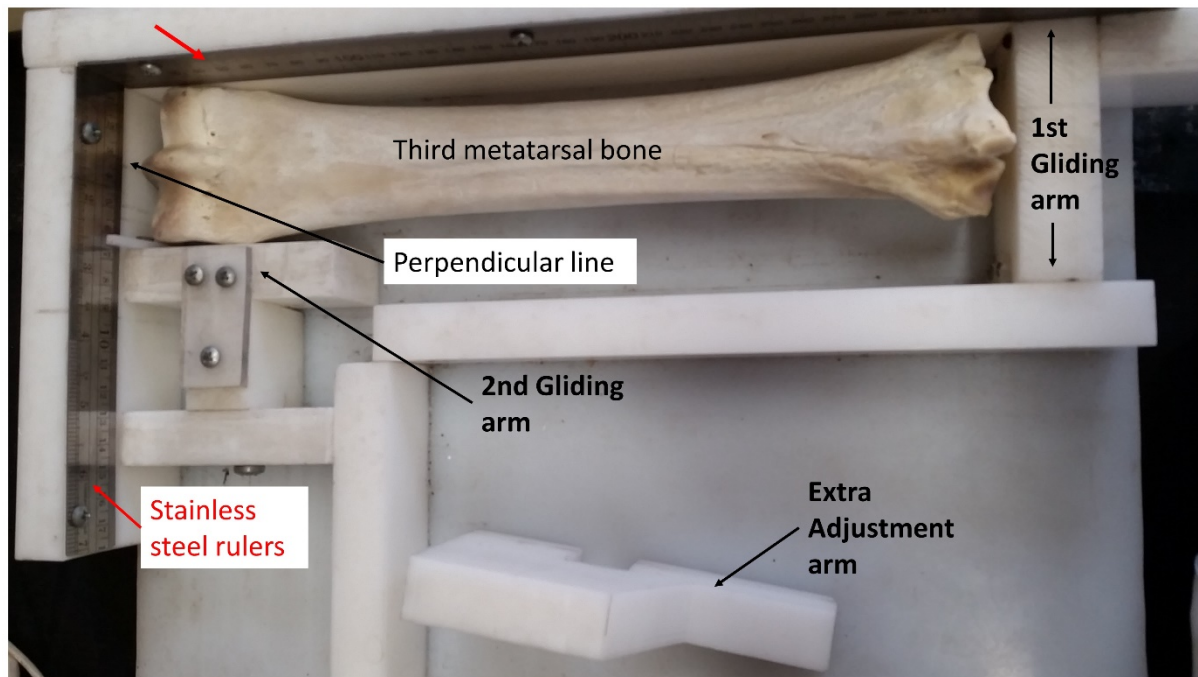


Figure 2. 1: The base of the custom-built morphometrical apparatus used for morphometric measurements (as right third metatarsal placed showing the plantar aspect with proximal extremity near 1st gliding arm) with the two gliding arms.

2.2.3 Medial and lateral heights of third metatarsal:

The consistency and difference of the heights of the medial and lateral landmarks was tested. Sixteen limbs were used in total from eight horses. All the bones were prepared by boiling after following routine bone preparation protocol. After preparation all the second and fourth metatarsals were detached from the third metatarsal bone. The ruler and gliding arm of custom-built apparatus were used for these measurements (Figure 2.11). Two perpendicular lines were used to ensure the good positioning and stability of the bones inside the apparatus during measurements.

Two measurements were taken from each Mt3, the medial and lateral heights. The medial height was measured from the medio-plantar edge of the facet for the second tarsal at the proximal articular surface of the third metatarsal where it articulated with the dorsomedial aspect of the second metatarsal to the medio-plantar edge of the articular surface of the medial condyle of the distal extremity of the bone. The lateral height was measured from the latero-plantar edge of the facet for the fourth tarsal at the proximal articular surface where it articulated with the fourth tarsal to the lateral edge of the articular surface of the lateral condyle of the distal extremity of the bone. All measurements were in centimetres (cm) as shown in Table 2.1.

2.2.4 Statistical analysis:

The bones measured 5 times with the operator blinded to previous measures, were used to enhance the reliability of bony measurements. A paired t-test was used to compare left and right limbs, and to compare medial and lateral sides in individual limbs. A P-value <0.05 was regarded as statistically significant.

2.3 RESULTS:

2.3.1 DISTAL EXTREMITY OF THE TIBIA:

The distal extremity of the tibia was much smaller compared to the proximal end, being quadrangular in form and having an articular surface (cochlea tibiae) which articulated with the trochlea of talus. It consisted of two grooves separated by a ridge directed obliquely craniolaterally in which the lateral groove was shallow and wider compared to the medial one and marked by a demarcation line or groove between where the tibia and fibula articulate. A shallow synovial fossa was located on the middle of the articular ridge in most of the samples.

The medial malleolus was much more distinct and formed the cranial border of a groove called sulcus malleolaris (for passage of the tendon of long digital flexor) but it was not as deep as compared to the groove of lateral malleolus. As the lateral malleolus was broader and also marked by a vertical groove (for passage of the tendon of the lateral digital extensor) it could also be used as a demarcation or separation point of the two lateral collateral ligaments (Figure 2.2 and 2.3) on the lateral malleolus.

The distal end of the tibia was bounded on either side by the latero-medial malleoli, to which the collateral ligaments of the tarsus attached. The tibia of the leg serves as a major functional component of the skeleton of the hind limb. Its shaft was thickly covered by muscle craniolaterally and caudally and was subcutaneous medially. The distal articular surface of the tibia is known as the cochlea (Figure 2.2) which had a craniolateral inclination, and which was comprised of two grooves separated by a ridge with thick support of the malleoli at each end of the lateral and medial aspects.

The fibula was a much-reduced long bone located at the lateral border of the tibia. The proximal part of the fibula remained isolated and only the head articulated with the tibia. The line of fusion of the distal end of the fibula with the tibia on the distal aspect of the lateral malleolus suggested the presence of the fibular bone with proximal and distal ends while the body completely fused within the corpus of the tibial bone.

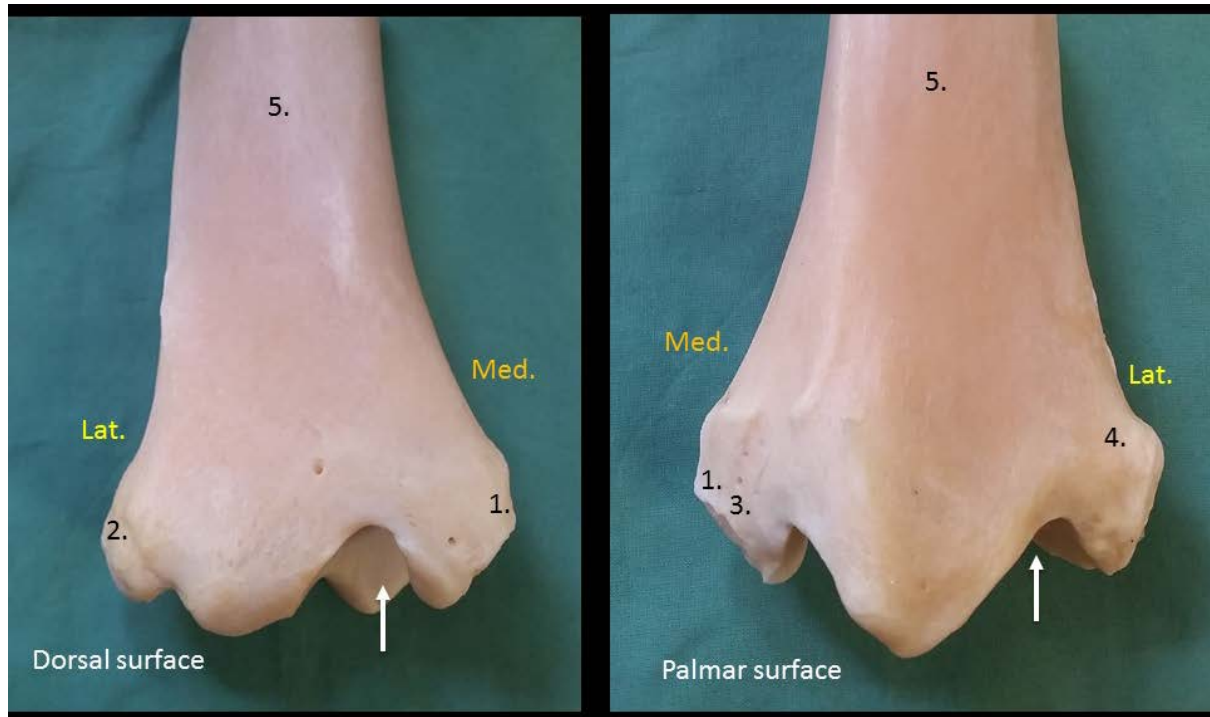


Figure 2. 2: Distal extremity of the right tibia dorsal aspect (left) and plantar aspect (right) surfaces of a Thoroughbred.

1, medial malleolus; 2, lateral malleolus; 3, popliteal notch; 4, groove for long digital flexor tendon; 5, body (shaft); Med., medial side; Lat., lateral side; arrows pointing towards the articular surface.

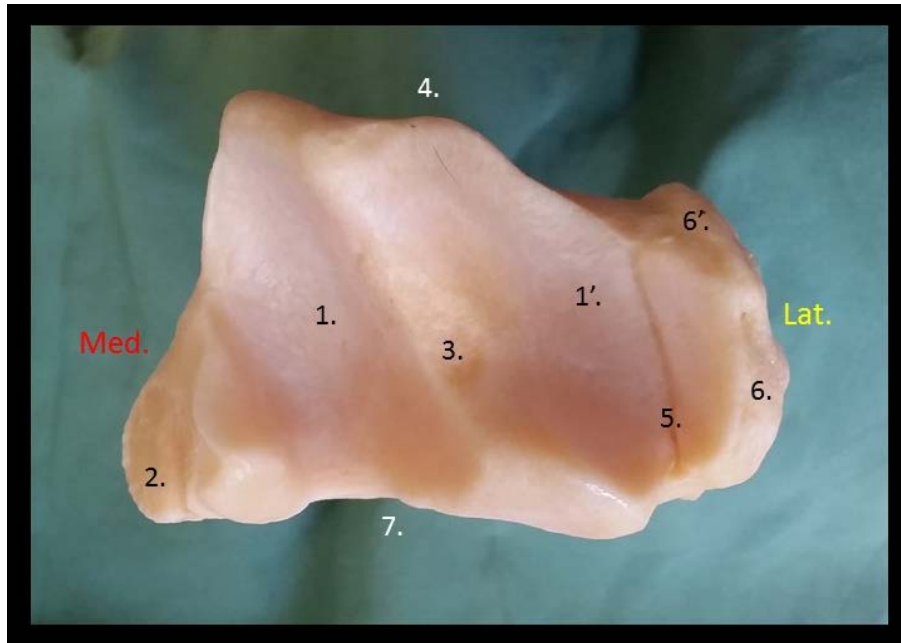


Figure 2. 3: Distal extremity of the Thoroughbred right tibia (and fibula) distal extremity view. 1, 1', articular grooves; 2, medial malleolus; 3, synovial fossa and intermediate ridge; 4, caudal border; 5, line of fusion of distal end of fibula with tibia; 6, 6', lateral malleolus; 7, cranial border; Med., medial side; Lat., lateral side.

2.3.2 TALUS TARSAL BONE:

The talus was extremely irregular in shape and lay medially in the proximal row. The proximal and dorsal surfaces were continuous and formed an articulation with the distal end of the tibia via a trochlea in an oblique direction. The direction of the head of the talus had only a moderate convex direction because of the angulation of the tarsal joint.

A shallow synovial fossa was usually present in the groove of the intermediate ridge of the trochlear. The talus was considered as having six surfaces for description. The proximal and dorsal surfaces formed the trochlea. Most of the distal surface was roughly oval in shape with the articular surface on the periphery and a non-articular groove in the middle of the distal surface. The articular surface mostly articulated with the central tarsal bone, but on the lateral side it had a facet for articulation with the fourth tarsal.

The plantar surface was quite oblique and very irregular having four facets for articulation with the calcaneus tarsal bone. The facets of articulations were separated by rough excavated areas and the largest fossa.

The medial surface bore two tuberosities, a large tuberosity on its distal part, and a small one on its proximal part, both for the attachment of the medial collateral ligament of the tarsal joint. The medial surface was much larger, wide and compact as compared with the lateral side, and the lateral surface was less rough but slightly marked by a wide rough fossa for the lateral collateral ligament attachment. On the proximal end it had a lateroplantar articulation facet for the processus cochlearis of calcaneus to articulate.

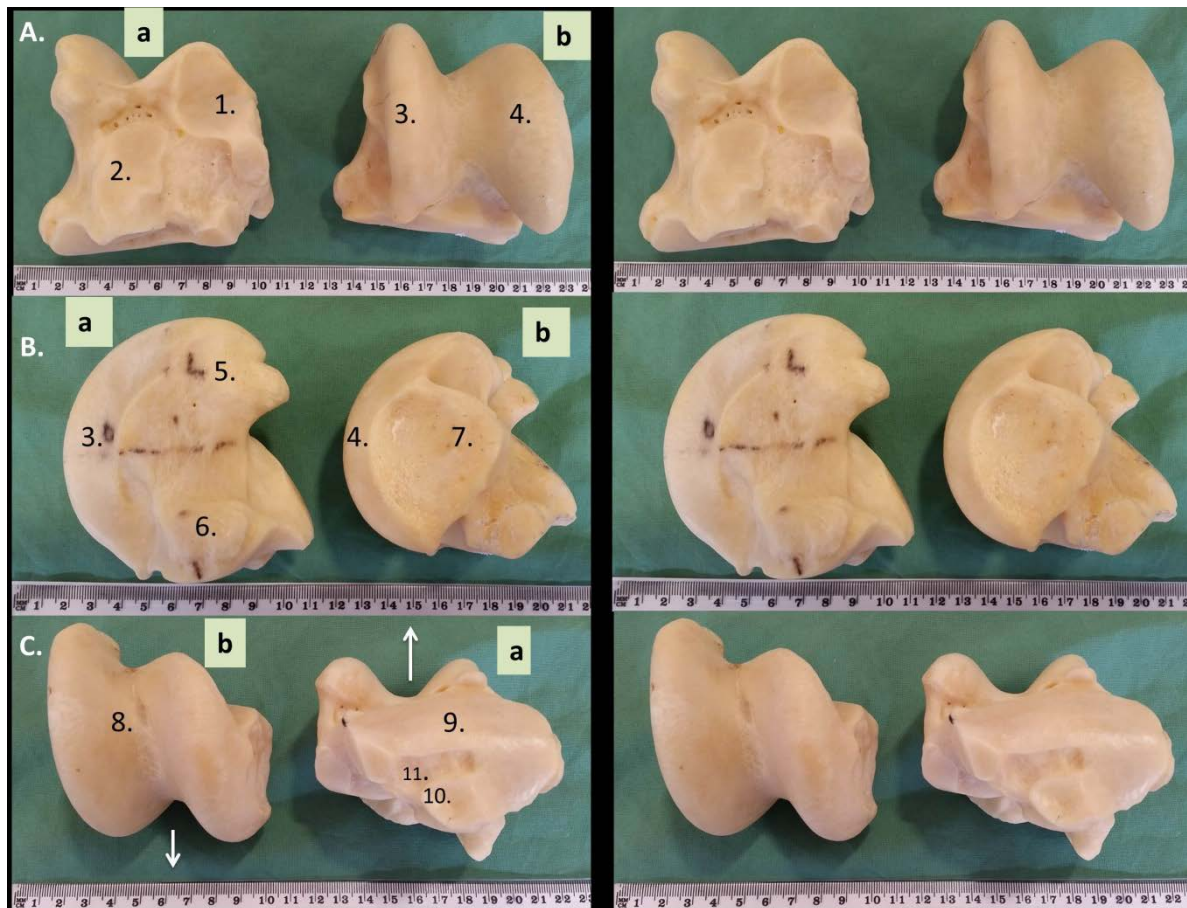


Figure 2. 4: Aspects of the talus tarsal bone of a Thoroughbred (In each image left side image is from left limb, while right side image was taken from right limb).

a: right side bone, b: left side bone A: Dorsal surface (right image) and plantar surface (left image). The plantar surface (left image): 1, 2, articular facets; 3, medial trochlear ridge of talus; 4, lateral trochlear ridge having a groove between these two ridges B: Medial and lateral surfaces. The medial surface (left image): 5, proximal tuberosity of talus; 6, distal tuberosity of talus; 7, lateral tuberosity of talus. C: Proximal and distal surfaces, distal surface (left image) of talus: 8, synovial fossa in between the medial and lateral trochlear ridges of talus; 9-10, articular facets for articulation with central tarsal bone; 11, non-articular depression; arrows, describe the dorsal sides of both talus bones.

2.3.3 CALCANEUS TARSAL BONE:

The calcaneus or fibular tarsal bone was the largest bone of the tarsus, being elongated, and flattened from side to side. The calcaneus lay mostly on the plantar aspect of the distal end of the tibia and talus tarsal bone but did not articulate with the distal end of the tibia. It consisted of a main body along with a bony articulating process on the medial aspect which is called the sustentaculum tali of calcaneus. There was a broad groove above this expanded medial process for the tendon of flexor hallucis longus muscle which passed through the tendinous sheath along this groove to the distal hind limb.

The main body or corpus was enlarged at its proximal end to form the tuber calcis or "point of the hock". The caudal part of this eminence gave attachment to the tendon of the gastrocnemius, while cranially and on each side, it furnished regions for the insertion of tendons of the superficial digital flexor, biceps femoris, and semitendinosus muscles.

The distal extremity bore a concave facet for articulation with the fourth tarsal bone. The medial surface of the body had on its lower part a strong process, the sustentaculum tali, which projected medially. This process had a large, oval, slightly concave facet cranially for articulation with the talus, and sometimes a small articular surface caudally for articulation with the central tarsal bone in most of the samples. With the smooth medial surface of the body its plantar surface formed a groove for the deep flexor tendon (Sulcus tarsi).

The calcaneus' medial surface had a prominence on the distal part for attachment of the medial collateral ligament. The lateral surface of the body was flattened, except distally, where there was a rough prominence for the attachment of the lateral collateral ligament. The dorsal border was concave throughout its length, smooth and rounded in its upper part. About its middle was a blunt pointed projection (Processus cochlearis) which was situated at

the level of the proximal end of the latero-planter aspect of the talus bone with its corresponding facet for articulation as described before (Figure 2.5, 1). There were multiple articular facets at the base of the calcaneus for articulation with the fourth tarsal bone and calcaneus also bore facets distally on the medial aspect for articulation with the talus tarsal bone. Proximal to these facets was a vast approximately triangular-shaped rough area medially for the attachment of intertarsal ligaments. These intertarsal ligaments not only provided attachments between talus and calcaneus but also attachments between calcaneus with the distal tarsal bones like the central tarsal bone. This extensive rough fossa was also involved in the formation of the sinus tarsi. The plantar border was straight and broad and widened a little at each end. It was rough and gave attachment to the long plantar ligament on its plantar aspect.

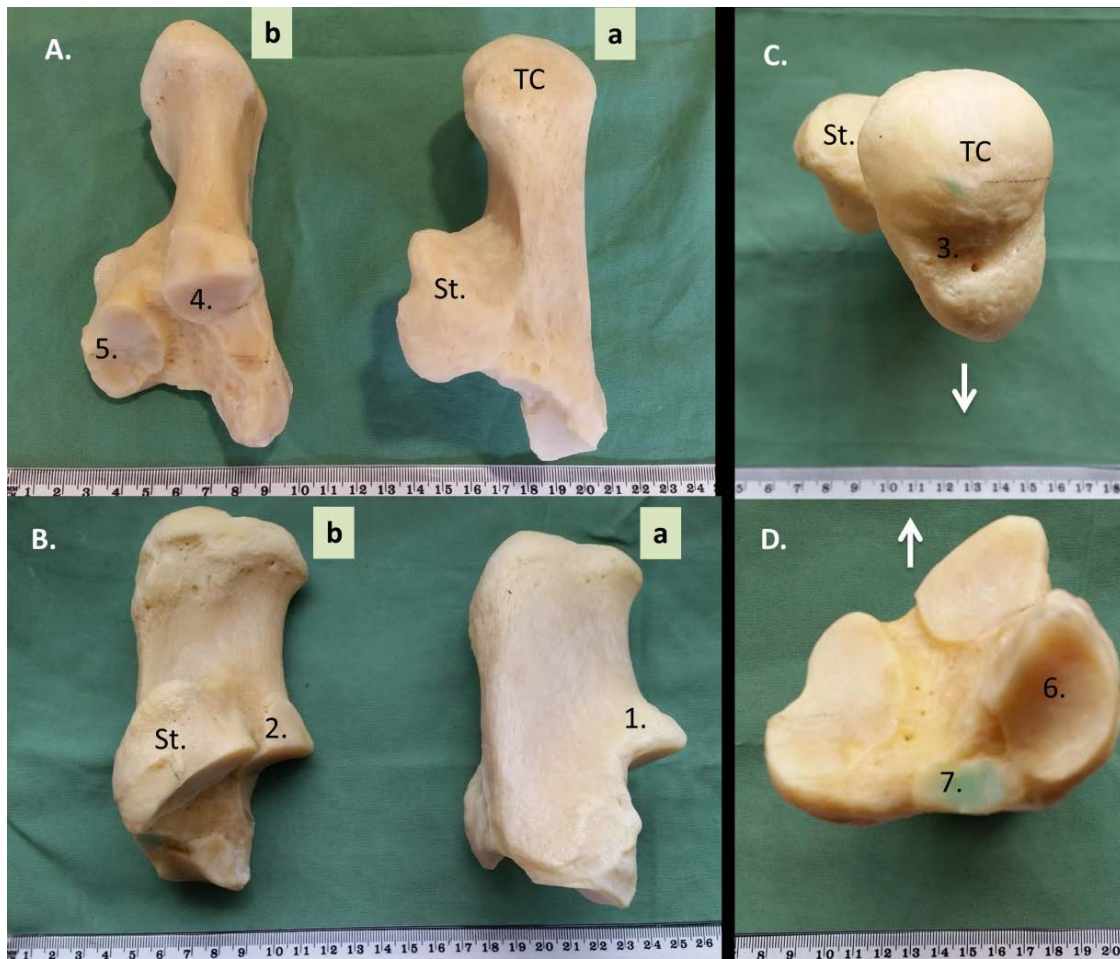


Figure 2. 5: Aspects of the calcaneal tarsal bone of a Thoroughbred. A: right side bone, B: left side bone.

The plantar surface of right calcaneal bone): 4, 5, articular facets; St, sustentaculum tali, TC, tuber calcaneus. B: Medial (left image) and (right image) lateral surfaces. The medial surface (left bone): 1, processus cochlearis; 2, medial articular facet with talus tarsal bone; 3, the communication between the two dorsal facets. C: proximal aspect of calcaneus with proximal surface of tuber calcaneus and other structures: 3, groove on tuber calcaneus. D: Distal surface of the base of calcaneus with arrow showing the dorsal border: 6-7, articular facets for articulation with fourth tarsal bone. Arrows: show the dorsal sides of the bone.

2.3.4 CENTRAL TARSAL BONE (CT):

The central tarsal bone (Os tarsi centrale) was an irregular rectangular or quadrilateral shape. It may be described as having two surfaces (proximal and distal) and four borders including lateral, medial, dorsal and plantar. This bone is called the central tarsal as it bears the central position in the tarsus and was situated between the talus proximally and the third tarsal bone distally, and also articulated with other tarsals like the 1st and 2nd tarsals on the medio-plantar aspect and with the fourth tarsal on the latero-plantar aspect. It articulated with talus by means of a shallow facet and with the third tarsal through two small facets distally. The proximal surface was concave from the dorsoplantar aspect, and most of this part articulated directly with the talus. This surface also bore a small groove at the center that opened at the lateral margin. This groove gave attachment to intertarsal ligamentous fibres between the central and talus tarsal bones. There was a facet for the calcaneus bone on the posterior raised part (Figure 2.6, 3).

The distal surface was convex, and this distal surface also bore a similar non-articular depression (groove) but much wider at the center, but its branches remained continuous with the plantar border and united with the groove at the plantar border and also extended to the lateral border.

The dorsal aspect of the central tarsal was almost flattened with almost equal height and not much variation in height at the dorsal border, as compared to the plantar border or aspect which was raised and a groove divided this raised region, providing a tunnel for passage of intertarsal ligaments uniting the distal bones to the proximal talus and calcaneal bones together. Thus, the plantar border bore a notch which separated two prominences.

This groove also separated the articular facets for the 3rd tarsal and the (fused) first and second tarsals. The lateral the medial borders of the central tarsal were rough, continuous

and convex. The lateral border of the central tarsal was oblique and a uniting point for proximal and distal grooves (Figure 2.6, 2) and this border also bore cranial and caudal articular facets for articulation with the 4th tarsal bone.

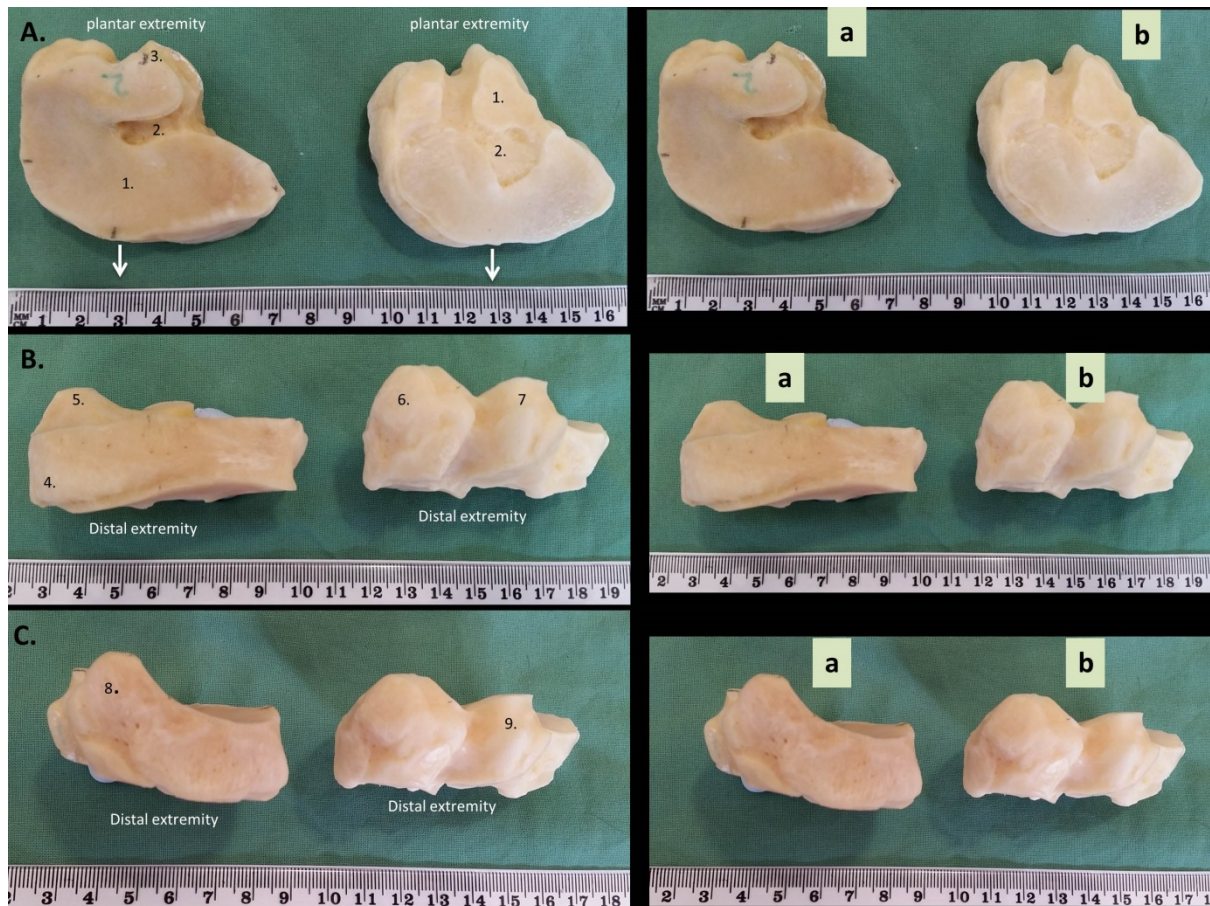


Figure 2. 6: Aspects of the central tarsal bone of a Thoroughbred. (In each image left side image is from left limb, while right side image was taken from right limb)

a: left side bone, b: right side bone. A. Proximal and distal surfaces of central tarsal bone: 1, articular surface for articulation with talus tarsal bone; 2, non-articular depression of central tarsal bone; 3, articular facet for calcaneus bone. B: Dorsal (left image) and plantar (right image) surfaces. The plantar surface: 4, non-articular tuberosity of dorsal surface; 5-6, plantar aspect of central tarsal; 7, plantar aspect of central tarsal C: Medial (left image) and lateral (right image) surfaces. The medial surface (left bone): 8, medial boundary of central; 9, plantar aspect of central tarsal bone. Arrows indicate the dorsal sides of bones.

2.3.5 FIRST AND SECOND TARSAL BONE (T1 and T2):

The first and second tarsal bones (*Os tarsale primum et secundum*) are usually found in a fused form as a single bone but in some cases can exist as separate bones with no fusion (Getty, 1975a). All specimens showed the fused form in the current study. The fused first and second tarsal bone shape was very irregular and roughly triangular shaped in a lateromedial orientation. When the first and second bones remain separated, the first tarsal is the larger of the two and plantar in position, but this presentation is not common (Getty, 1975a; Nickel et al., 1986a).

The first tarsal bone articulated with the central tarsal proximally, and distally with the second metatarsal. It also articulated with the third tarsal dorso-laterally, with the help of a small facet. The fused bone was situated on the medio-plantar part of the tarsus in the distal row, distal to the central and plantar to the third tarsal. The 2nd tarsal is the smallest bone among the tarsal bones if it remains separated. Even when fused with the 2nd tarsal as found in these specimens, the fused 1st and 2nd tarsal bone remained smaller in size as compared to the other tarsal bones.

The plantar surface of the fused tarsal bone was convex and faced medioplantarly. Its cranial part was ridged and gave attachment to the medial collateral ligament while its caudal part contained an imprint for the insertion of the medial tendon of the tibialis anterior (cunean) muscle. The dorsal articular surface had a deep notch which represented the division of the first and second tarsal bones and its cranial part contained a facet for articulation with the third tarsal. Its proximal surface was quite concave and also bore two articular facets for articulation with the central tarsal bone; a prominent border separated this surface from the medial surface. The distal surface was broad dorsally and articulated with the large (third) and medial small (second) metatarsal bones.

The lateral extremity comprised the 2nd tarsal bone and contained a ridge or tubercle. The second tarsal looked quadrangular in shape having articular facets on the lateral edge for articulation with the third tarsal bone while the medial extremity was like a pointed flattened blunt point (more likely a discoid shape) and was comprised of the first tarsal bone.

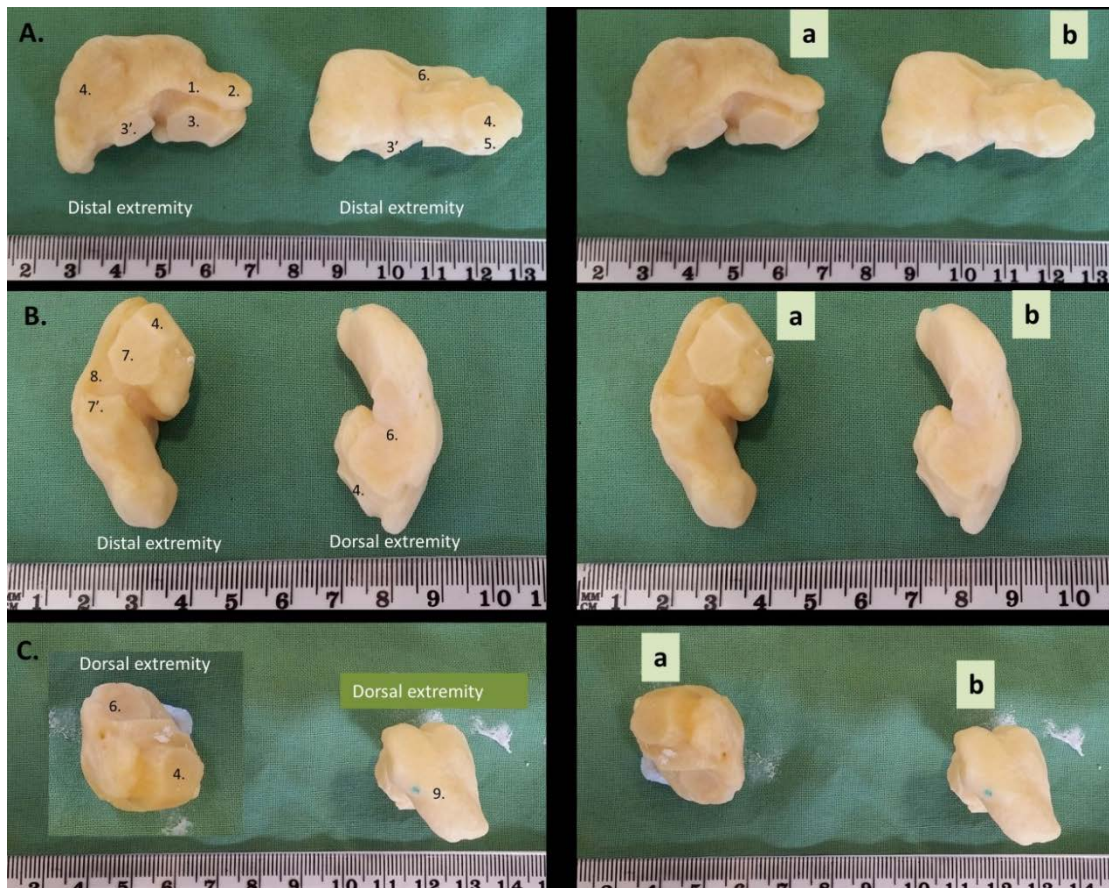


Figure 2. 7: Aspects of the first (T1) and second (T2) fused tarsal bone of a Thoroughbred (In each image left side image is from left limb, while right side image was taken from right limb). a: left side bone, b: right side bone.

A: The plantar surface; 1, body or corpus of first tarsal; 2, dorsal ridge of first tarsal; 3, 3', articular facet for second metatarsal bone; 4, body of second tarsal bone along its extended plantar portion; 4, articular facet for third tarsal bone. The dorsal surface (right image in A); 5, articular facet for third tarsal bone; 6, articular facets for central tarsal bone. B: Proximal (right image) and distal (left image) surfaces; 7, 7', articular facet for second small metatarsal bone; 8, non-articular depression. C: Medial (left image) and lateral (right image) surfaces. The lateral surface: 9, body of first tarsal bone along its extended plantar portion.

2.3.6 THIRD TARSAL BONE (T3):

The third tarsal bone (*Os tarsale tertium*) was wedge shaped and triangular in outline. The third tarsal bone was larger in size than the fused first and second tarsal. It resembled the central tarsal bone to some extent because it mostly articulated with the central tarsal through its proximal surface. It was situated in the distal row between the central tarsal at its proximal end and the large third metatarsal bone at the distal end.

The third tarsal possessed two surfaces and three borders. Its proximal surface articulated with the central tarsal and was quite concave and bore a non-articular shallow groove (depression) with a rough area which divided the proximal surface into two unequal facets, a dorsal larger facet and small plantar articular facets to articulate with the central tarsal. The distal surface was slightly convex in shape and articulated with the large third metatarsal bone. It also had an extensive rough excavation in the central portion which was much deeper than its proximal surface groove.

The dorsal border was convex and contained a ridge on its dorso-medial portion. This ridge was quite thick and rough suitable for attachments sites for fibres of ligaments or joint capsule. The medial border contained a small articular facet for the first and second tarsal on its cranial part and bore a deep notch. The lateral border could be divided into two parts by a shallow notch between two diagonally opposite articular facets for articulation with the fourth tarsal bone.

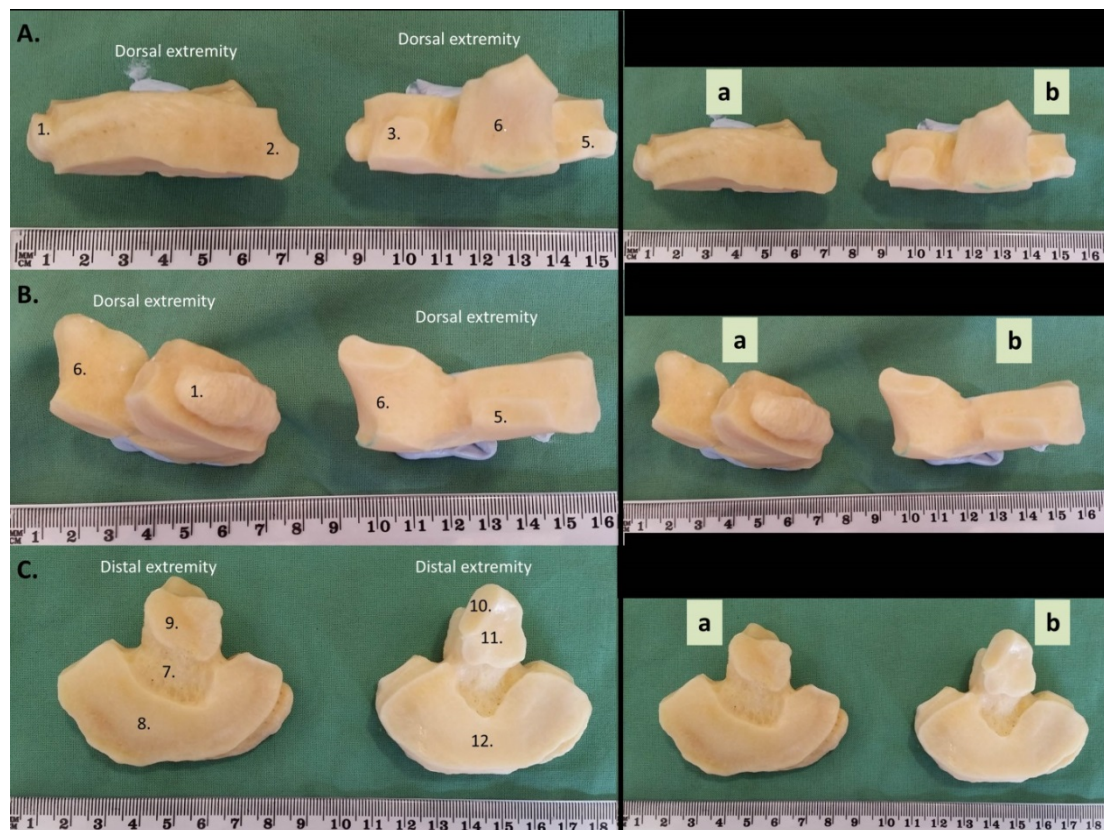


Figure 2. 8: Aspects of the third tarsal (T3) bone of a Thoroughbred (In each image left side image is from left limb, while right side image was taken from right limb).

a: left side bone, b: right side bone. A: Dorsal (left image) border. 1, dorsal ridge of dorsal non-articular surface; 2, lateral boundary of third tarsal bone; The plantar border (right image in A): 3, articular facet for first and second fused tarsal; B: Medial surface, showing a deep notch. Lateral surface. 5, facets for fourth tarsal bone; 6, plantar boundary. C: Proximal surface of third tarsal bone; 7, non-articular depression and non-articular surface; 8, articular facet for proximal articular surface of third metatarsal bone; 9, plantar articular facet for third metatarsal bone; The distal surface (left image of C) surfaces 10, facets for fourth tarsal bone; 11-12, articular facets for central tarsal bone.

2.3.7 FOURTH TARSAL BONE (T4):

The fourth tarsal bone (*Os tarsale quartum*) was the lateral bone of the distal row; and was cuboidal in shape. It was in the distal row and unlike the other bones. The fourth tarsal bone was present on the latero-plantar part of the tarsus and along with the first and second tarsal present on the medio-plantar part of the tarsus. This bone attained an intermediate position for articulation proximally with the proximal tarsal bones (talus and calcaneus) and also occupied both the intermediate and the distal rows (located on the plantar aspect of the central and third tarsals), because of its greater depth, and articulated distally with the proximal end of the fourth metatarsal.

The proximal surface was convex and articulated with both proximal tarsals, mostly with the calcaneal tarsal and also to a smaller extent with the tibial tarsal.

The distal surface articulated with the larger metatarsal (Mt3) and lateral small metatarsal bone (Mt4). The medial surface contained four articular facets, for articulation with the central and third tarsal bones. This surface was crossed dorsoplantarly by a smooth groove which, by apposition with the other adjacent bones formed a tarsal canal (*Canalis tarsi*). This canal of the tarsus gives passage to the proximal perforating branch of the larger cranial tibial artery which passes between the tarsal bones to anastomose with branches of the saphenous artery on the plantar aspect of the limb. The other surfaces like the dorsal, lateral and plantar surfaces were rough and continuous. A latero-plantar tuberosity provided a rough pointed area as an attachment site for the plantar ligament.

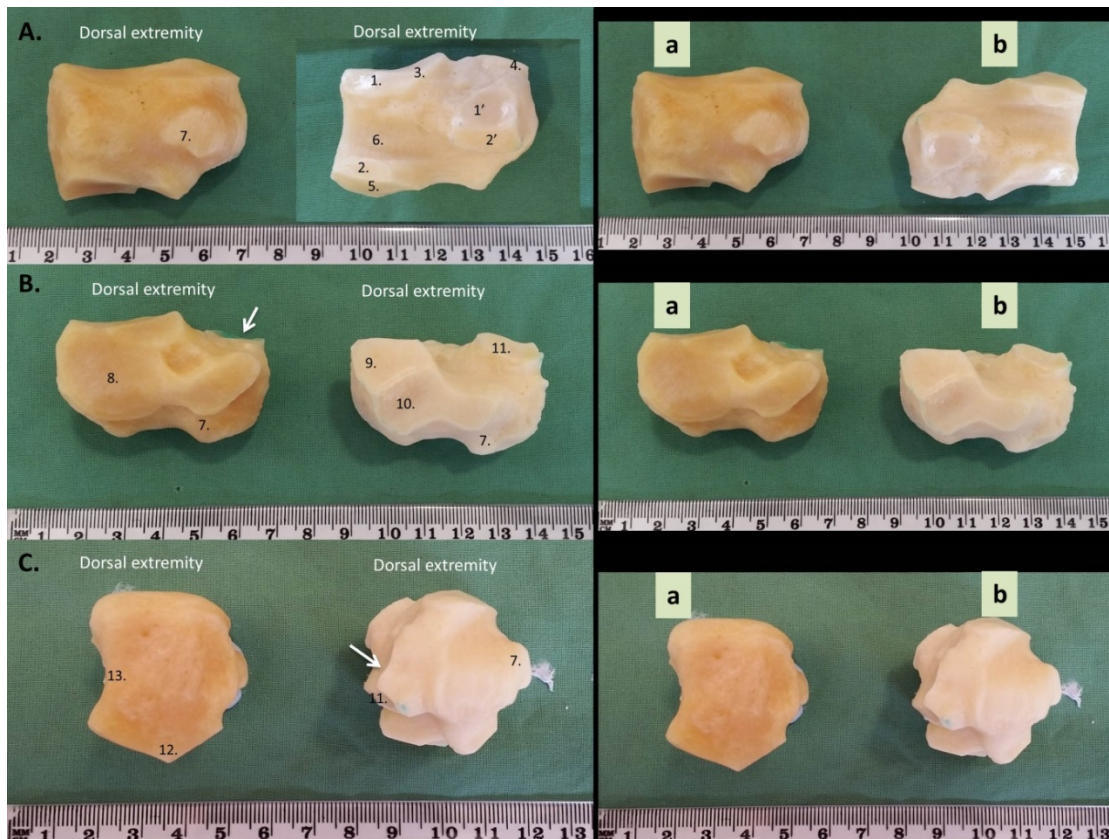


Figure 2. 9: Aspects of the fourth tarsal (T4) bone of a Thoroughbred (In each image left side image is from left limb, while right side image was taken from right limb). a: left side bone, b: right side bone.

A: The medial surface; 1,1', articular facets for central tarsal; 2,2', articular facets for third tarsal; 3, articular facet for talus; 4, articular facet for calcaneus; 5, facet for third metatarsal bone; 6, groove which associates with the central and third tarsal bones in formation of the vascular tarsal canal; The plantar surface. 7, latero-plantar tuberosity. B: Proximal surface, distal surface of fourth tarsal bone; 8, articular facet for calcaneus; The distal surface 9, plantar articular facet for third metatarsal bone; 10, facets for fourth metatarsal bone; C: the medial extremity. 11, articular facets for third tarsal bone. The lateral extremity (left image of C): 12, dorsal edge point having a combination articulation with the third and fourth metatarsal bones; 13, medial boundary of vascular canal of tarsus; arrows, articular facet for central tarsal bone.

2.3.8 PROXIMAL EXTREMITY OF METATARSAL BONES:

Among the three metatarsal bones (*Ossa metatarsalia*) only the third or large metatarsal bone (cannon bone) is fully developed and carries a digit while the other two, the second and fourth are quite reduced and commonly called the small metatarsals (splint bones). The third metatarsal bone is located centrally while the second metatarsal is on the medio-plantar and the fourth metatarsal is on the latero-plantar aspect of the third metatarsal.

2.3.8.1 The third (dorsal) metatarsal bone (Mt3):

The third or large fully developed metatarsal bone (*Os metatarsal tertium*) was longer than the corresponding metacarpal by about one sixth of the length. The cylindrical body or shaft was almost circular on cross-section, except in its distal part.

The proximal articular surface of the third metatarsal (Mt3) was slightly concave along with a large central non-articular depression, continued laterally by a deep notch. The major portion of this surface articulated with the third tarsal but there was a lateral facet for the fourth tarsal, and usually a small facet postero-medially for the second tarsal bone. On the plantar aspect there were couple of articular facets for articulation with the small metatarsal bones. The nutrient foramen was present in the proximal third part of the bone in the plantar aspect.

The dorsal surface was crossed by a rough ridge for insertion of the tibialis cranialis, which became larger and turned distally on the lateral side. At the proximal part of its lateral surface there was a groove for the third metatarsal artery, directed obliquely distal and plantar, and which was continued by the furrow which was formed by the apposition of the lateral small metatarsal bone. A shallow impression in a similar place on the medial aspect indicated the position of the corresponding vein.

Table 2. 1: Average medial and lateral heights (cm) of Sixteen third metatarsal bones from eight horses.

Horse	Right		Left	
	Lateral height	Medial height	Lateral height	Medial height
H1	31.3	31.2	31.3	31.1
H2	29.8	29.6	29.8	29.6
H3	30.7	30.4	30.8	30.6
H4	31.3	31.1	31.3	31.1
H5	31.4	31.2	31.5	31.3
H6	30.6	30.3	30.6	30.2
H7	29.8	29.6	29.9	29.6
H8	30.5	30.2	30.5	30.2

In both right and left hind limbs, the length to the medial proximal end of Mt3 was added to the length to the lowest point of the medial articular condyle to obtain the medial length of Mt3, and the same length of the lateral side were obtained for the lateral length of Mt3 respectively. These medial and lateral lengths showed a significant difference within individual limbs, for instance the right Mt3 had significantly larger mean lateral length ($30.69 \pm 0.62\text{cm}$) than its medial side ($30.43 \pm 0.63\text{cm}$) with P value <0.00 (Figure 2.10).

The left Mt3 also had a similar difference between lateral and medial heights. The lateral height of the left Mt3 was significantly larger with mean lateral length ($30.77 \pm 0.59\text{cm}$) than its medial side ($30.72 \pm 0.63\text{cm}$) with P value <0.00 (Figure 2.11). The comparison between the lateral heights of right and left bones showed no significant differences ($P = 0.09$) (Figure 2.12) and similarly, the medial heights of right and left bones showed no significant differences ($P = 0.105$) (Figure 2.13).

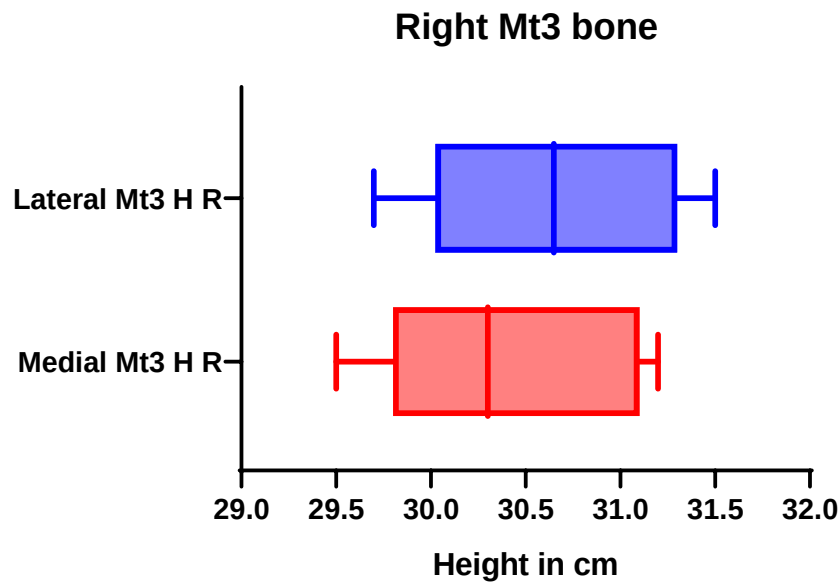


Figure 2. 10: Box plots of lateral and medial heights of right third metatarsal (Mt3). Solid lines in the box indicate median. Abbreviations; H. Height (length) R, Right.

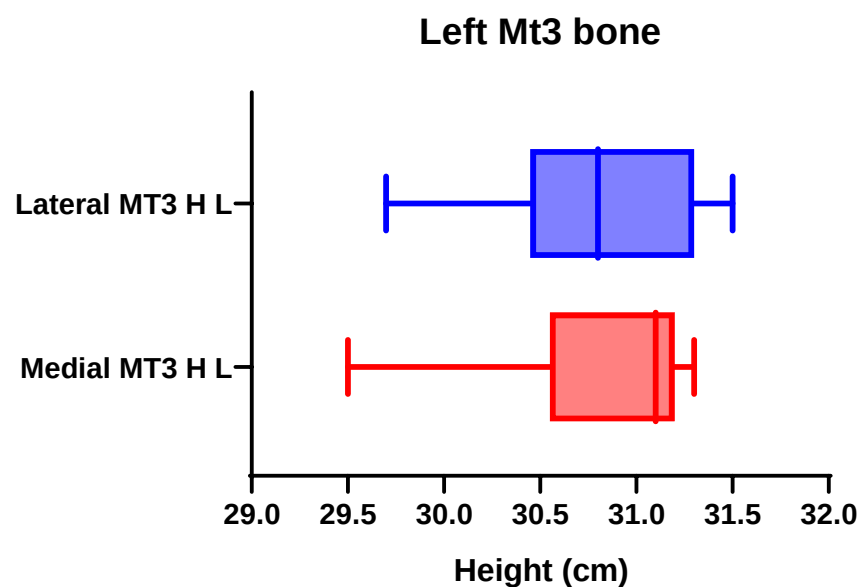


Figure 2. 11: Box plots of lateral and medial heights of left third metatarsal (Mt3 or MT3). Solid lines in the box indicate median. Abbreviations: H. Height (length) L, Left.

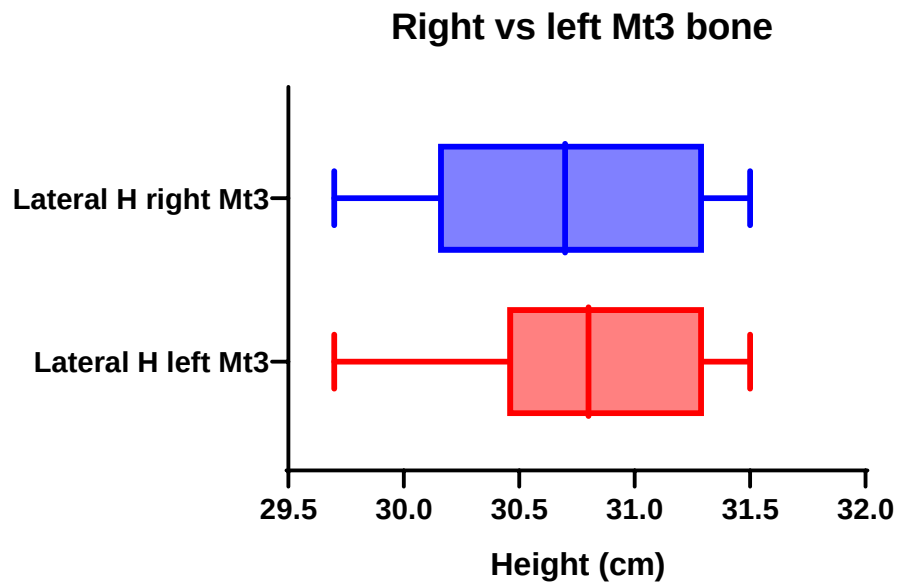


Figure 2. 12: Box plots of lateral heights of right (in blue) and left side (in red) third metatarsals (Mt3). Solid lines in the box indicate median. Abbreviation; H. Height (length).

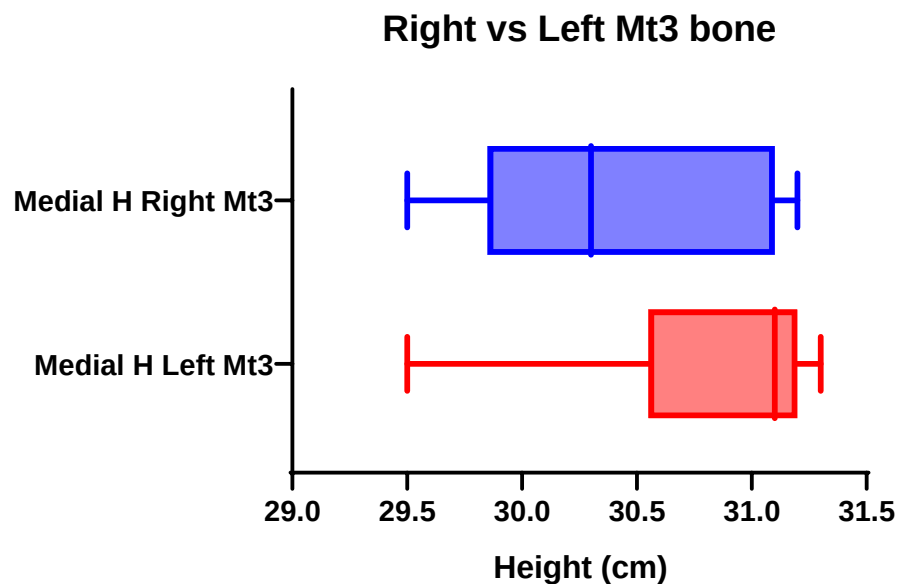


Figure 2. 13: Box plots of medial heights of right (in blue) and left side (in red) third metatarsals (Mt3). Solid lines in the box indicate median. Abbreviation; H. Height (length).

The fourth (lateral) metatarsal bone (Mt.4):

It was larger and quite massive compared to the corresponding metacarpus (of forelimb) and second smaller metatarsal, especially in its dorsal part (Getty, 1975). It articulated proximally with the fourth tarsal and medially with first and second tarsals. The base or proximal extremity was larger bearing one or two articular facets proximally for the fourth tarsal, and two dorsomedially for articulation with the large metatarsal while elsewhere, it was roughened for ligament, interosseous, or tarsal joint sheath attachment.

2.3.8.2 The second (medial) metatarsal bone (Mt.2):

It was not as massive and slenderer than the fourth metatarsal, especially in its proximal part. The base bore two articular facets proximally for the fused first and second tarsals, along with sometimes another one for the third tarsal bone and dorsolaterally two articular facets for the third metatarsal bone.

Finally, the general morphology of the bones of the tarsal joints were similar with many significant morphometrical variations between the medial and lateral sides of the same bone. There were many bony landmarks which could potentially help in assessment of alignment and orientational changes of the tarsal joint.

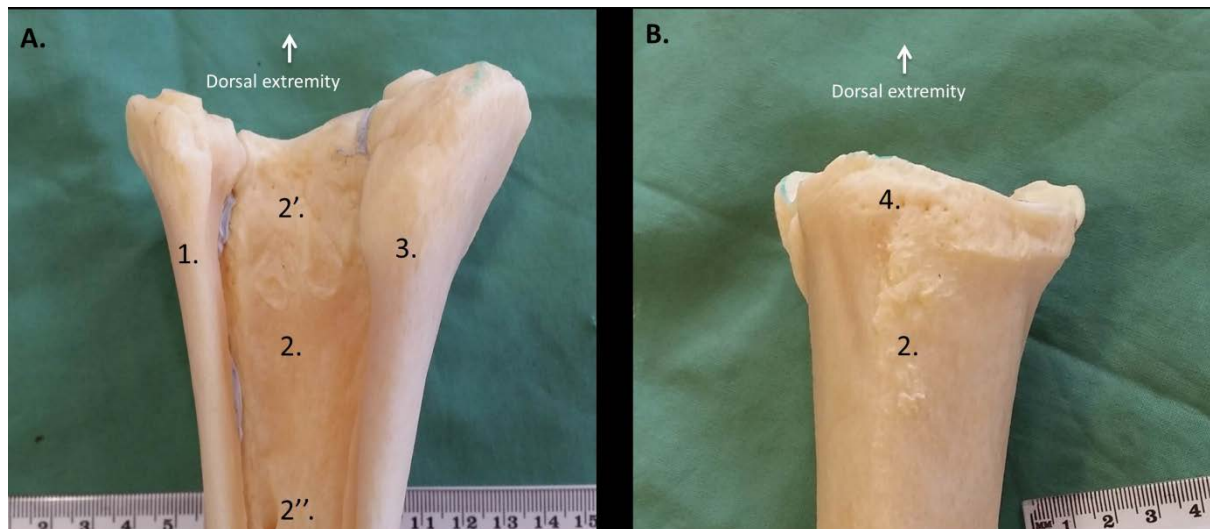


Figure 2. 14: The dorsal and plantar aspects of the proximal one third portions of the right metatarsal bones of a Thoroughbred.

A: plantar surfaces; 1, second metatarsal bone; 2, third metatarsal bone; 2', rough area for the attachment of interosseous m.; 2'', nutrient foramen, 3, fourth metatarsal bone. B: dorsal surfaces of metatarsal bones, 4, proximal rough area on dorsal surface of third metatarsal bone.

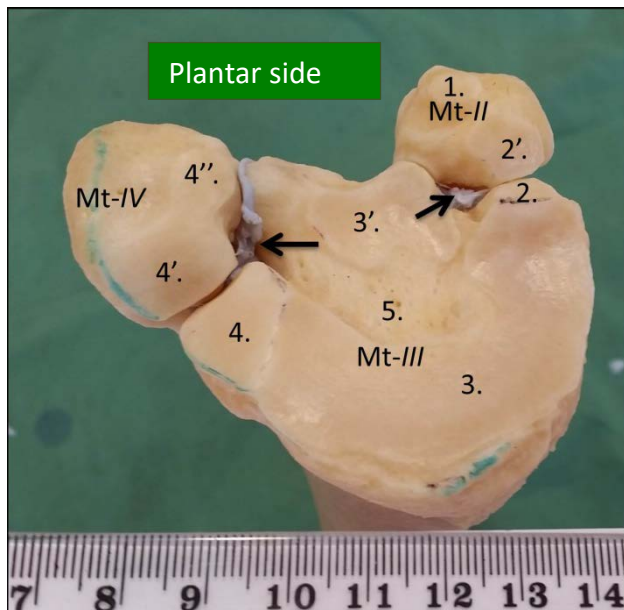


Figure 2. 15: Proximal aspect of metatarsal bones (right side bone) of a Thoroughbred.

Features include; 1, articular facet for first tarsal bone; 2, articular facet for T2 bone on the medioplantar side of third metatarsal (Mt3); 2', articular facet for second tarsal bone on dorsomedial side of second metatarsal (Mt2); 3, articular larger facet for third tarsal bone on the dorsal side of proximal surface of the third metatarsal bone; 3', articular facet for third tarsal bone on plantar side of the proximal surface of the third metatarsal bone 4, articular facets for fourth tarsal bone on lateroplantar side of the proximal surface of the third metatarsal bone; 4', 4'', articular facets for fourth tarsal bone on laterodorsal side of the proximal surface of the fourth metatarsal (Mt4) bone; 5, non-articular depression on proximal aspect of third metatarsal; arrows, non-articular depressions in-between metatarsal bones.

2.4 DISCUSSION:

Radiographic measurements can be variable and change according to the inclination of the X-ray beam, and any changes in distance or alignment of the subject with the radiography machine. Therefore, radiographic measurements cannot be considered as depicting real values. However, such imaging techniques are frequently used for conformational analysis for diagnosis of any pathology and assessment of future performance such as in presale or purchase examinations. This morphometric analysis specifies the characteristics of the constitutive tarsal bones to better understand their alignment and identify specific landmarks that might be used to locate regions of interest in the complex tarsal joint. In addition to this, the detailed shape of tarsal bones and morphometrical variations of these bones within the same breed could help to clarify the normal conformation, alignment change under loading and pathogenetic mechanism of tarsus disorders.

This study has indicated that there are many morphometrical bony features of tarsal bones within Thoroughbreds, between right and left hindlimbs within the same breed and also reported some variations between the medial and lateral sides in the individual bones. Similarly, biometric analysis of the talonavicular joint in a human study was helpful to specify the characteristics of the constitutive bone components of this joint (Bonnell et al., 2011).

The distal end of the tibia and the proximal aspect of the talus articulate together to form the tarsocrural joint (TT). The talus and calcaneus articulate with each other to form the talocalcaneal joint (TC). The central tarsal and talus bone articulate to form the proximal inter-tarsal joint (PIT), and the joint formed by the central tarsal bone and the distal row of tarsal bones is called the distal inter-tarsal joint (DIT). The distal row of tarsal bones articulates with

the proximal aspect of the metatarsal bones to form the tarso-metatarsal joint (TMT) (Kraus-Hansen et al, 1992; Dyson and Romero 1993; Bell et al, 1993).

In the current study, dimensional parameters were measured using a custom-built apparatus, calliper and plastic tape. The caudal border was raised in the middle of the thick ridge combining the two grooves at the distal caudal border, which was thicker than the cranial part. Thus, adding to this these raised parts on both cranial and caudal borders and lateromedial malleoli provided an extra bony strength at these four points to keep secure the trochlea of the talus bone for better articulation during large movements of this joint. Its compacta of third metatarsal is also much thickened on its dorsal aspect which has been suggested to be due to excessive loading or weight bearing because of its oblique position (Getty, 1975a; Nickel et al., 1986b).

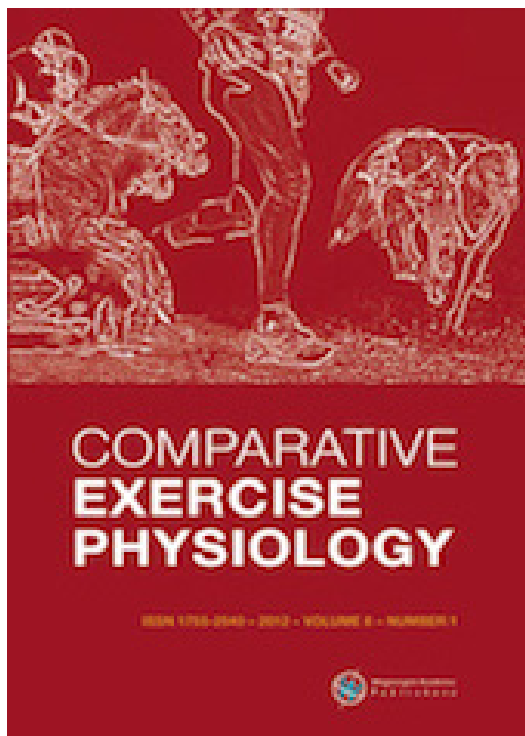
The length of the lateral side of Mt3 in thoroughbred was significantly longer than the length of the medial side of Mt3 in the individual limbs. In previous findings, the length to the lateral condyle was higher than the length to the medial condyle of equine third meta carpus (Mc3) and the medial condyles of Mc3 having greater width and depth as compared to lateral condyles in all groups (Alrtib et al., 2013). The long lateral side of the limb support the findings of previous research that the hoof more commonly lands towards the lateral side (Van Heel, Barneveld, Van Weeren, & Back, 2004).

Moreover, the differences between medial and lateral sides of the metatarsal bones. The differences in the lengths, widths and depths can be used to distinguish the lateral and medial sides of the bone successfully. The differences can also be consequently helpful in differentiation between the right and left bones or in fragments of bone in archaeological specimens.

It can be concluded that the morphometrical studies can give opportunities for more improvement of clinical exploration, understanding the real 3D construction of this complex tarsal joint. Different characteristics of tarsal bones were specified, and certain landmarks can be used for the identification of any misalignment of tarsus. Further, these landmarks should be useful to assessment of conformation or alignment of tarsus on acquired radiographic images for any diagnostic or therapeutic protocols in future studies. Over the long term, this study can be helpful in developing the notion of individual morphotypes under particular loading and other pathologies by enhancing the understanding of factors such as loading on selected parts of bone surfaces.

Chapter 3: Radiographic method for evaluation of tarsus morphometry (Published article)

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Running header: **Radiographic tarsus morphometry**

Key words: Radiography, equine, tarsus, conformation

3.1 Abstract:

The equine tarsus is one of the most common area of the hind limb associated with lameness. Tarsal function is probably directly related to its conformation. Previous conformational measurement methods and radiological data are either limited or too specific to explain the complex tarsus conformation in different loading conditions. This study aimed to develop a consistent method to evaluate equine tarsal conformation from lateromedial (LM) radiographs. Twenty cadaveric hind limbs from 15 adult horses of different breeds were cut at the distal one third of the tibia. Hind limbs mounted in a loading rig and positioned with the metatarsus vertical were digitally radiographed. The zero-degree lateromedial (ZLM) was defined by vertical and horizontal landmarks including overlapping of the lateral and medial trochlea of the talus and a contact point between the dorsal edges of lateral and medial borders of the distal central tarsal bone. Radiographs missing these features were retaken to achieve consistent ZLM views. Specific radiographic features were selected as landmarks to develop tarsal parameters based on their clarity and their being consistently identifiable. The intra-rater repeatability of ten measurable morphometric parameters was evaluated with each radiograph measured twice with an interval of at least one month and Bland-Altman plots developed from this data. Repeat measurements did not differ significantly (Intraclass correlation coefficients (ICC) ranged from 0.731-0.966). This study provides a base for evaluation of the tarsal conformation by radiography.

3.2 Introduction:

The tarsus of horses usually comprises six bones; talus and calcaneus in the proximal row, a central tarsal bone in the intermediate row, separate third, fourth and fused first and second tarsal bones together forming a single irregular bone (Dyce, Sack, & Wensing, 1996), but occasionally there are seven bones as the first and second tarsal bones may remain separated (Yoon et al., 2002). The articulations are held together by multiple ligaments and are enveloped by one continuous fibrous joint capsule (Getty, 1975c). Diagnostic imaging is routinely applied to this region because of the complexity of this area (Vanderperren, Raes, Hoegaerts, & Saunders, 2009), for determination of normal geometry and diagnosis of any abnormalities or diseases (Stashak, 1987; Dahn & Ueltschi, 1989; Schebitz, 1965a), and presale or purchase radiographic examination for subsequent performance (Cohen et al., 2006) as the tarsus of an equine is one of the most commonly affected area for hind limb lameness or other problems (Vanderperren, Raes, Hoegaerts, et al., 2009).

Different diagnostic imaging methods like radiography, magnetic resonance imaging (MRI), and computed tomography (CT) allow non-invasive and repeated assessment of joints for evaluations of different abnormalities or morphological changes (Ley, Björnsdóttir, Ekman, Boyde, & Hansson, 2016). These methods are quite helpful for clinicians in the diagnosis of early stages of any developing deformities (Luyten, Denti, Filardo, Kon, & Engebretsen, 2012). However, radiography is still frequently used as a quick diagnostic tool in most clinical practices (Vanderperren, Raes, Van Bree, et al., 2009).

Different projections can be considered for the radiographic evaluation of the tarsus; lateromedial (LM), dorsoplantar (DP), dorso-lateral plantaro-medial oblique (DL-PIMO), plantaro-lateral dorso-medial oblique (PIL-DMO) as well as two additional views:

lateromedial of the flexed tarsus and dorsoplantar of the calcaneus and talus with the tarsus flexed (J. A. Butler, C. M. Colles, S. J. Dyson, S. E. Kold, & P. W. Poulos, 2008b; Shively & Smallwood, 1980). For a better evaluation, the direction of the x-ray beam should be along a tangent to the tarsal bones for each of the four projections (Per Eksell, Uhlhorn, & Carlsten, 1999). The optimum position of the leg, accurate projections, and high-quality radiography have great importance for a reliable diagnosis (Butler, Colles, Dyson, Kold, & Poulos, 1993; Laverty et al., 1991; Shelley & Dyson, 1984).

A more specific technique has been established to assess neonatal angular deformity in foals. This can occur as a consequence of supporting soft tissue laxity and/or hypoplasia and/or incomplete ossification of the central tarsal and third tarsal bones, or as configurational abnormalities of the epiphysis and metaphysis (J. Auer, Martens, & Morris, 1982; Caron, 1988; Leitch, 1985). However, this method also has no clarity in the identification of landmarks, did not ensure normal conformation of the tibia or Mt3, did not include any tarsal bones conformational or morphological details nor did it allow for detection of any deviations in tarsal bones which can impact such changes. In addition, no evaluation of the effects of the orientation of lateromedial radiographs on radiographic measurements was considered.

Despite a wide usage of radiography, there are only a few radiographic parameters described in the literature to evaluate the tarsus conformation, like some tarsal bone dimensions in the LM view in normal tarsals (Dahn & Ueltschi, 1989) but in that study there was no information regarding landmarks identification, effects of rotations, nor the repeatability and reliability of their radiographic parameters. The lateromedial view is one of the more commonly taken standard views (Audigie et al., 1993; P Eksell et al., 1998; Laverty

et al., 1991) but there are still insufficient geometrical parameters and methods for better evaluation of the complex tarsus conformation. Thus, there is a need to develop more radiographic morphometric parameters and for their validation. Determination of repeatability and reproducibility of specific landmarks of the parameters in radiographic projections, addressing the effects of rotations on such geometric parameters all need to be further investigated. Studies investigating the structural geometry of joints are useful because they have the potential to provide objective measurements that can be used to assess joint instability and susceptibility to breakdown. In theory, an unstable conformation of the tarsal joint can either be congenital or acquired over time in response to skeletal loading. Furthermore, investigation of joint geometry enables quantitative assessment of any potential structural changes that may occur prior to the clinical manifestation of joint disease. Hence reliable ways of measuring archival and field radiographs are likely to be useful. This shortage of data on the conformation of the tarsus, which could have an important association with tarsal function and pathology, has promoted the study described in this paper. It was hypothesised that tarsal internal geometry can be assessed through LM radiographic view by developing some reliable measurements. The aim was to develop some consistent measurements of the equine tarsus considering both horizontal and vertical axes that might be applied in the field to assess tarsal morphometry as a first step in understanding the biomechanics of this complex joint.

3.3 Materials and methods:

3.3.1 Lateromedial view of the Tarsus

In this study, a specific LM view of the tarsus was determined and considered as the zero-degree lateromedial view or ZLM. The lateromedial radiograph is a two-dimensional image. This means that its radiographic measurements could be affected by two directions of rotation, that is rotations around vertical and horizontal axes (Major, Johnson, Hesse, & Glover, 1996). In a number of cephalometric studies, it has been shown that rotation around the vertical axis of the skull during radiography would affect horizontal parameters while rotation around the horizontal axis would affect vertical parameters (Ghafari, Cater, & Shofer, 1995; Malkoc, Sari, Usumez, & Koyuturk, 2005). Based on this fact, evaluating the equine tarsal lateromedial view, was done by detecting and assessing the presence of any rotation around these axes with the help of locations and superimpositions of certain landmarks. The acquired LM radiographic images might be affected by three main axes of tarsal rotations, so the measurements were taken considering vertical and transverse axes, while the sagittal axis was ignored because it would only affect the object position and have no effect on measurements (Y. J. Yoon et al., 2002). Therefore, determination of the zero degree was dependent on the specific position around vertical and sagittal axes.

3.3.1.1 Radiographing tarsal joints in Ex-vivo

3.3.1.1.1 Animals

Twenty cadaveric right and left hind limbs were collected from fifteen adult horses of known age, sex, and breed that were euthanized or died for reasons not related to this study. All the hind limbs used in this experiment were collected from the Department of Pathology, Faculty of Veterinary and Agricultural Sciences (FVAS), The University of Melbourne. There were 15

hindlimbs from 10 Thoroughbreds, and 5 from mixed breeds, including 2 Standardbred and 3 Warmblood Horses. Their ages ranged from 3 to 18 years. The horses selected for this study did not have known tarsal injuries or clinically apparent locomotor disorders of the hind limbs. The limbs were cut at the distal one-third of the tibia.

3.3.1.1.2 Equipment

The loading apparatus was designed to hold the cut end of the distal tibia and allow adjustment via screws in the aluminium cup to position the limb so that the metatarsal could be placed in a near-vertical position. The aluminium loading cup was attached to a bar that could be moved up or down with a mechanical pump to further position and hold the limb in as normal a posture as possible (Figure 3.1). The marked limb base was a 32.5 x 29.3 cm plate of thick transparent plastic. The rectangular iron weight with a V-shaped cut to restrain the hoof provided additional stability (Figure 3.1). The transparent radiographic machine perspex holding plate marked on its lower surface (Figure 3.2) was designed to control the rotation of the portable atomscope HF80/15+ Ultralight radiography machine and was fixed on the stable marked radiographic base (flat wooden base 42.5 x 35.3 cm stable plate) on the same axis of rotation (Figure 3.2A).

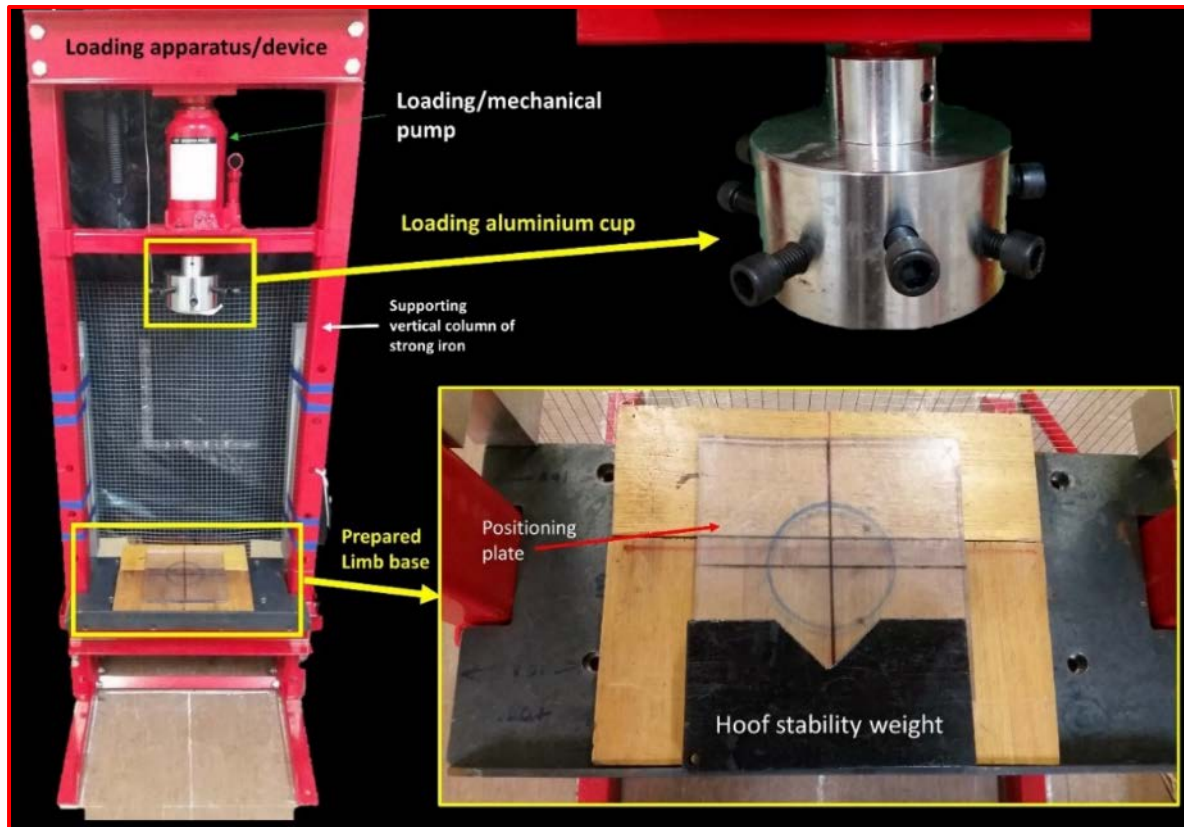


Figure 3. 1: Loading apparatus for positioning the limbs for radiography of cadaveric hindlimbs. The aluminum cup was designed to fit and hold securely onto the cut end of the distal tibia. The marked limb base (positioning plate) included a circle to assist with hoof placement and a hoof stabilizing weight.

3.3.1.1.3 Preparation of limbs

The cut edge of the skin was sutured where necessary. The solar surface of the hoof was fixed on the limb base in the center with the lateral surface of the limb facing the head of the radiography machine and the radiographic base was put on to be at the same height as the tarsus. The limb was held with the metatarsus vertical and secured by tightening the screws around the loading capsule (Figure 3.2B). It was helpful to angle the x-ray beam 10° proximodistally centering at the approximate level of the centrodial joint OR alternatively the x-ray beam was centred on the talus which was then marked by a pointer to be held

thorough transparent tape to provide a landmark after manual palpation for proper positioning of the center of the x-ray beam parallel to an imaginary line joining the lateral and medial malleoli of the tibia, or to a line tangential to the heel of the hoof, thus making use of the divergence of the x-ray beam through the lower joints of hock joint to get good superimposition of the distal intertarsal joints.

3.3.1.1.4 Radiographing prepared limbs

The digital radiographic cassette was placed in a cassette holder and positioned adjacent to the prepared limb with third metatarsal in vertical position to be radiographed so that the surface which faced the radiography machine was perpendicular to its head. The radiography machine was placed within 100 cm away from the prepared limb at the level of tarsus. The radiography machine holding plate and base was placed on the table and wooden boxes of different heights used to adjust the level of radiography machine in relation to tarsus level. For an appropriate exposure, the kilovolt peak (kVp) was estimated by utilizing an equation known as Santos's rule. This kVp was varied according to the lateromedial thickness of the tarsus using the following equation (Lavin, 1994).

$$(2 \times \text{cm [measurement thickness]}) + 40 = \text{kVp}$$

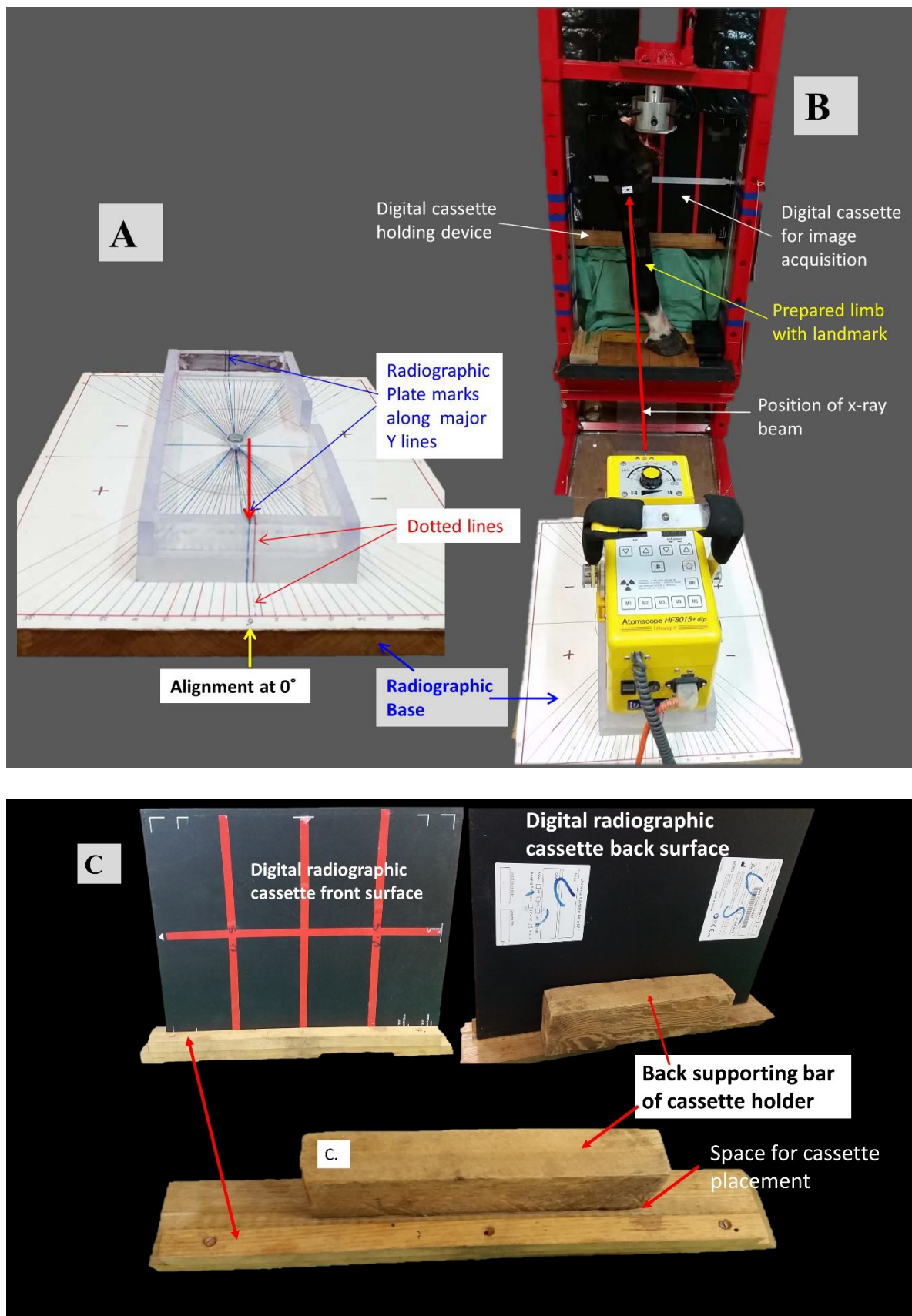


Figure 3. 2. Radiographing a right tarsus of a Thoroughbred showing the alignment of radiographic plate at zero degrees with the radiographic base (A). The hindlimb was fixed

in the loading apparatus. The x-ray beam was centred at the level of the talus (tibiotarsal bone). The radiography machine was fitted onto a radiographic holding plate (B). Digital cassette holder device (C).

3.3.1.2 Identifying vertical and horizontal tarsal axes

1. Generally for the ZLM radiographs the lateral and medial trochlea of talus overlapped completely, and talocalcaneal joint was clearly visible due to overlapping of lateral and medial articular facets of the talocalcaneal joint along with morphometric measurements that showed an equal distance from the intertrochlear groove to the dorsal surface of each trochlear ridge. However, some radiographs had insufficient superimposition of the lateral and medial trochlea of the talus, then those limbs were re-manipulated dorsoplantarly using the limb base and re-radiographed until the desired alignment was achieved. The vertical axis was then represented by a proximodistal imaginary line extending perpendicular to the x-ray beam (Figure 3.3). The limb position was then marked on hoof and the limb base. The dorsal mark was on the front while plantar mark was on the back of the limb base. These marks were used to maintain the correct position of the vertical axis while the horizontal axis was found.
2. At the level of the distal inter-tarsal joint on the radiographs, the aim was to have a contact point between the dorsal edge of the lateral and medial borders of the distal end of the central tarsal and the overlapping of the dorsal borders of the second and fourth metatarsus. The limbs radiographs that lacked this feature, were re-manipulated lateromedially and re-radiographed until the contact point between the borders was achieved. After attaining this feature, the horizontal tarsal axis was represented by an imaginary line extending dorsoplantarly perpendicular to the x-ray beam. The dorsal and plantar borders of this horizontal axis were identified in the distal intertarsal joint and were approximately parallel

to the line between the dorsal and plantar borders of the distal aspect of the central tarsal (Figure 3.3).

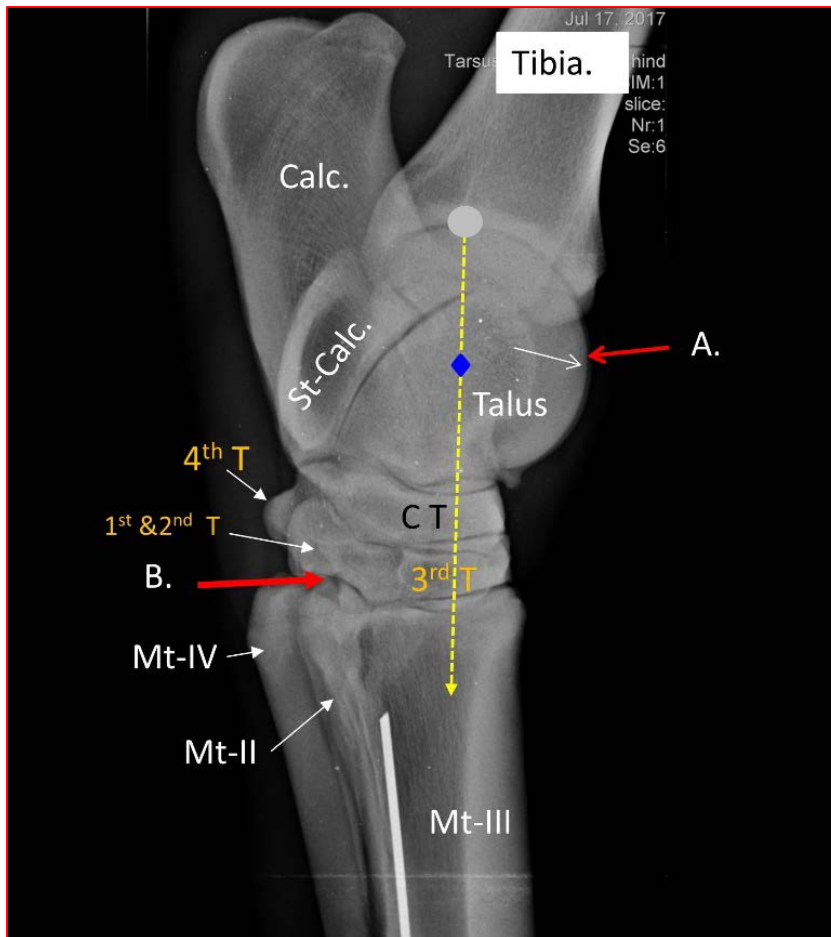


Figure 3. 3. The ZLM view of the right tarsus. The overlapping of the lateral trochlear dorsal border and the medial trochlea of the talus (A) was used for vertical evaluation. The overlapping of the dorsal borders of the second and fourth metatarsus (B) was used for horizontal evaluation to produce a consistent alignment.

The center of the x-ray beam (blue dot) was positioned perpendicular to the talus tarsal bone for vertical axis (dotted line). Calc. Calcaneus tarsal bone; St-Calc. Sustentaculum tali of calcaneus; CT. Central tarsal bone; 3rd T. Third tarsal bone; 4th T. Fourth tarsal, 1st and 2nd T; First and second tarsal fused; Mt-II. Second metatarsal bone; Mt-III. Third metatarsal bone; Mt-IV. Fourth metatarsal bone.

3.3.1.3 Tarsal measurable parameters:

In this study, tarsal parameters were developed using specific features selected based on their possible pathological significance in the tarsal joint. In order to avoid any effect of size variation among horses, all the developed parameters were angles and ratios. The selected tarsal parameters were:

Reference line:

The long axis of Mt3 was defined by taking the midpoints of its dorsoplantar diameter at 5 to 6 and 10 to 12 cm distal to the tarsometatarsal joint, then joining those two points with a straight line to represent the long axis of the third metatarsus (Figure 3.4A/1).

1. *Distal intertarsal-Metatarsus joint angle/Individual angular parameter # 1 (IAP-1)*: this angle was measured at the intersection point between a line extending along the distal intertarsal joint and the reference line (RL, Figure 3.4) which is the long axis of the third metatarsus (Mt3).
2. *Proximal intertarsal-Metatarsus joint angle/Individual angular parameter # 2 (IAP-2)*: this angle was at the intersection point between the RL and a line extending along the proximal intertarsal joint (Figure 3.4A/2).
3. *Dorso-distal edge of calcaneus- Talocalcaneal angle/ Individual angular parameter # 3 (IAP-3)*: this angle was at the intersection point between a line extending along the dorso-distal edge/incisura calcanei of the calcaneus and the proximal and distal end points of the curved talocalcaneal joint (Figure 3.4A/3).
4. *Sustentaculum tali of calcaneus-Metatarsus joint angle/ Individual angular parameter # 4 (IAP-4)*: this angle was at the intersection point between the RL and the long axis of sustentaculum tali of the calcaneus (St-Calc) which was found by joining two midpoints

between the dorsal and plantaro-distal broad edges of St-Calc similar to the Mt3 (Figure 3.4A/4).

5. *Distal intertarsal- tarsometatarsal joint/ Correlated angular parameter # 5 (CAP-5)*: this was the angle between a line perpendicular to the distal intertarsal joint and a line perpendicular to the best fit line of the tarsometatarsal joint (Figure 3.4B/5).
6. *Proximal intertarsal-distal intertarsal joint/ Correlated angular parameter # 6 (CAP-6)*: This was the angle between a line perpendicular to the best fit line for proximal intertarsal joint and a line perpendicular to the best fit line of distal surface of the central tarsal or the distal intertarsal joint (Figure 3.4B/6). No splitting of difference was used in these measurements.
7. *Calcaneoquartal-Proximal intertarsal tarsal joint/ Correlated angular parameter # 7 (CAP-7)*: This was the angle between a line perpendicular to the calcaneo-quartal joint and a line perpendicular to the proximal intertarsal joint (Figure 3.4B/7).
8. *Dorsal height of central tarsal/ dorsal height of 3rd tarsal ratio parameter (Ratio parameter # 8 (RP-8))*: The dorsal height of the central (Figure 3.4C/dHC) and third (Figure 3.4C/dH3rd) tarsal bones were extended from the most dorsal proximal extremity to their distal extremities.
9. *Length of sustentaculum tali/width of talus ratio (Ratio parameter # 9 (RP-9))*: The craniocaudal length of St-Calc was taken by extending the line joining the midpoints of the proximo-distal diameter measured near to the cranial and caudal ends of St-Calc (Figure 3.4C/L-stCalc). The width of the talus (Figure 3.4C/WT) was extended from where the dorsodistal edge of calcaneus crosses the proximal talocalcaneal joint to the maximum dorsoplantar width from the curved dorsal border of the superimposed lateral and medial trochlea of talus.

10. *Width of calcaneus/ width of talus ratio (Ratio parameter # 10 (RP-10))*: The width of the calcaneus was taken from middle of cranial height and caudal heights extended from dorsal border to plantar border (Figure 4C/WCalc) to the dorsoplantar width of the talus as described above (Figure 4C/ WT).

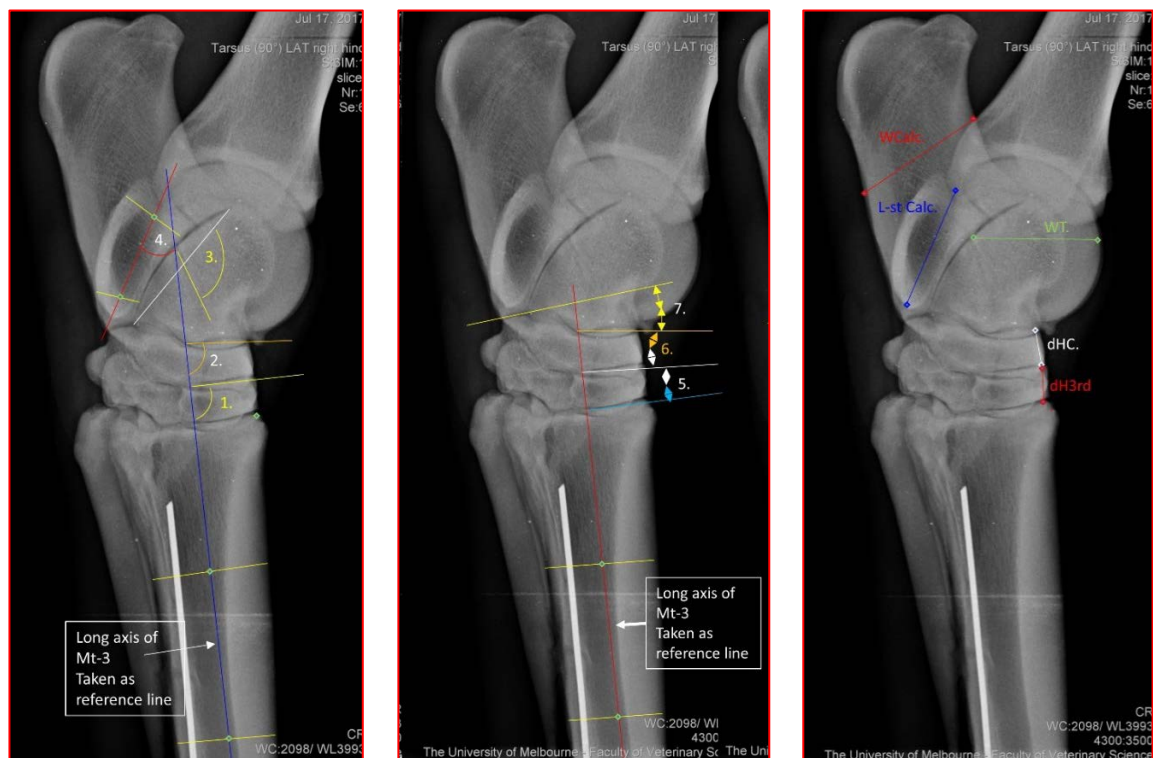


Figure 3. 4. ZLM radiographs of a right tarsus showing the lines used for ratios and angular parameters.

R1: long axis of metatarsus (Mt-3) taken as the R1 reference line, 1. Distal intertarsal-Metatarsus joint angle, 2. Proximal intertarsal-Metatarsus joint angle, 3. Dorso-distal TaloCalcaneal junction angle, 4. Sustentaculum tali (St.)-Metatarsus angle, 5. Distal intertarsal-Tarsometatarsus (DI-TMT) joint angle, 6. Proximal intertarsal-Distal intertarsal (PI-DI) joint angle, 7. Calcaneoquartal-Proximal intertarsal (CalQ-PI) tarsal joint, WCalc. Width of calcaneus, WT. Width of talus, L-St-Calc. Length of sustentaculum tali of calcaneus, dHC. Dorsal height of central tarsal, dH3rd. Dorsal height of third tarsal.

3.3.1.4 Intra-Rater Repeatability

Radiographs and Measurements

The digital radiographic cassettes were scanned (FireCR+ Veterinary 80 CR reader) and imported into DicomPACS®DX-R X-Ray acquisition software (Digital Imaging and Communications in Medicine, Picture Archiving and Communication System). This acquisition software caters for the magnification of acquired images viewed either at normal size or zoomed in or out, which does not affect the measurements as was tested prior to the measurements were being taken. All measurements were done under the same laboratory environment. Each radiograph was measured twice with an interval of at least one month between repeated measurements. All the measurements were taken by the author to minimize error (Kublashvili, Kula, Glaros, Hardman, & Kula, 2004).

3.3.1.4.1 Statistical analysis:

The Normal distribution of combined measurements from the first and second measurements was assessed by the Shapiro-Wilk test for Normality. The mean values with standard deviations, 95% confidence interval (CI) of mean, median, skewness, and kurtosis were calculated. Intra-tester repeatability of repeat measurements was evaluated by calculating intraclass correlation coefficients (ICC). Lin's concordance correlation coefficient (Lin's CCC) was calculated. Tentative suggestions for the interpretation of Lin's CCC are: 0.21–0.40 Fair; 0.41–0.60 Moderate; 0.61–0.80 Substantial; 0.81–1.00 Almost perfect. The 95% CI of Lin's CCC were also measured (Lawrence & Lin, 1989, 2000; Tedeschi, 2006). The mean difference between first and second readings with standard deviations, Bland-Altman plots of first and

second radiographic measurements of each radiographic parameters were developed using 95% limits of agreement of Bland-Altman plots. Significance was set at $p < 0.05$. Bland-Altman plots provide a way of displaying the data for each parameter in which differences between the repeat measurements for each subject were plotted on the Y axis against their means on the X-axis. The plots also show any extreme or outlying observations. The 95% limits of agreement were added to these plots to provide a summary picture for each parameter. The curve-fitting technique was not used for the means of IAP, CAP and RP data in figure 3.5 because the Bland-Altman approach was conducted directly on the observations (no statistical model involved). The repeatability of a method from replicated measurements obtained by the same method can be analysed by the agreement between two measurements quantified using the differences between observations made on the same subjects. The 95% limits of agreement, estimated by mean difference ± 1.96 standard deviation of the differences, provides an interval within which 95% of differences between measurements are expected to lie. Each standard deviation can be used to calculate limits within which we expect the differences between two measurements by the same method to lie (Bland & Altman, 1999).

3.4 Results

3.4.1 Lateromedial (ZLM) view at zero angle for tarsus conformation:

The ZLM view was achieved as described in methods (Figure 3.3) for both vertical and horizontal landmarks in all specimens.

Tarsal parameters and Intra-Rater repeatability

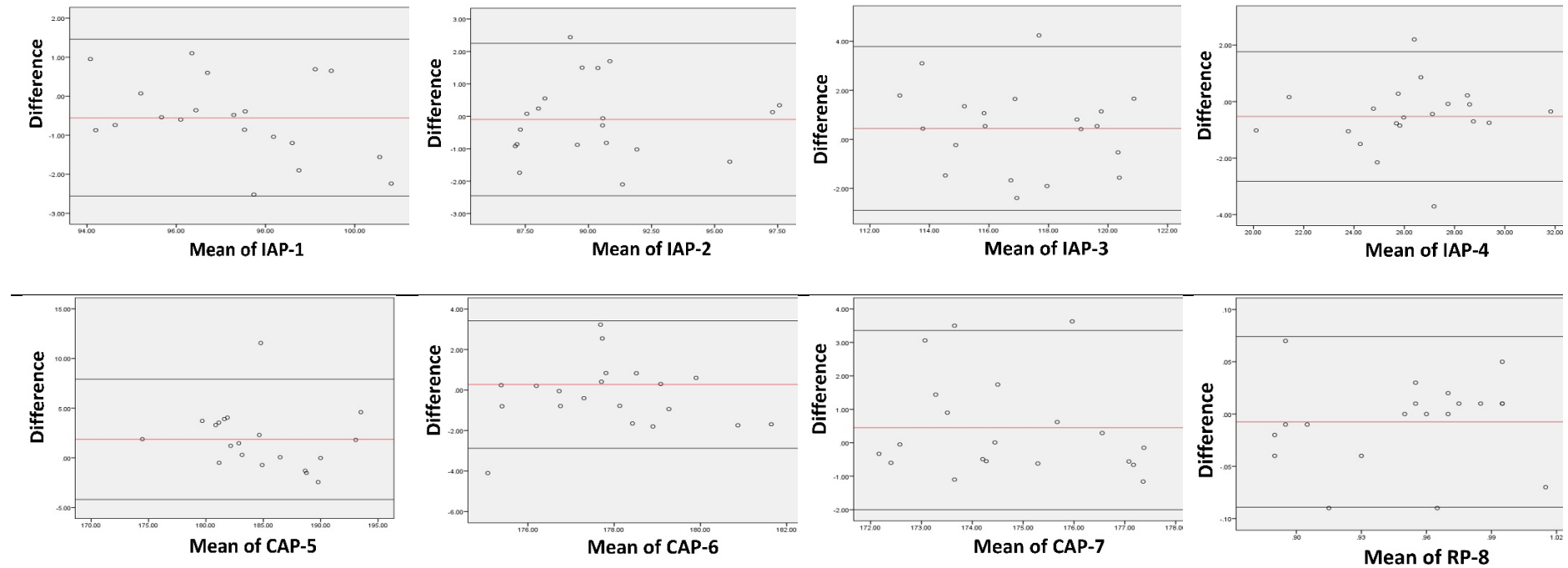
The ICC and Lin's CCC coefficients are reported in Table 3.1. The difference between repeated measurements was variable among the parameters and ranged between 0.73 to 0.97. The greatest difference between the repeated measurements was in L-st Calc/WT ratio with an ICC of 0.731, whereas the least difference was in the Proximal intertarsal-Metatarsus joint angle with an ICC of 0.966. According to Lin's CCC in the comparison between the two measurements, the agreement of the measurements was generally substantial to almost perfect. The highest agreement was in Proximal intertarsal-Metatarsus joint angle with a CCC of 0.93. The poorest agreement was in the L-St-Calc/WT ratio with a CCC of 0.563. Bland-Altman plots for first eight tarsal parameters were developed (Figure 3.5).

Table 3. 1. Means $\pm$ standard deviations of each radiographic parameters during each readings, ICC and Lin's CCC coefficients in 20 tarsi

Sr. No.	Measurable parameters (n=20)	Readings	Mean $\pm$ SD	Intraclass Correlation (ICC)	95 % CI of ICC		Lin's CCC	95 % CI of Lin's CCC	
					Lower	Upper		Lower	Upper
1	Distal intertarsal-Metatarsus joint angle (IAP-1)	1 st	96.97 $\pm$ 1.82	0.916	0.752	0.969	0.838	0.667	0.925
		2 nd	97.53 $\pm$ 2.23						
2	Proximal intertarsal-Metatarsus joint angle (IAP-2)	1 st	90.35 $\pm$ 3.23	0.966	0.914	0.986	0.930	0.834	0.972
		2 nd	90.46 $\pm$ 3.22						
3	Dorso-distal calc-TaloCALCane junction angle (IAP-3)	1 st	117.33 $\pm$ 2.48	0.878	0.698	0.951	0.774	0.522	0.901
		2 nd	116.88 $\pm$ 2.72						
4	St. calcani-Metatarsus angle (IAP-4)	1 st	25.97 $\pm$ 2.81	0.946	0.854	0.979	0.893	0.756	0.955
		2 nd	26.50 $\pm$ 2.70						
5	DI-TMT Joint angle (CAP-5)	1 st	177.79 $\pm$ 1.92	0.794	0.482	0.918	0.646	0.302	0.841
		2 nd	178.06 $\pm$ 1.94						
6	PI-DI Joint angle (CAP-6)	1 st	174.93 $\pm$ 1.75	0.811	0.536	0.925	0.671	0.356	0.850
		2 nd	174.49 $\pm$ 2.02						

7	CalcQ-PI Joint angle (CAP-7)	1 st	185.60 ± 4.72	0.868	0.599	0.951	0.758	0.515	0.888
		2 nd	183.74 ± 5.33						
8	dHC/dH3rd ratio (RP-8)	1 st	0.94 ± 0.11	0.912	0.780	0.965	0.831	0.627	0.928
		2 nd	0.94 ± 0.10						
9	L-st Calc/WT (RP-9)	1 st	0.95 ± 0.05	0.731	0.324	0.893	0.563	0.182	0.797
		2 nd	0.95 ± 0.04						
10	WCalc/WT ratio (RP-10)	1 st	1.02 ± 0.05	0.958	0.866	0.985	0.915	0.812	0.963
		2 nd	1.01 ± 0.04						

Figure 3. 5: Bland-Altman plots for first eight tarsal parameters; Difference, the difference between first and second readings. Mean of IAP-1 to IAP-4, Means of Individual angular parameters 1-4. Mean of CAP-5 to CAP-7, Means of correlated angular parameters 5, 6 and 7. Mean of RP-8, Mean of ratio parameter number 8.



3.5 DISCUSSION:

The present study defines a radiographic zero-degree lateromedial view (ZLM) of the equine tarsus for morphometrical purposes. The achievement of a consistent ZLM was based on some reliable landmarks and their correct identification in a specific orientation in respect to the vertical, sagittal and horizontal axes of the tarsus. The potential effects on measurements of rotations around the vertical and horizontal axes of the tarsus needs to be considered. In the horizontal evaluation, overlapping of the dorsal border of the fourth metatarsus beneath the dorsal border of the second metatarsal was selected because it was relatively small and was very sensitive to any minor rotation around the tarsal horizontal axis.

Most clinical images are more focused on particular areas for diagnostic importance rather than maintaining a proper alignment for conformation. The vertical and horizontal landmarks have great significance for accurate orientation of the tarsus to achieve the ZLM in the field. In the normal LM view in clinical radiographs the lateral trochlear ridge of the talus appears to extend more dorsally than the medial ridge, and the proximal extremities of the splint bones directly overlap so this would control for displacements of the tarsometatarsal joint in an individual limb but does not allow for comparisons between different limbs because of the likelihood of individual variations in the shape and position of the splint bone heads. In contrast the ZLM requires complete overlapping of lateral and medial trochlear ridges of the talus. The complete overlapping of the lateral trochlea over medial trochlea of talus tarsal was used as the vertical landmark in this study and was very sensitive to any minor rotation around the vertical axis of tarsus. This kind of evaluation has not been addressed in the commonly used lateromedial view which has been described in

the literature (Butler, J.A., C. M. Colles, S. J. Dyson, S. E. Kold, & P. W. Poulos, 2008a; Shively & Smallwood, 1980; Verschooten & Schramme, 1994). Thus, any changes in orientation can be monitored through vertical and horizontal landmarks of structures that are likely to be intrinsic to the functioning of the tarsocrural joint. Thus, identification of the view can be considered a fundamental starting point in studying tarsal morphometry for biomechanical studies.

Achieving the ZLM may be difficult *ex-vivo*, however, it is possible to radiograph this accurately in field radiography with some care and with the help of vertical and horizontal landmarks. In tarsus radiography, the long axis of the third metatarsus was defined and its suitability was considered as a reference line. In a similar study of radiographic evaluation of carpal conformation, the degree of flexion and extension of the carpus was calculated from radiographs by defining the axes of the radius and third metacarpus. The axes were calculated by joining the mid points at a particular distance (6cm-12cm) from their respective joints (Barr, 1994).

In a geometric evaluation of the foal tarsus, long axes were drawn by bisecting lines along the tibia and the third metatarsal bones (Caron, 1988). However, the long axis of the third metatarsal bone cannot be standardized in any radiographic evaluation protocol or study in angular deformities when a foal stands in an unnatural position (Caron, 1988; Clegg, 2003). This illustrates the difficulties such as lateromedial midpoints to be joined by a straight line and superimposition of bony structures to define its orientation in the same frontal plane on representative LM radiographs (Caron, 1988; Clegg, 2003; Vanderperren, Raes, Van Bree, et al., 2009).

As the articular surfaces of the joint are curved, complete overlapping becomes difficult especially if there is a slight oblique orientation, thus use of best fit line provides a better way to standardize the joint measurements.

The proximal inter tarsal-distal intertarsal joint, and Distal intertarsal-metatarsal joint parameters were designed to identify the relationship between the morphometry of the articular surfaces of the central and third tarsal bones and to find any displacements. Such displacements of the distal intertarsal joint, were reported and diagnosed (Düsterdieck, May, Pleasant, & Howard, 2002). Furthermore, some authors have reported displacements concerning the intertarsal joint (Gross, 1964; Moll, Slone, Humburg, & Jagar, 1987; Wheat & Rhode, 1964). It has been suggested that the distal intertarsal joint is less likely to be displaced as compared to the proximal intertarsal or tarsometatarsal joints, because the fourth tarsal bone height is equal to the combined central and third tarsal bones and therefore bridges and stabilizes the distal intertarsal joint (Moll et al., 1987). The displacement of the tarsometatarsal joint is associated with ligament disruption and tarsal and metatarsal fractures (Bolt, Williams, & Burba, 2003; Moll et al., 1987).

The repeatability of the parameters was generally higher for the individual angles (table 3.1). All individual angular parameters had an intraclass correlation coefficient (ICC) of higher than 0.88. They also had higher values of Lin's CCC and both (1st and 2nd) readings were within the range of almost perfect agreement (0.81–1.00). The individual angular parameter # 3 (Dorso-distal edge of calcaneus- Talocalcaneal angle) was considered in substantial agreement (0.61–0.80). Such results indicate that the individual angular parameters were measured consistently.

The consistency between the measurements could be due to the use of easily located tarsal landmarks which consequently reduced errors in landmark identification. In addition, all the measurements were taken by one examiner which would also minimize the variations between repeated readings (Kublashvili et al., 2004). However, the ICC and the Lin's CCC for the correlated angular parameters were not as high as for the individual angles. In the ratio parameters, the results of the repeatability were quite variable. Although the ICC and Lin's CCC were relatively higher (> 0.9) for 2/3 ratios, they were moderate (0.8-0.7) for 1/3 ratios. This higher variation in ratios seems to be due to their small absolute values as compared to other parameters.

3.5.1 Conclusion

This study has provided a base for the measurement of tarsal conformation using radiography. This included identification of some landmarks within the joint to evaluate any rotation around tarsal axes, establishing the ZLM with the help of landmarks, locating the RL and developing ten consistent and repeatable measurable parameters that indicate some of the detailed morphometry of the tarsus. Some of developed parameters were especially promising and have relatively higher reliability ($ICC > 0.95$). These parameters were Proximal intertarsal-Metatarsus joint angle (IAP-2), St. calcani-Metatarsus angle (IAP-4) and WCalc/WT ratio (RP-10) which may be helpful in determination of the relative alignment, geometry and changes in positions of tarsal bones within this complex joint. In order to use these parameters in archival lateromedial radiographs, they should be evaluated on radiographs oriented around the tarsal axes. The effects of changes in joint angle and rotations are important in the radiographic evaluation of detailed conformation and morphometry of the

tarsus. Although the project was aimed to include all morphological details of the bones, some details were very difficult to locate and consequently excluded.

Acknowledgment

The authors would like to thank the University of Melbourne for financial support. Brendan Kehoe and the staff of the Pathology and Radiology departments for their assistance.

Chapter 4: Developing measurable radiographic parameters of the tarsus on the DP view.

4.1 INTRODUCTION:

The conformation of the tarsus is one of the important factors given serious deliberation when purchase evaluation is conducted for a Thoroughbred racehorse (Love et al., 2006), and faulty conformation may decrease a horse's value considerably. Tarsus conformation is considered as essential for the diagnosis of diseases (Schebitz, 1965b; T. Stashak, 1987) and presale or purchases often include radiographic examination for subsequent performance (Cohen et al., 2006). Radiography is still frequently used as a quick diagnostic tool (Vanderperren, Raes, Hoegaerts, et al., 2009). The most significant cause of wastage in the Thoroughbred racing industry is musculoskeletal injury (Jeffcott et al., 1982; Love et al., 2006; Rosedale, Hopes, & Digby, 1985), thus the identification of potential predisposing factors such as differentiation of specific normal and abnormal conformations, has great significance. However, the detailed description of conformation of the tarsus is not well defined in the literature.

In equine radiography, a complete radiographic examination of the tarsus or hock joint can be performed with a minimum of four standard views: latero-medial, dorso-plantar, dorsolateral-plantaromedial oblique, and dorsomedial-plantarolateral oblique (Butler, Colles, Dyson, Kold, & Poulos, 2000). In some selected cases, flexed lateromedial and dorsoplantar views can provide additional valuable information. Accurate projections with high quality radiography and careful positioning of the object have also significant importance (Butler et al., 1993; Laverty et al., 1991; Shelley & Dyson, 1984). In clinical

situations all four projections should be included to maximize the accuracy of the radiographic evaluation. When the number of projections that can be realistically taken is limited, however, less than four projections have been used for evaluation of the tarsus (Audigie et al., 1993; P Eksell et al., 1998; Per Eksell et al., 1999; Laverty et al., 1991), and dorsoplantar (DP) view is one of most commonly taken view for clinical evaluation, because it can provide most of the morphological details.

There is no information available on the prevalence of specific tarsus conformational traits or assessment of internal geometry of the tarsus in Thoroughbreds or other breeds despite the established importance of conformation in the assessment of young racehorses or other professional athletes. There are still insufficient geometrical parameters available in the literature for better evaluation of the complex conformation of the tarsus. This might reflect the lack of enough available morphometric information in the data base (Beeman, 1973; Love et al., 2006).

The evaluation of tarsal conformation in relation to its susceptibility to injury was also considered to be difficult despite being a common site for injury and associated fractures. In a study by Love et al. 2006, weak associations were found between performance and conformation (weak hocks trait) that could not be accounted for by the very strong relationship between pedigree and conformation, while including nothing to describe the internal geometry of the tarsus. This indicates the difficulties in assessing conformation, rather than an absence of an association between conformation, performance and susceptibility to injury. There is a shortage of data on the conformation of the tarsus, which could have an important association with tarsal pathology. Although DP clinical radiographs are used mostly for diagnostic purposes rather to evaluate any morphometry and/or

conformational changes of the tarsus this view seems likely to provide a useful way of assessing tarsal conformation and has promoted the hypothesis in this chapter was “Can the tarsus internal geometry be assessed through the DP radiographic view by developing some reliable measurements?

To test this hypothesis the following aims were developed:

1. Identify a consistent tarsal dorsoplantar view considering both horizontal and vertical axes.
2. Identify a reference structure on dorsoplantar radiographs.
3. Develop reliably tarsal measurable parameters on dorsoplantar radiographs.
4. Validate the repeatability of these parameters.

4.2 MATERIALS AND METHODS:

4.2.1 Dorsoplantar view of hock at zero angle (ZDP):

In this study, radiographs in the dorsoplantar view of the equine tarsus were taken, and a specific profile was determined and called the zero-degree dorsoplantar view or ZDP. Although there were three main axes for rotations of the tarsus in the dorsoplantar view, the measurements were taken considering the vertical and horizontal axes as they can affect the radiographic measurements considerably, while the sagittal axis has the least effect. Therefore, establishing the ZDP was dependent on the correct orientation around the vertical and horizontal axes. The materials and procedure adopted for this ex-vivo experiment was as follows.

4.2.1.1 Animals:

Fifteen right and left cadaveric hind limbs were collected from twelve adult horses (table 4.1). All the hind limbs (cadavers) in this experiment were collected from the department of Pathology, FVAS, The University of Melbourne (Werribee campus). The horses were euthanized or died for reasons not related to this study and were not associated with the locomotor disorders nor tarsal injuries. The hind limbs were sectioned at the distal third or fourth of the crus through the soft tissues and tibia.

Table 4. 1: Details of horses from which tarsus specimens were collected

No.	Horse breed	Used limb/s	Age (in years)	Gender
1	Thoroughbred	Right	14	Female
2	Thoroughbred	Right	3	Female
3	Thoroughbred	Left	5	Male
4	Thoroughbred	Right and Left	3	Female
5	Thoroughbred	Right and Left	7	Male
6	Thoroughbred	Right and Left	9	Female
7	Thoroughbred	Right	8	Male
8	Standardbred	Right	30	Female
9	Thoroughbred	Right	4	Female
10	Thoroughbred	Right	6	Male
11	Thoroughbred	Left	3	Female
12	Thoroughbred	Right	5	Male

All other materials, including the loading device/apparatus, limb base, radiographic base with radiographic plate, radiography machine, digital cassettes, cassette holder, digital radiographic processor, were described in the previous chapter 3, section 3.3.1.1 in “Equipment section”.

4.2.1.2 Preparation of limbs:

1. The cut edge of the skin was sutured around the cut edge of the tibia to avoid skin movement during limb adjustments for radiography (if necessary).

The lateromedial width of the dorsal surface of the third metatarsus was measured twice at intervals of 12 cm with a Vernier’s caliper distal to the tarsometatarsal joint and midpoints of these diameters were marked. The two midpoints were then joined with a straight thick

wire to create a middle line (imaginary vertical axis) and to align with the vertical axis of the radiographic beam, helpful in radiography beam alignment during radiography process (Figure 4.1).

2. The solar surface of the hoof was fixed onto the limb base and centered with the dorsal surface of the limb facing the head of radiography machine. The limb was held in an extended position and this position was secured by putting the proximal end of the remaining tibia into the loading cup of the loading apparatus.

3. The limb was placed with the cannon (third metatarsal) positioned perpendicular to ground (vertically) in loading apparatus.

4. Palpation was carried out to identify the approximate levels of the centrodistal joint and the x-ray beam was centered on marked point (Figure 4.1). If the x-ray beam was horizontal and the medial side appear abnormally narrow then the x-ray beam was angled 5-10° proximodistally, so the medial side of the centrodistal joint may look more normal.

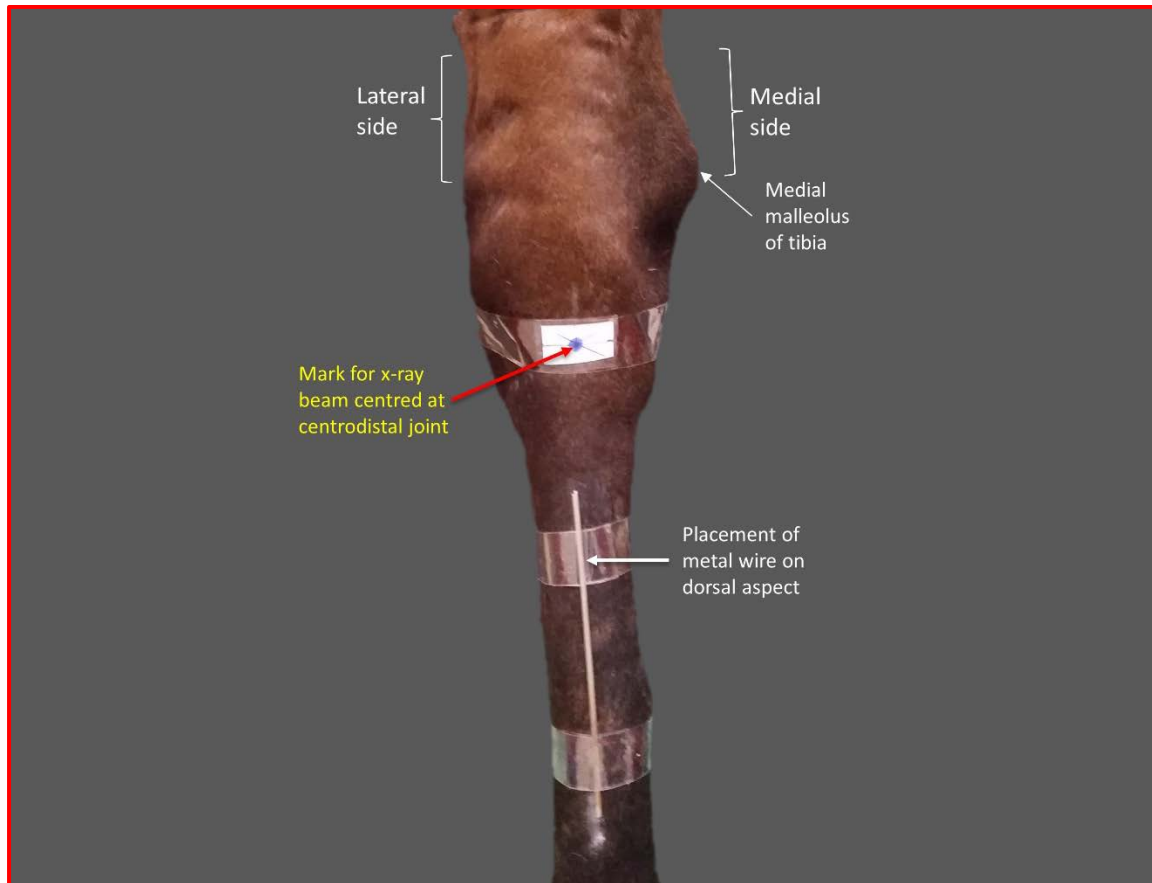


Figure 4. 1: Right hind limb of a Thoroughbred showing the placements of the marker for the center point for the x-ray beam on the distal intertarsal joint and the straight metallic wire on the dorsal aspect of the metatarsus distal to the tarsometatarsal joint to help with the positioning of the x-ray beam of the radiography machine.

4.2.1.3 Radiographing prepared limbs:

1. The cassette holder which contained a digital cassette was held parallel with third metatarsal (perpendicular to the ground) to the plantar aspect of the tarsus and the horizontal x-ray beam was directed at the distal intertarsal joint.
2. It was fixed adjacent to the radiographic apparatus in such a way that the surface which faced the radiography machine was perpendicular to the head of the radiography machine.
3. The limb base (with the prepared limb) was adjusted on the marked base of the radiographic base.

4. The radiography machine was placed at a distance of 100 cm away from the prepared limb. The center of the head of the radiography machine was at the level of the centrodistal joint for DP radiographs.
5. The amount of radiation varied according to the dorsoplantar thickness of the tarsus according to Lavin's formula (Lavin, 1994).

$$\text{KVs} = (2 \times \text{cm thickness of desired area of interest}) + 40$$



Figure 4. 2: Radiographing a right tarsus of a thoroughbred. The cadaveric articulated distal hind limb was fixed in the limb base. The radiographic machine was placed on the radiographic plate. The central x-ray beam was directed perpendicular to the talus as indicated by putting landmark on the limb.

4.2.2 Identifying vertical and horizontal tarsal axes:

1. Generally, for the ZDP radiographs, superimposition of lateral borders of the tuber calcaneus and distal tibia along with a same ratio of the dorsal widths of the central, and fourth tarsals taken at middle of dorsal aspect were considered in the vertical evaluation. However, the lateral edges of calcaneus and distal tibia did not align with each other and lacked a suitable ratio of the dorsal widths of the central, and fourth tarsals in some radiographs, so those limbs were re-manipulated lateromedially and re-radiographed until the desired alignment was achieved. The vertical tarsal axis was then represented by a proximodistally imaginary line extending perpendicular to the x-ray beam (Figure 4.3).
2. The position of the limb was marked by some dorsal, lateral and plantar marks on the limb base. The mark positions were on the toe of hoof for dorsal mark and back side for heels mark on the limb base (which was kept in a constant position) to maintain the correct position of the vertical axis while the horizontal axis was found.

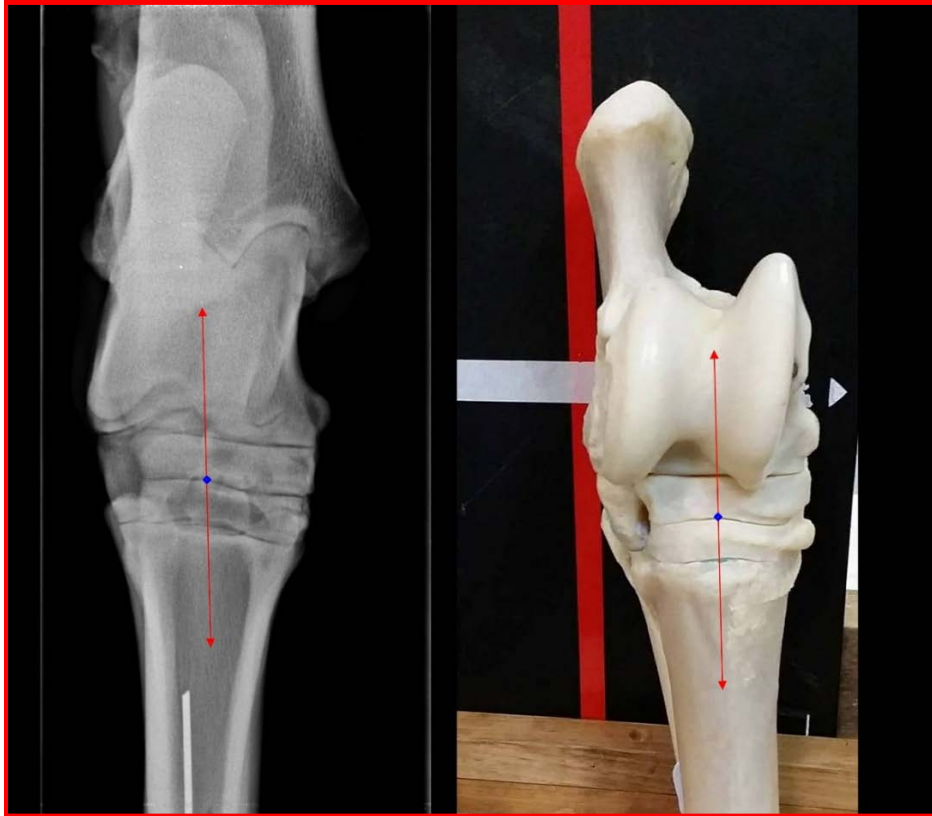


Figure 4. 3: A dorsoplantar radiograph of an intact right tarsus with a photograph of a bone preparation of a right tarsus. The figure illustrates the location of vertical axis (red line). The center of the x-ray beam (blue dot) was positioned perpendicular to the distal intertarsal joint between the central and third tarsal bones.

3. At the level of the proximal tibiotalar/tarsocrural joint on the radiographs, the aim was to have a contact point between the proximal borders of the lateral and medial trochlear ridges of the talus. The radiographs lacking this feature, were re-radiographed by changing the position of radiography machine and digital cassette until the required alignment between the proximal lateral and medial trochlear ridge borders was achieved. After attaining this feature, the horizontal tarsal axis was represented by an imaginary line extending lateromedially perpendicular to the x-ray beam. The proximal borders of this horizontal axis were located in the proximal intertarsal joint and approximately parallel to

the line which was between proximal borders of the lateral and medial trochlear ridges of talus (Figure 4.4).

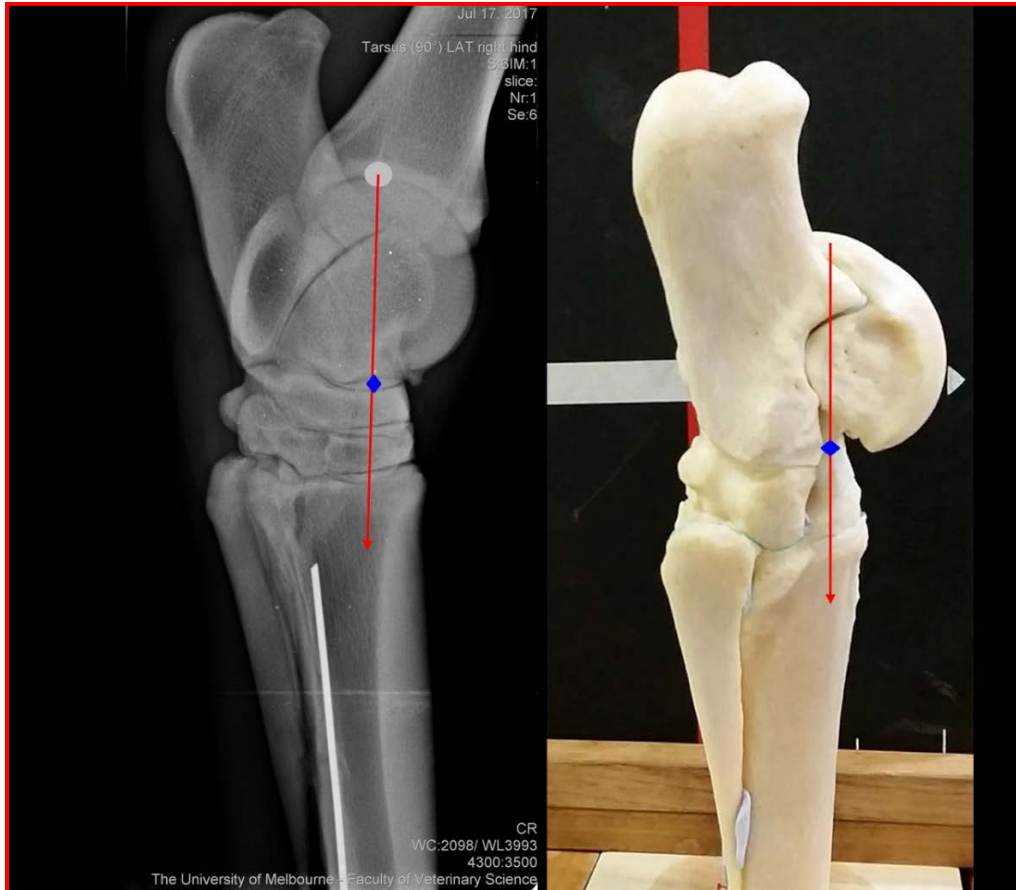


Figure 4. 4: A lateromedial radiograph and photograph of the lateral aspect of a right tarsus. The figure illustrates how the location of the horizontal axis (blue dot) was found in the proximal intertarsal joint by extending a vertical line from the proximal borders of lateral (and medial) trochlear ridges of the talus. The proximal borders of the lateral and medial trochlear ridges were superimposed in the radiograph as highlighted above.

4.2.3 Reference line:

The bone surfaces were correlated to another structure or a reference line to create some measurable parameters such as angles or ratios to produce quantitative data. These parameters could be referred to each other for the purpose of developing correlated

parameters. In this study, this reference line was designed to meet a number of conditions:

- Its landmarks should be easy to identify on the radiograph.
- It showed minimal or no morphometrical variations between and within horses.
- It had minimal or no incidence of lesions.
- It was a part of the tarsal bony components.

Based on these conditions in a lateromedial plane, the proximal extremity of the third metatarsus was selected to be the most suitable reference in measuring tarsal angular parameters. This reference or Reference Line (RL) was extended between a medial and a lateral anatomical landmark.

4.2.3.1 Landmarks of the RL:

The reference line was a straight line extended between two landmarks on the lateral and medial side of the distal tarsus. The lateral landmark was the point where the dorso-lateral edge of distal articular surface of the fourth tarsal, and the dorso-lateral edge of the articular facet on the proximal surface of the third metatarsal for articulation with fourth tarsal came together.

The medial landmark was the point where the dorso-medial edge of the distal articular surface of the second tarsal, and the dorso-medial edge of the articular facet on the proximal surface of the third metatarsal for articulation with second tarsal came together (Figure 4.5).

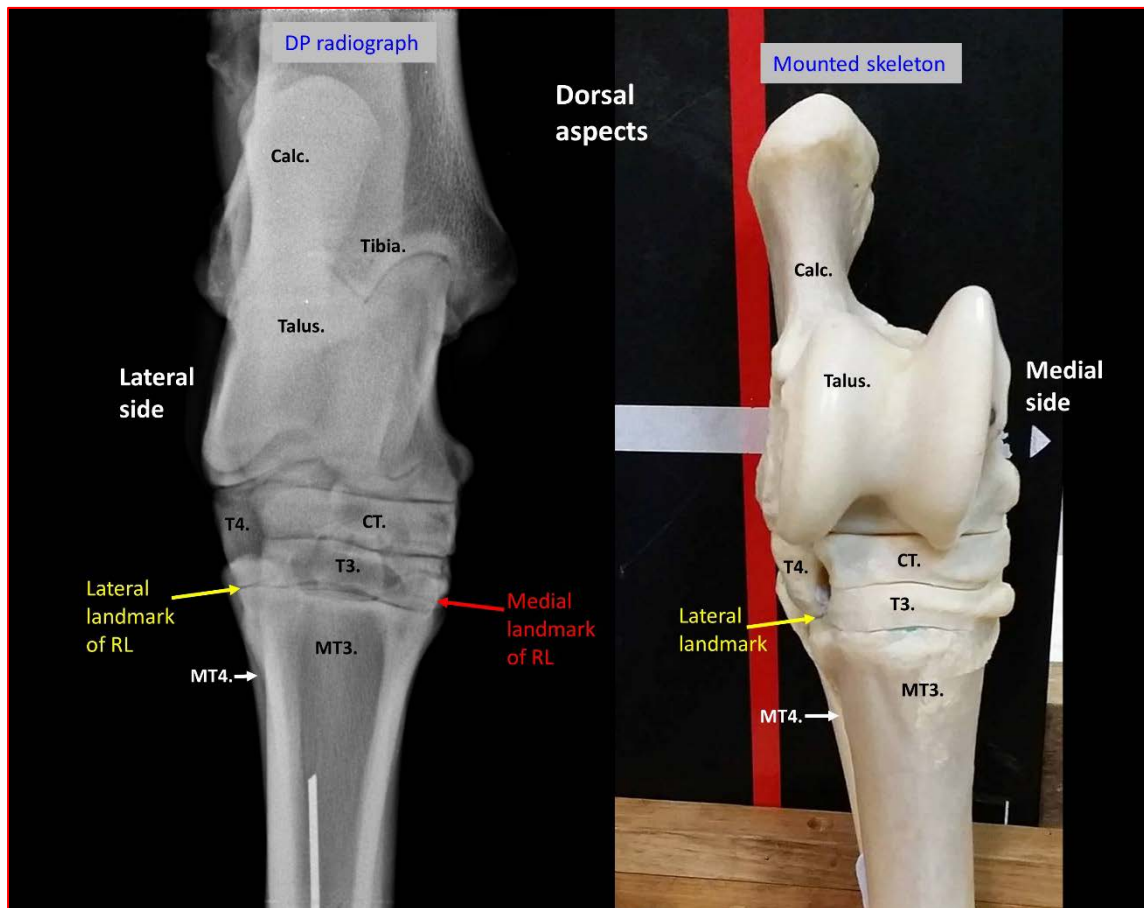


Figure 4. 5: Dorsal aspects of two different right tarsi in a mounted skeleton and a dorsoplantar radiograph. The figure illustrates the locations of both lateral and medial landmarks used for the reference line (RL); Calc. Calcaneus tarsal; CT, Central tarsal; T3, third tarsal; T4, fourth tarsal; MT3, Third metatarsal; MT4, Fourth metatarsal.

4.2.4 TARSAL MEASURABLE PARAMETERS:

Measurable tarsal parameters have been optimized based on the possibility and accuracy of detecting their landmarks on the DP radiographs. These parameters were designed to represent detailed tarsal morphometrical conformation.

In the tarsal dorsoplantar radiographs, anatomical details of tarsal bones appeared

superimposed on each other either in individual bones or between bones. Therefore, some tarsal preparations were required to get a better understanding of this complicated region and to identify landmarks of the measurable parameters.

4.2.4.1 Mounted tarsi:

Three boiled tarsi from two horses were used as a preparation step for radiography. Gross studies of tarsal morphology were performed in detail for each single bone and for relationships between the bones after being mounted into the three tarsi. The main details that were noted were the shape of the articular surfaces including concavity and convexity, extension of articular surfaces, facets, tubercles and fossae (As described in detail in Chapter 2). The three tarsi were then radiographed lateromedially and dorsoplantarly. A comparison was made between the radiographs and the mounted tarsi to select the most measurable radiographic parameters. This additional analysis was performed on mounted tarsi to observe the landmarks on acquired images along with comparison with radiographs marked by either thin metal threads and/or small metal balls depending on the feature size, location and surface of the selected landmarks to ascertain their exact locations on the acquired images.

4.2.4.2 Dissected specimen:

Although radiographing the mounted tarsi was helpful in determining the measurable parameters on the dorsoplantar radiographs, removing their cartilage and soft tissues especially the ligaments, affected the normal articulation and alignment of the bones and consequently the accuracy of locating landmarks on radiographs.

A right hindlimb from a Standardbred horse was collected from the Pathology department of The University of Melbourne, Veterinary Science, to be used as a model in the study. All soft tissues, except ligaments, in the tarsal region were removed. Both lateral and medial collateral ligaments and intertarsal ligaments were partially retained in order to keep the tarsal, tibia and metatarsal bones as close as possible to the normal articulation and alignment. Desired parameters were indicated and marked by either thin metal threads and/or small metal balls depending on the feature size, location and surface.

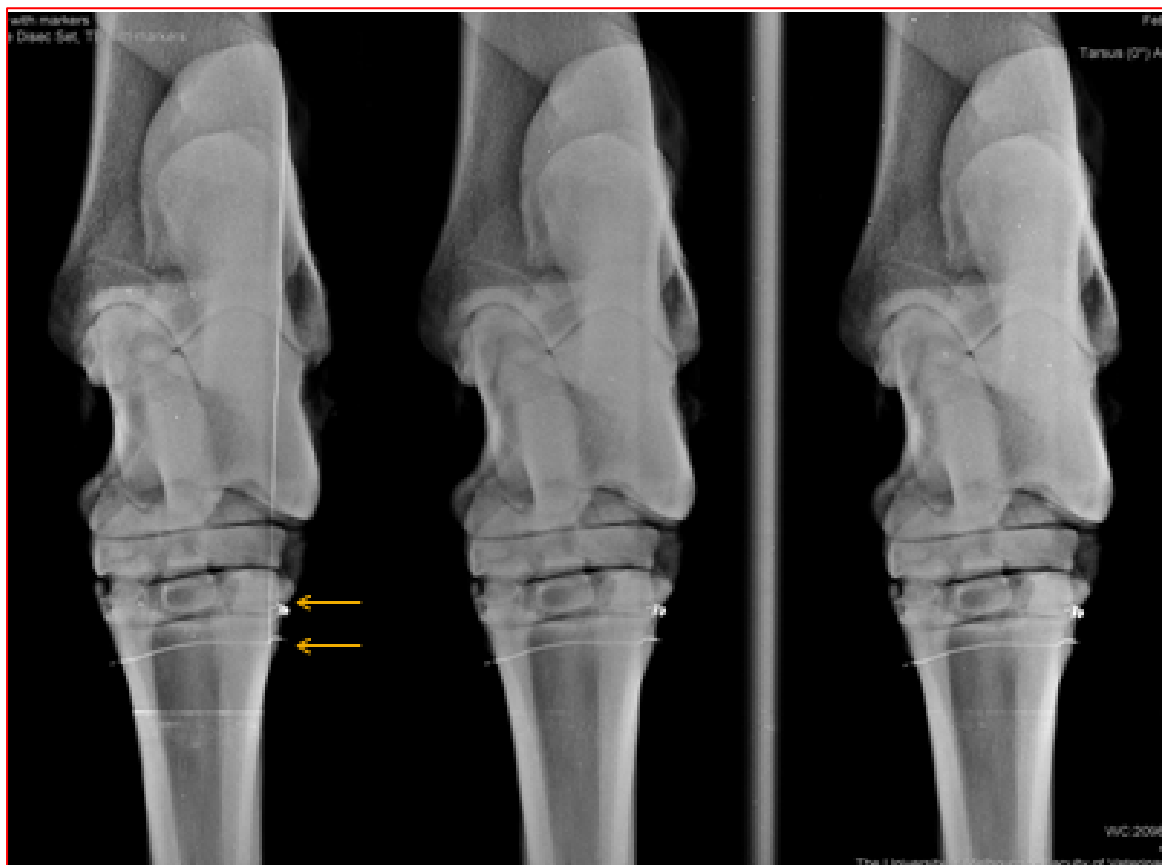


Figure 4. 6: Use of metal wire for identification of some landmarks.

4.2.5 INTRA-RATER REPEATABILITY:

4.2.5.1 Animals:

Fifteen hind limbs were collected from 12 adult horses. The horses were euthanized or died for other reasons not related to this study and were not associated with the locomotor disorders and injuries. The hind limbs were sectioned at the distal third or fourth of the tibia bone. The information including horse breed, gender and age was collected for each horse (Table 4.1).

4.2.5.2 Limb preparation, radiographing and measurements:

Each tarsus was prepared and radiographed by using procedures described in Section 3.3.1.1 to get the DP view using an Atomscope HF80/15+ Ultralight radiography machine. Radiographic cassettes were scanned (FireCR+ Veterinary 80 CR reader) and imported into DicomPACS®DX-R X-Ray acquisition software for measurements. Each tarsus was radiographed and measured twice with an interval of at least one month. All the measurements were taken by the author to minimize inconsistency of the measurements (Kublashvili et al., 2004).

4.2.5.3 Statistical analysis:

The Normal distribution of combined measurements from the first and second measurements was assessed by the Shapiro-Wilk test for Normality. The mean values with standard deviations, 95% confidence interval (CI) of mean, median, skewness, and kurtosis

were calculated, and graphs were plotted for each of the developed radiographic parameters.

Intra-tester repeatability of repeat measurements was evaluated by calculating intraclass correlation coefficients (ICC). The mean values with standard deviations, 95% CI of the mean of first and second readings were calculated, ICC of the first and second measurements was calculated within the same rater and 95% CI of ICC were also calculated.

Lin's concordance correlation coefficient (Lin's CCC) was calculated by the following method. The coefficient (R_c) shows how well the relationship between the measurements is represented by a line through the origin at an angle of 45 degrees (as would be generated if the two measurements generated identical results) as derived by Lin (1989) and described in more detail with the associated scale in the previous chapter.

The mean difference between first and second readings with standard deviations, Bland-Altman plots of first and second radiographic measurements of each radiographic parameters were calculated using 95% limits of agreement of Bland-Altman plots. The 95% limits of agreement provide an interval within which 95% of differences between measurements by the two repeat measurements were expected to lie and was estimated by mean difference 1.96 standard deviation of the differences. Significance was set at $p < 0.05$. Agreement between the first and second measurements was also quantified by using the differences between observations on the same subjects made using the two repeat measurements after an interval.

4.3 RESULTS:

4.3.1 Dorsoplantar (DP) view for tarsus conformation:

The DP view was achieved by two evaluations. The view was considered to be at the DP when there was almost complete overlapping of the lateral edges of the distal tibia and calcaneus and the ratio of widths from the middle of the third and fourth tarsal bones (vertical landmark) in the vertical evaluation. While in the horizontal evaluation, the view was considered to be at the DP when there was a distal border of the lateral trochlea ridge of talus which was above the calcaneoquartal joint (Figure 4.7).

4.3.1.1 Reference line:

In order to produce quantitative data, surfaces of bone were correlated to another structure or a reference to create some of the parameters such as angles. There was a slight difference observed in the lateromedial heights of the third metatarsal, as the medial height and lateral height landmarks of the RL in the Mt3 bones showed significant differences ($p = 0.00$). The mean of the medial heights was 30.56 cm ($SE \pm 0.18$) while mean of the lateral heights was 30.78 cm ($SE \pm 0.18$) as described in detail in previous chapter 2, section 2.3.8.

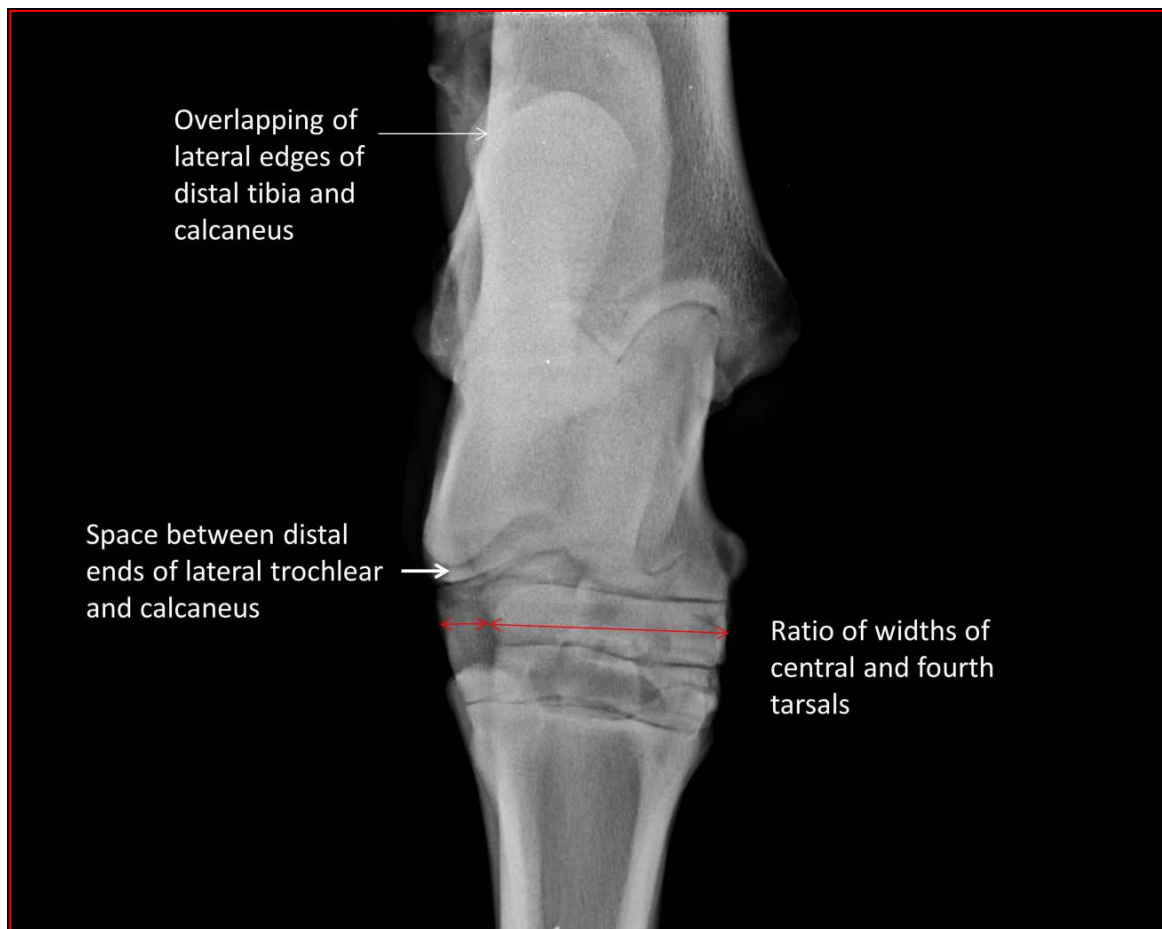


Figure 4. 7: The DP view of a right tarsus of a Thoroughbred. The ratio of dorsal widths of the central and fourth tarsals is indicated along with the overlapping of the lateral borders of the distal tibia and calcaneus which were used for evaluation of the vertical alignment. The space between the distal end of the lateral trochlear ridge of talus and the distal end of calcaneus where it articulates with the proximal surface of the fourth tarsal was used for evaluation of the horizontal alignment.

4.3.2 TARSAL MEASURABLE PARAMETERS:

Using the above-mentioned mounted tarsi and tarsal preparation set, 49 parameters were selected to be evaluated in this study. Angles and ratios were selected because they should be least affected by different sizes of samples and distances with radiography apparatus, so they should be useful to evaluate archival radiographs in due course. There were 30 angles and 19 ratios.

4.3.2.1 Angular parameters:

The angular parameters were either individual angular parameters (Figure 4.8) or correlated angular parameters (Figure 4.9). All angular parameters were taken with reference to the reference line on the third metatarsus. The angles were towards different positions according to their locations in the tarsal joint.

4.3.2.1.1 Individual Angular parameters:

The individual angular parameters were mostly defined as the angle which was formed between a certain feature of interest and the metatarsal reference line (RL). They were:

1. Tibial dorso-distal inter ridges angle (Talus ridge angle):

This angle was measured at the intersection point between two lines extending along (best fit with) the medial and lateral sides of the intermediate ridge of the distal extremity of the tibia (Figure 4.8/1). The tarsocrural joint is located between the distal extremity of the tibia and the proximal surface of the trochlea of talus and this joint is a typical ginglymus formed by the trochlea of the talus and the corresponding surface of the distal end of the tibia.

2. Tibial metaphyseal/malleolar angle (Tmp angle):

This angle was measured at the intersection point between a line perpendicular to the medial and lateral ends of the tibial metaphysis and a line perpendicular to the RL (Figure 4.8/2 and RL).

3. Tibio-talus joint angle (Tt angle):

This angle was measured at the intersection point between a line perpendicular to a line joining the medial and lateral distal ends of the distal articular surface of the tibia and a line perpendicular to the RL (Figure 4.8 /3 and RL).

4. Proximal intertarsal joint angle (PIT angle):

This angle was measured at the intersection point between a line perpendicular to the proximal intertarsal joint and a line perpendicular to the RL (Figure 4.8/4 and RL). The proximal intertarsal joint line was extended between two landmarks, the first landmark was medial and the most distal extremity of the dorsal central facet of talus at its distal articulation with the central tarsal. The second landmark was at the lateral end of the calcaneal facet of the fourth tarsal at its dorsal articulation with calcaneus.

5. Proximal central joint angle (PC angle):

This angle was measured at the intersection point between a line perpendicular to the proximal talocentral joint and a line perpendicular to the RL (Figure 4.8/5 and RL). The proximal talocentral joint line was extended between two landmarks, the first landmark was medial and at the most distal end of the dorsal central facet of talus at its distal articulation

with the central tarsal. The second landmark was the lateral edge of the talus facet of the central tarsal at its dorsal articulation with talus.

6. Distal intertarsal joint angle (DIT angle):

This angle was measured at the intersection point between a line perpendicular to the distal intertarsal joint and a line perpendicular to the RL (Figure 4.8/6 and RL). The distal intertarsal joint line was extended between two landmarks, the first landmark was medial and at the most distal edge of the third tarsal facet of the central tarsal at its distal articulation with the third tarsal. The second landmark was at the lateral edge of the central tarsal facet of the third tarsal bone at its proximal articulation with the central tarsal bone.

7. Tarsometatarsal joint angle (TMT angle):

This angle was measured at the intersection point between a line perpendicular to the tarsometatarsal joint and a line perpendicular to the RL (Figure 4.8/7 and RL). The tarsometatarsal joint line was extended between two landmarks, the first landmark was the medial distal edge of the dorsal third metatarsal facet of the third tarsal at its distal articulation with the third metatarsal. The second landmark was at the lateral edge of the distal third metatarsal facet of the fourth tarsal at its distal articulation with the third metatarsal. The line joining the lateromedial ends of this joint was found to be a more consistent and reliable representation for this joint because it is a more curved joint and the best fit line was not found to be consistent.

8. Third tarsal-metatarsal joint angle (TtM angle):

This angle was measured at the intersection point between a line perpendicular to the third tarsal-metatarsal joint and a line perpendicular to the RL (Figure 4.8/8 and RL). The third

tarsal-metatarsal joint line was extended between two landmarks, the first landmark was medial at the most distal edge of the dorsal third metatarsal facet of third tarsal at its distal articulation with the third metatarsal. The second landmark was lateral end of distal third metatarsal facet of third tarsal at its distal articulation with the third metatarsal.

9. The calcaneoquartal tarsal joint angle (Pcq angle):

This angle was measured at the intersection point between a line perpendicular to the calcaneoquartal joint and a line perpendicular to the RL (Figure 4.8/9 and RL). The calcaneoquartal joint line was extended (best fit line) between two landmarks, the first landmark was the medial edge of the proximal calcaneal facet of the fourth tarsal at its proximal articulation with the calcaneal tarsal. The second landmark was the lateral edge of the proximal calcaneal facet of the fourth tarsal at its articulation with the calcaneus.

10. Distal metatarsoquartal joint angle (dMq angle):

This angle was measured at the intersection point between a line perpendicular to the distal metatarsoquartal joint and a line perpendicular to the RL (Figure 4.8/10 and RL). The distal metatarsoquartal joint line was extended between two landmarks, the first landmark was the medial edge of the distal third metatarsal facet of the fourth tarsal at its distal articulation with the third metatarsal. The second landmark was the lateral edge of the distal third metatarsal facet of the fourth tarsal at its distal articulation with the third metatarsal.

11. Long calcaneal axis-RL joint angle (Calc-RL angle):

This angle was measured at the intersection point between the long axis of calcaneus from the dorsoplantar aspect and the RL. The long axis of calcaneus was defined by a line joining two mid-points (landmarks), the proximal mid-point was between the most dorsal part of

the tuber calcaneus and the distal point was the most lateral edge of the calcaneo-quartal joint (Figure 4.8/11 and RL). The line joining both proximal and distal points represented the long axis of the calcaneus in the dorsoplantar view.

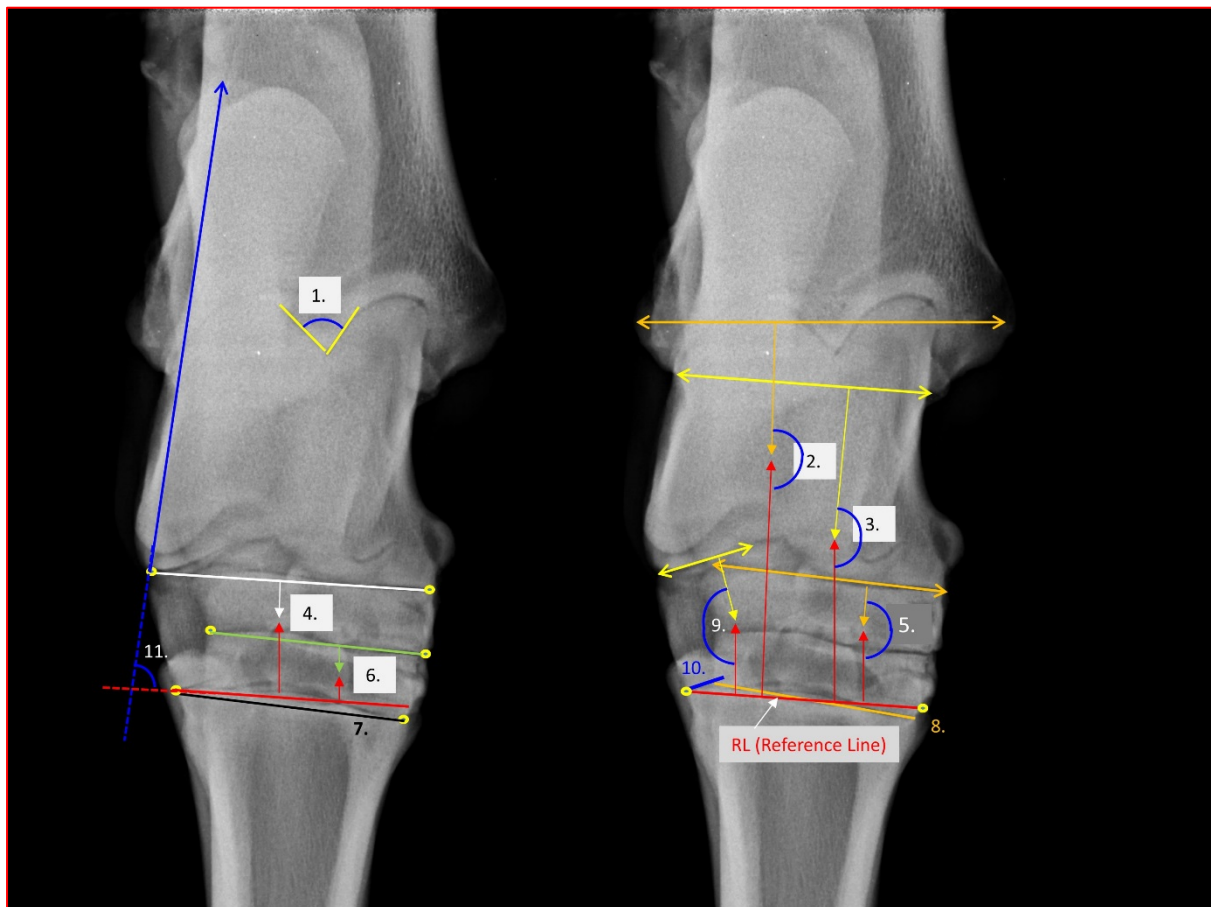


Figure 4. 8: DP radiographs of a right tarsus of a thoroughbred. The individual angular parameters are shown in this figure. The reference line is shown in red in both images.

They are 1. Tibial dorso-distal inter ridges angle, 2. Tibial metaphyseal/malleolar angle, 3. Tibio-talus joint angle, 4. Proximal intertarsal joint angle, 5. Proximal central joint angle, 6. Distal intertarsal joint angle, 7. Tarsometatarsal joint angle, 8. Third tarsal-metatarsal joint angle, 9. The calcaneoquartal tarsal joint angle, 10. Distal metatarsoquartal joint angle, 11. Long calcaneal axis-RL joint angle.

4.3.2.1.2 INTRA-RATER REPEATABILITY OF INDIVIDUAL ANGULAR PARAMETERS:

The p values were >0.05 for 9 of 11 individual angular parameters and 2 parameters had p-values of 0.009, and 0.001 for the normality of parameters (Table 4.2). These were the Tibial metaphyseal /malleolar angle (Tmp angle) and Tibio-talus (Tt) angle. There were two further p-values that were only slightly above 0.05. The Normality Q-Q plots were plotted for each parameter (Figure 4.9) to check their approximate agreement with a straight line as in real data sets like these, the natural variations need consideration such as individual variations, breed, age, sex, locations of landmarks and other factors which can cause data points not to be in a perfect agreement on a straight line.

The repeatability statistical analysis was summarized in Tables 4.3 and 4.4. From the tables, it can be realized that generally, the difference between repeated measurements was smaller for the individual angles. In the individual angular parameters, all the differences between the repeated measurements had higher Intraclass coefficients (0.661-0.949). The greatest difference between the repeat measurements was in the Tarsometatarsal (TMT) angle with an ICC of 0.661 whereas the least difference was in the Proximal intertarsal (PIT) angle with an ICC of 0.949. Lin's concordance correlation coefficient (CCC) was also generally higher in the individual angular parameters in the comparison between the two repeated measurements. Almost perfect agreement, with a Lin's CCC range of 0.81-1.00 was found in 6 out of 11 parameters, these parameters were the Tibial dorso-distal inter ridges angle, Tibial metaphyseal /malleolar angle (Tmp angle), Tibio-talus (Tt) angle, Proximal intertarsal (PIT) angle, Proximal central (PC) angle and Long Calc axis-RL angle. Substantial agreement was found in the Distal intertarsal angle, Third tarsal-metatarsal (TtM) angle, Proximal

calcaneal joint (Pcq) angle and Distal metatarsoquartal (dMq) joint angle with a Lin's CCC of 0.749 and 0.768 respectively. The scatter plots of Lin's CCC presented in figure 4.10.

The mean difference between the first and second measurements were not significantly different from each other (p values ranged from 0.127 to 0.796) in all individual angular parameters. Bland-Altman plots of each individual angular parameter were plotted using 95% limits of agreement (Figure 4.11).

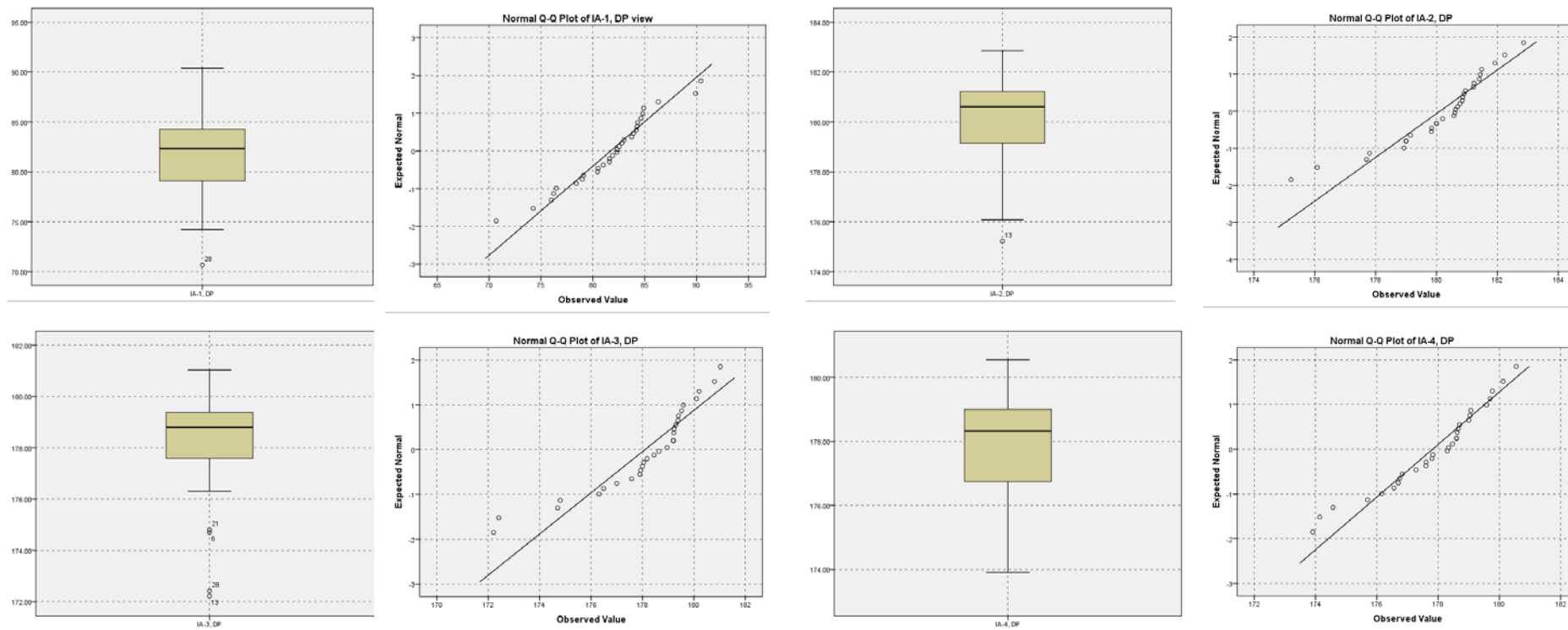
Table 4. 2: Individual angular parameters measured in fifteen samples for equine tarsal conformation

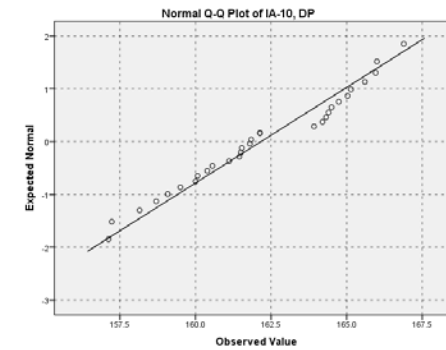
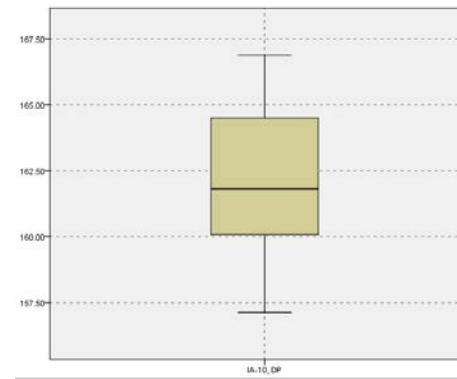
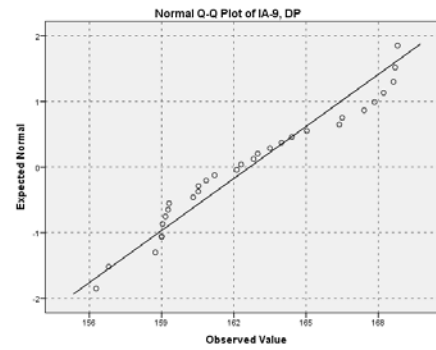
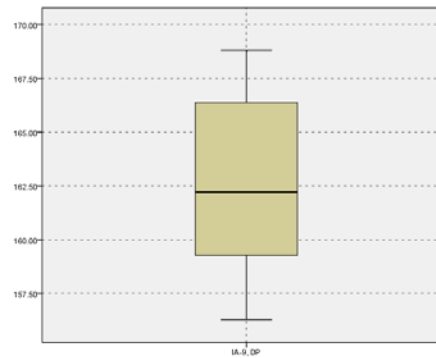
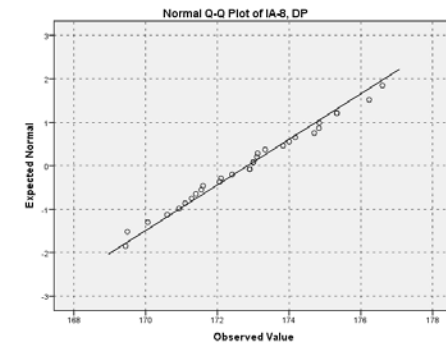
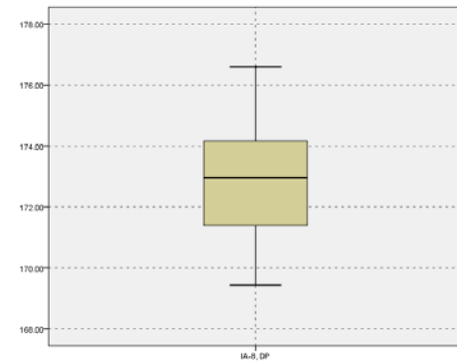
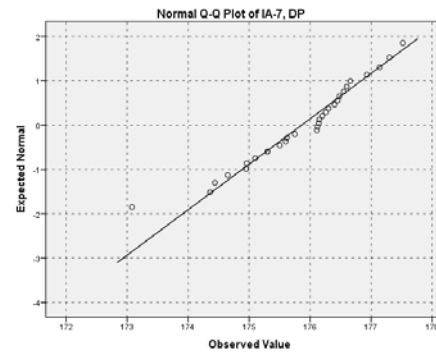
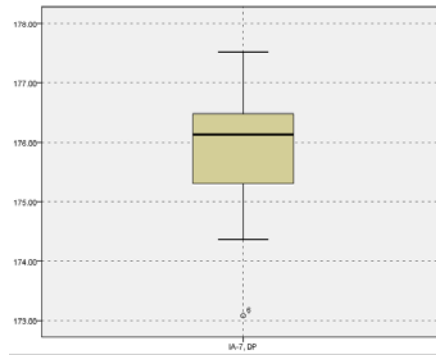
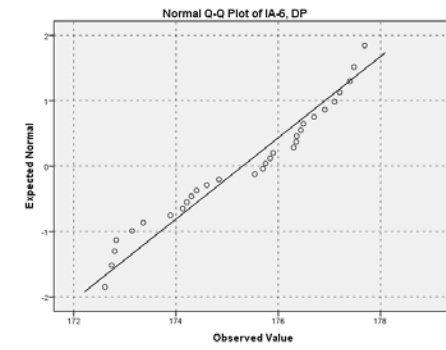
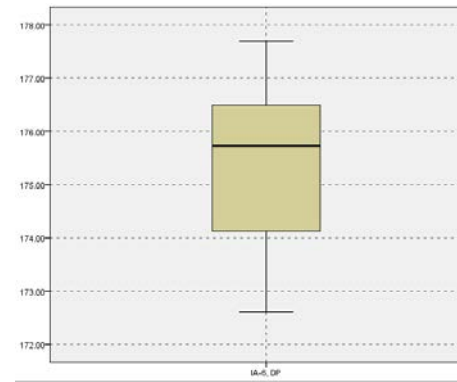
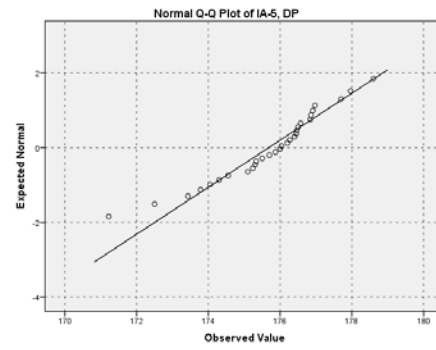
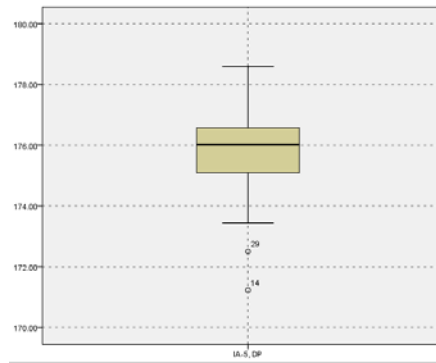
SR. NO.	MEASURABLE PARAMETERS	MEAN $\pm$ SD	MEDIAN	95 % CI OF MEAN		SKEWNESS (KURTOSIS)	TEST OF NORMALITY (SHAPIRO-WILK) SIG.
				Lower	Upper		
1	IA-1, Tibial dorso-distal inter ridges angle	81.72 $\pm$ 4.24	82.34	80.72	83.30	-0.426 0.864	0.345
2	IA-2, Tibial metaphyseal /malleolar angle (Tmp angle)	180.11 $\pm$ 1.70	180.61	179.48	180.74	-1.254 1.835	0.009*
3	IA-3, Tibio-talus (Tt) angle	178.10 $\pm$ 2.18	178.80	177.29	178.91	-1.406 1.775	0.001*
4	IA-4, Proximal intertarsal (PIT) angle	177.82 $\pm$ 1.70	178.32	177.18	178.45	-0.772 0.170	0.100
5	IA-5, Proximal central (PC) angle	175.69 $\pm$ 1.59	176.02	175.09	176.28	-0.869 1.192	0.164
6	IA-6, Distal intertarsal angle (m)	175.30 $\pm$ 1.61	175.73	174.70	175.90	-0.287 -1.221	0.051
7	IA-7, Tarsometatarsal (TMT) angle	175.86 $\pm$ 0.98	176.13	175.50	176.23	-0.762 0.891	0.296
8	IA-8, Third tarsal-metatarsal (TtM) angle	172.84 $\pm$ 1.91	172.95	172.13	173.55	0.088 -0.627	0.826
9	IA-9. Proximal calcaneal joint (Pcq) angle	162.65 $\pm$ 3.79	162.21	161.24	164.07	0.266 -1.115	0.065
10	IA-10, Distal metatarsoquartal (dMq) joint angle	162.17 $\pm$ 2.76	161.82	161.13	163.20	-0.111 -1.006	0.284

11

IA-11, Long Calc axis-RL angle	78.37 ± 3.01	77.54	77.24	79.49	0.497 -0.795	0.059
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Figure 4. 9: Normality distribution (Normal Q-Q) and mean box plots for individual angular parameters.





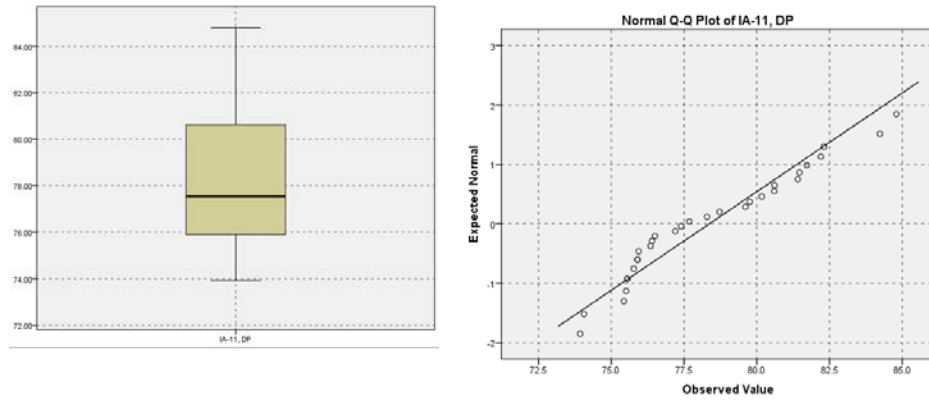
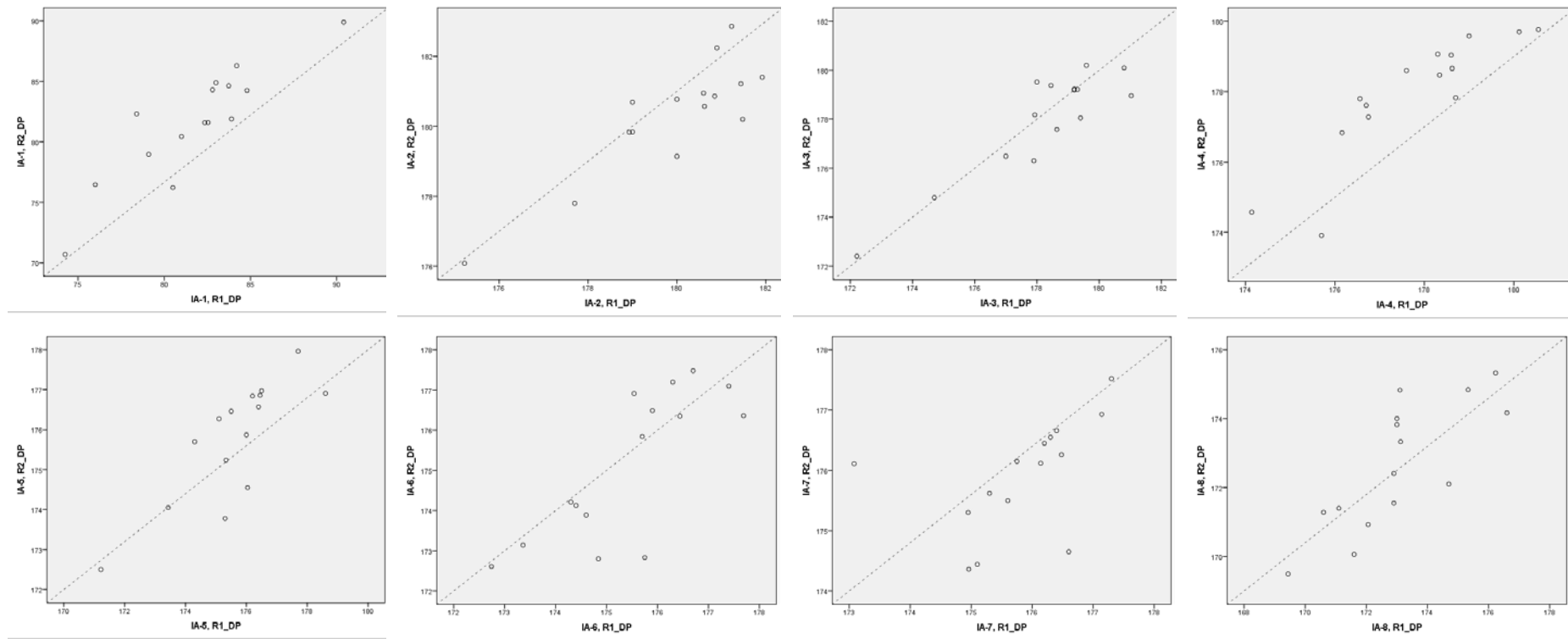


Table 4. 3: Intra-rater repeatability of the individual angular parameters using ICC and Lin's CCC coefficients in 20 tarsi

Sr. No.	Measurable parameters (n=15)	measurements	Mean $\pm$ SD	Intraclass Correlation Coefficient (ICC)	95 % CI of ICC		Lin's CCC	95 % CI of Lin's CCC	
					Lower	Upper		Lower	Upper
1	IA-1, Tibial dorso-distal inter ridges angle	1 st	81.80 $\pm$ 3.89	0.939	0.817	0.979	0.877	0.697	0.953
		2 nd	81.64 $\pm$ 4.70						
2	IA-2, Tibial metaphyseal /malleolar angle (Tmp angle)	1 st	179.93 $\pm$ 1.75	0.922	0.767	0.974	0.846	0.615	0.943
		2 nd	180.30 $\pm$ 1.68						
3	IA-3, Tibio-talus (Tt) angle	1 st	178.22 $\pm$ 2.26	0.949	0.853	0.983	0.897	0.727	0.964
		2 nd	177.98 $\pm$ 2.17						

4	IA-4, Proximal intertarsal (PIT) angle	1 st	177.72 ± 1.72	0.940	0.825	0.980	0.879	0.682	0.957
		2 nd	177.91 ± 1.74						
5	IA-5, Proximal central (PC) angle	1 st	175.60 ± 1.74	0.896	0.694	0.965	0.801	0.524	0.925
		2 nd	175.77 ± 1.48						
6	IA-6, Distal intertarsal angle (m)	1 st	175.44 ± 1.41	0.866	0.611	0.954	0.750	0.447	0.899
		2 nd	175.16 ± 1.82						
7	IA-7, Tarsometatarsal (TMT) angle	1 st	175.82 ± 1.06	0.661	-0.050	0.887	0.476	-0.017	0.783
		2 nd	175.91 ± 0.92						
8	IA-8, Third tarsal-metatarsal (TtM) angle	1 st	173.05 ± 2.01	0.876	0.645	0.958	0.768	0.455	0.912
		2 nd	172.64 ± 1.84						
9	IA-9. Proximal calcaneal joint (Pcq) angle	1 st	162.49 ± 3.72	0.865	0.596	0.955	0.749	0.408	0.907
		2 nd	162.82 ± 3.97						
10	IA-10, Distal metatarsoquartal joint angle (dMq)	1 st	162.23 ± 2.84	0.868	0.601	0.956	0.754	0.413	0.910
		2 nd	162.10 ± 2.79						
11	IA-11, Long Calc axis-RL angle	1 st	78.60 ± 3.34	0.933	0.806	0.977	0.867	0.680	0.948
		2 nd	78.13 ± 2.74						

Figure 4. 10: Scatter plots of Lin's CCC showing the distribution of measurements and level of agreement and reproducibility between first and second measurements of thirteen individual angular parameters (IA); R-2nd, second measurement.



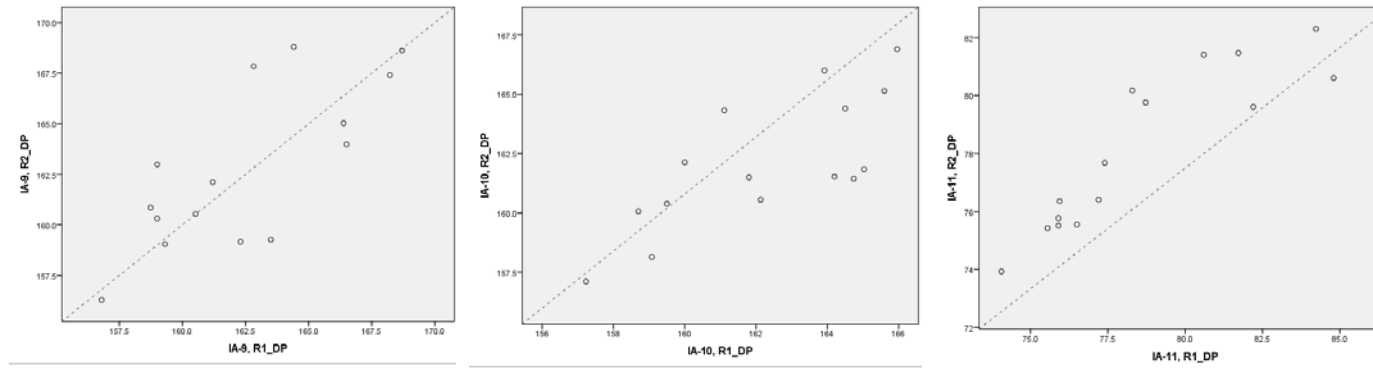
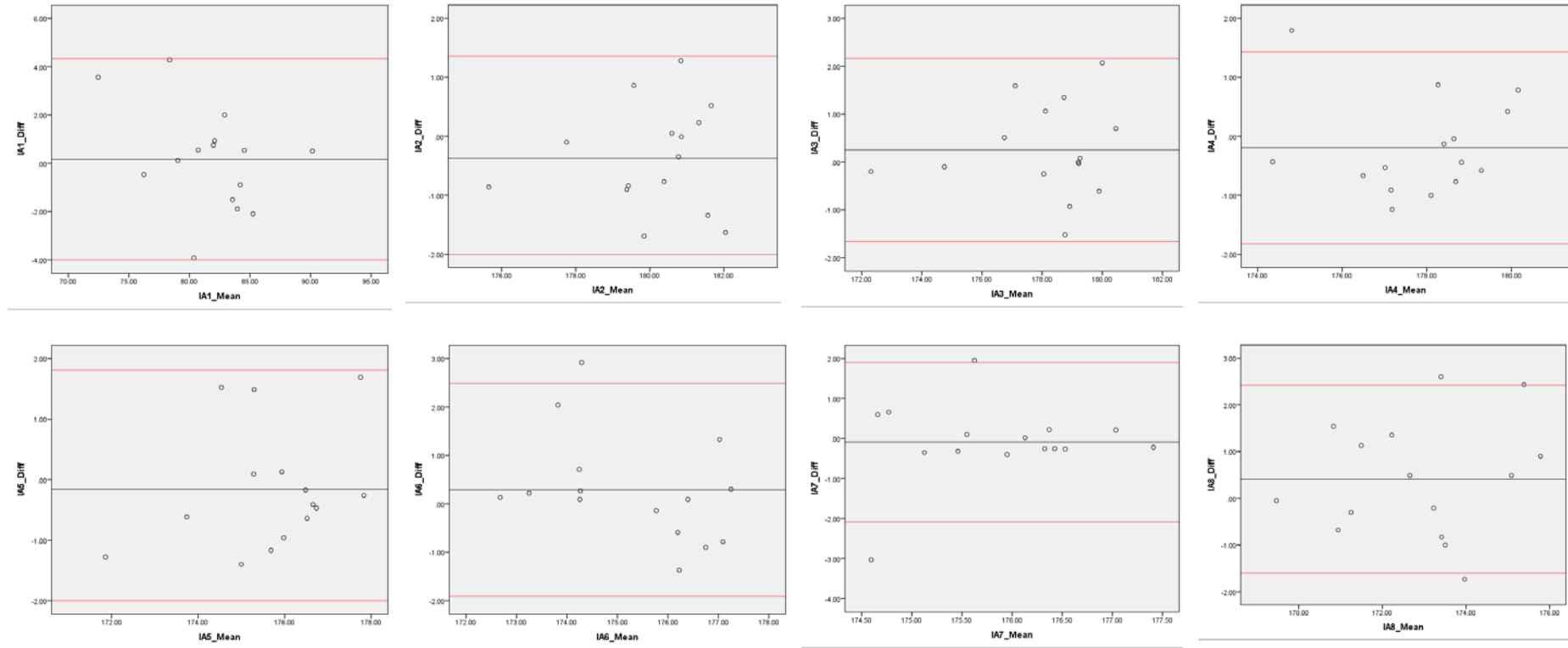


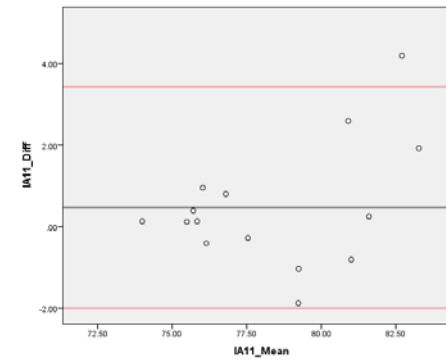
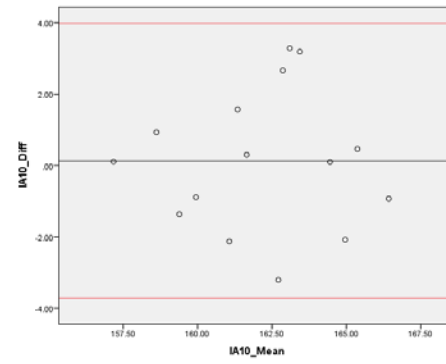
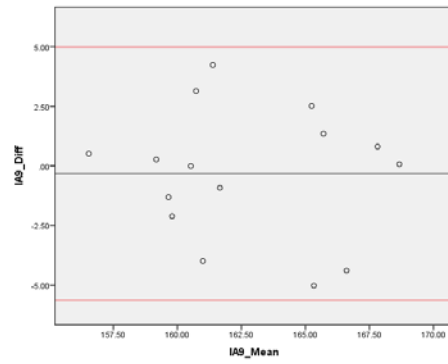
Table 4. 4: Intra-rater repeatability of the individual angular parameters in 15 tarsi using mean difference 95% (CI) limit of agreement between repeated measurements for the Bland-Altman Plots.

Sr. No.	Measurable parameters (n=20)	measurements	Mean $\pm$ SD	Mean Difference $\pm$ SD	Sig. level ($p < 0.05$)	Calculation of 95 % Limits of agreement of Bland-Altman plots	
						Lower	Upper
1	IA-1, Tibial dorso-distal inter ridges angle	1 st	81.80 $\pm$ 3.89	0.16 $\pm$ 2.13	0.771	-4.02	4.34
		2 nd	81.64 $\pm$ 4.70				
2	IA-2, Tibial metaphyseal/malleolar angle (Tmp angle)	1 st	179.93 $\pm$ 1.75	-0.37 $\pm$ 0.88	0.127	-2.10	1.36
		2 nd	180.30 $\pm$ 1.68				

3	IA-3, Tibio-talus (Tt) angle	1 st	178.22 ± 2.26	0.25 ± 0.97	0.340	-1.66	2.16
		2 nd	177.98 ± 2.17				
4	IA-4, Proximal intertarsal (PIT) angle	1 st	177.72 ± 1.72	-0.19 ± 0.83	0.385	-1.82	1.43
		2 nd	177.91 ± 1.74				
5	IA-5, Proximal central (PC) angle	1 st	175.60 ± 1.74	-0.16 ± 1.00	0.538	-2.14	1.81
		2 nd	175.77 ± 1.48				
6	IA-6, Distal intertarsal angle (m)	1 st	175.44 ± 1.41	0.29 ± 1.12	0.337	-1.91	2.49
		2 nd	175.16 ± 1.82				
7	IA-7, Tarsometatarsal (TMT) angle	1 st	175.82 ± 1.06	-0.09 ± 1.01	0.742	-2.08	1.90
		2 nd	175.91 ± 0.92				
8	IA-8, Third tarsal-metatarsal (TtM) angle	1 st	173.05 ± 2.01	0.41 ± 1.26	0.229	-1.60	2.42
		2 nd	172.64 ± 1.84				
9	IA-9. Proximal calcaneal joint (Pcq) angle	1 st	162.49 ± 3.72	-0.32 ± 2.71	0.652	-5.63	4.99
		2 nd	162.82 ± 3.97				
10	IA-10, Distal metatarsoquartal (dMq) joint angle	1 st	162.23 ± 2.84	0.13 ± 1.97	0.796	-3.72	3.99
		2 nd	162.10 ± 2.79				
11	IA-11, Long Calc axis-RL angle	1 st	78.60 ± 3.34	0.47 ± 1.51	0.248	-2.49	3.43
		2 nd	78.13 ± 2.74				

Figure 4. 11: Intra-rater/ repeatability of the individual angular parameters in 15 tarsi using mean difference 95% (CI) limit of agreement between repeated measurements for the Bland-Altman Plots





4.3.2.2 Correlated angular parameters:

A correlated angular parameter was presented by the angle which was formed between two different individual angles or points of interest. The features or landmarks of interest may be adjacent on the similar bone or these may be in different bones of the same joint.

The correlated angles were obtained by combining the two features of interest or different individual angular parameters together without any effect of reference line angulation, but all the landmarks used for individual angles of interest were similar for the correlated angles.

The following parameters were included:

1. Proximal intertarsal- proximal calcaneoquartal joint (PIT-Pcq Joint angle):

This was the angle that was measured between a line perpendicular to the Proximal intertarsal joint (PIT) and a line perpendicular to the proximal calcaneoquartal (Pcq) joint as previously described by some landmarks (Figure 4.12/1).

2. Proximal intertarsal- tibial metaphyseal/malleolar joint (PIT-Tm Joint angle):

This was the angle that was measured between a line perpendicular to the proximal intertarsal joint and a line perpendicular to the widest aspect of lateral and medial malleolus of the distal extremity of the tibia (Figure 4.12/2).

3. Proximal intertarsal-tibiotalus joint (PIT-Tt Joint angle):

This was the angle that was measured between a line perpendicular to the proximal intertarsal (PIT) joint and a line perpendicular to the tibiotalus or tarsocrural joint (Figure 4.12/3).

4. **Proximal central- tibial metaphyseal/malleolar joint (PC-Tm Joint angle):**

This was the angle that was measured between a line perpendicular to the proximal central joint and a line perpendicular to widest aspect of the lateral and medial malleolus of the distal extremity of the tibia (Figure 4.12/4).

5. **Proximal central- tibiotalus joint angle (PC-Tt Joint angle):**

This was the angle that was measured between a line perpendicular to the proximal central (PC) joint and a line perpendicular to the tibiotalus or tarsocrural joint as previously described by some landmarks (Figure 4.12/5).

6. **Tarsometatarsal- proximal calcaneoquartal joint (TMT-Pcq Joint angle):**

This was the angle that was measured between a line perpendicular to the tarsometatarsal (TMT) joint and a line perpendicular to the proximal calcaneoquartal joint as previously described by some landmarks (Figure 4.12/6).

7. **Tarsometatarsal- tibial metaphyseal/malleolar joint (TMT-Tm Joint angle):**

This was the angle that was measured between a line perpendicular to the tarsometatarsal joint and a line perpendicular to the lateral and medial malleolus of the distal extremity of the tibia as previously described by some landmarks (Figure 4.12/7).

8. **Tarsometatarsal- tibiotalus joint (TMT-Tt Joint angle):** This was the angle that was measured between a line perpendicular to the tarsometatarsal and a line perpendicular to the tibiotalus or tarsocrural joint as previously described by some landmarks (Figure 4.12/8).

9. Tarsometatarsal - proximal intertarsal joint (TMT-PIT Joint angle):

This was the angle that was measured between a line perpendicular to the tarsometatarsal and a line perpendicular to the proximal intertarsal joint (Figure 4.12/9).

10. Tarsometatarsal - proximal central joint (TMT-PC joint angle):

This was the angle that was measured between a line perpendicular to the tarsometatarsal and a line perpendicular to the proximal central joint (Figure 4.12/10).

11. Tarsometatarsal – distal metatarsoquartal joint (TMT-DMq joint angle):

This was the angle that was measured between a line perpendicular to the tarsometatarsal and a line perpendicular to the distal metatarsoquartal joint (Figure 4.12/11).

12. Distal intertarsal – tibial metaphyseal/malleolar joint (DIT-Tm Joint angle):

This was the angle that was measured between a line perpendicular to the distal intertarsal joint and a line perpendicular to the lateral and medial malleolus of the distal extremity of the tibia (Figure 4.12/12).

13. Distal intertarsal - tibiotalus joint (DIT-Tt Joint angle):

This was the angle that was measured between a line perpendicular to the distal intertarsal and a line perpendicular to the tibiotalus or tarsocrural joint (Figure 4.12/13).

14. Distal intertarsal - proximal intertarsal joint (DIT-PIT joint angle):

This was the angle that was measured between a line perpendicular to the distal intertarsal and a line perpendicular to the proximal intertarsal joint (Figure 4.12/14).

15. Distal intertarsal - proximal calcaneoquartal joint (DIT-Pcq joint angle):

This was the angle that was measured between a line perpendicular to the distal intertarsal (DIT) joint and a line perpendicular to the proximal calcaneoquartal joint (Figure 4.12/15).

16. Distal intertarsal - proximal central joint (TMT-PC joint angle):

This was the angle that was measured between a line perpendicular to the tarsometatarsal and a line perpendicular to the proximal central joint (Figure 4.12/16).

17. Distal intertarsal - tarsometatarsal joint (DIT-TMT Joint angle):

This was the angle that was measured between a line perpendicular to the distal intertarsal and a line perpendicular to the tarsometatarsal joint (Figure 4.12/17).

18. Proximal calcaneoquartal-proximal central joint (Pcq-PC Joint angle):

This was the angle that was measured between a line perpendicular to the proximal calcaneoquartal joint and a line perpendicular to the proximal central (PC) joint (Figure 4.12/18).

19. Proximal calcaneoquartal- distal metatarsoquartal joint (Pcq-DMq Joint angle):

This was the angle that was measured between a line perpendicular to the proximal calcaneoquartal joint and a line perpendicular to the distal metatarsoquartal joint (Figure 4.12/19).

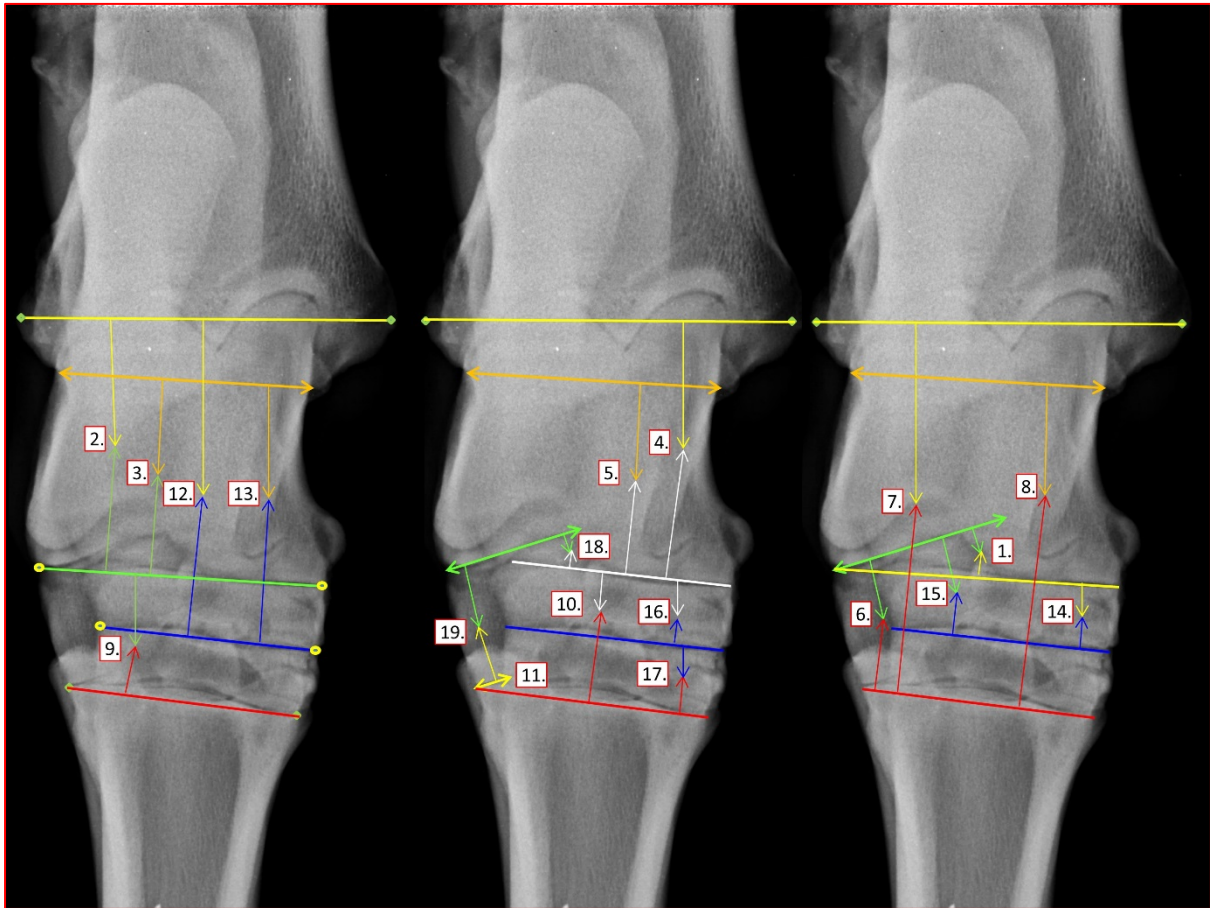


Figure 4. 12: DP radiographs of a right tarsus of a Thoroughbred. The correlated angular parameters are shown in this picture.

1. Proximal intertarsal- proximal calcaneoquartal joint; 2. Proximal intertarsal- tibial metaphyseal/malleolar joint; 3. Proximal intertarsal- tibio-talus joint; 4. Proximal central-tibial metaphyseal/malleolar joint; 5. Proximal central- tibio-talus joint; 6. Tarsometatarsal- proximal calcaneoquartal joint; 7. Tarsometatarsal- tibial metaphyseal/malleolar joint; 8. Tarsometatarsal-tibial tibio-talus; 9. Tarsometatarsal- proximal intertarsal joint; 10. Tarsometatarsal-proximal central joint; 11. Tarsometatarsal- distal metatarsoquartal joint; 12. Distal intertarsal- tibial metaphyseal/malleolar joint; 13. Distal intertarsal-tibio-talus joint; 14. Distal intertarsal-proximal intertarsal joint; 15. Distal intertarsal-proximal calcaneoquartal; 16. Distal intertarsal-proximal central joint; 17. Distal intertarsal-tarsometatarsal joint; 18. Proximal calcaneoquartal-proximal central joint; 19. Proximal-distal metatarsoquartal joint.

4.3.2.2.1 INTRA-RATER REPEATABILITY OF CORRELATED ANGULAR PARAMETERS:

The p values were >0.05 for normality check of all 19 correlated angular parameters (0.071-0.982) (Figure 4.13 and Table 4.5).

The repeatability statistical analysis is summarized in Tables 4.6 and 4.7. From the tables, it can be realized that generally, the difference between repeated measurements was smaller for the correlated angles. In the correlated angular parameters, all the differences between the repeated measurements had higher Intraclass coefficients (0.779 - 0.980). The greatest difference between the repeat measurements was in the DIT-TMT Joint angle with an ICC of 0.779 whereas the least difference was in the Pcq-DMq Joint angle with an ICC of 0.980.

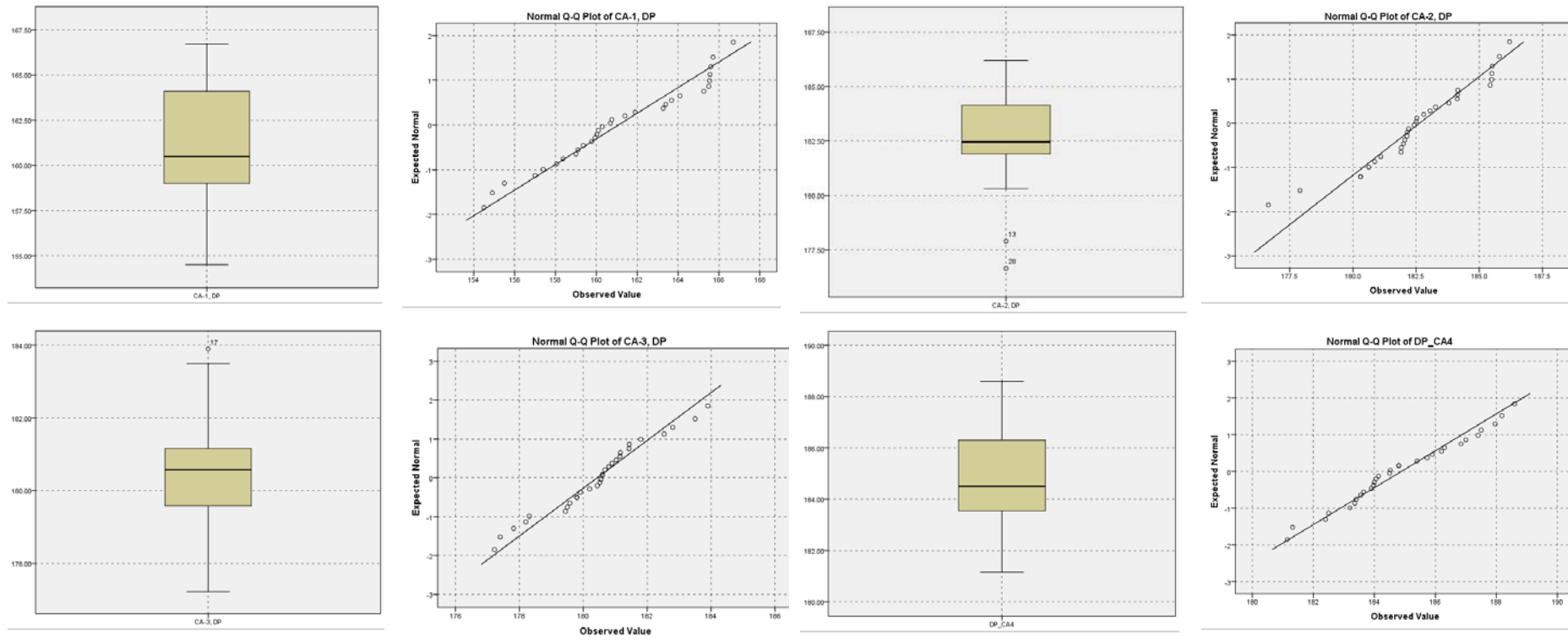
Lin's concordance correlation coefficient (CCC) was also generally higher in the correlated angular parameters in the comparison between the two repeated measurements. Scatter plots of Lin's CCC presented in figure 4.14. Almost perfect agreement, with a CCC range of 0.81-1.00 was found in 13 out of 19 parameters, these parameters were the PIT-Pcq Joint angle, PIT-Tm Joint angle, PC-Tt Joint angle, TMT-Pcq Joint angle, TMT-Tm Joint angle, TMT-Tt Joint angle, TMT-PIT Joint angle, TMT-DMq joint angle, DIT-Tm Joint angle, DIT-Tt Joint angle, TMT-PIT Joint angle, TMT-DMq joint angle, DIT-Tm Joint angle, DIT-Tt Joint angle, DIT-Pcq joint angle, Pcq-PC Joint angle and Pcq-DMq Joint angle. Substantial agreement (0.61 – 0.80) was found in 6 out of 19 correlated angular parameters, among these PIT-Tt Joint angle, PC-Tm Joint angle, DIT-PIT joint angle, TMT-PC joint angle were at higher levels (0.79 - 0.80), while the two parameters (DIT-TMT Joint angle and TMT-PC joint angle) were at a lower level of agreement (0.62 – 0.63).

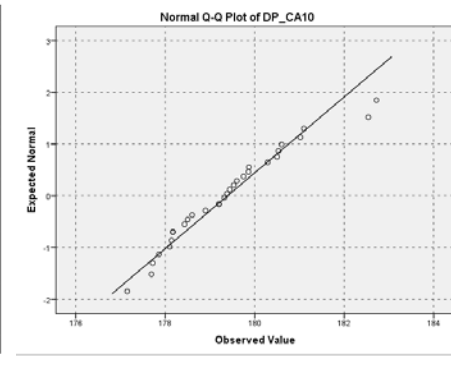
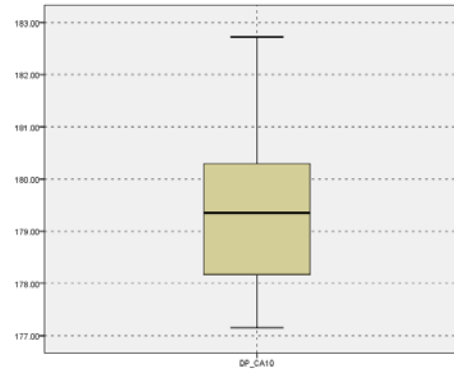
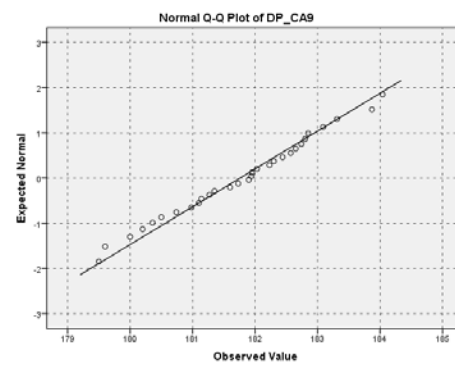
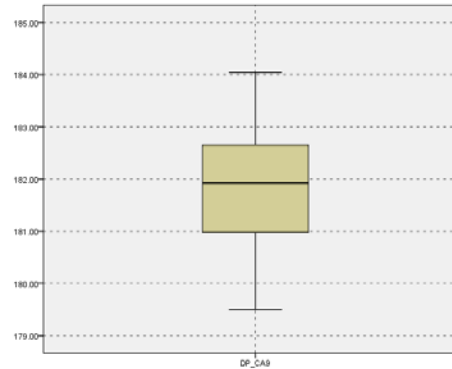
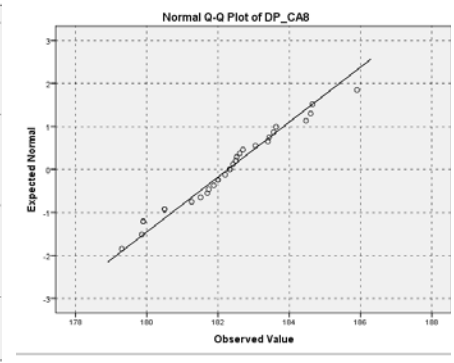
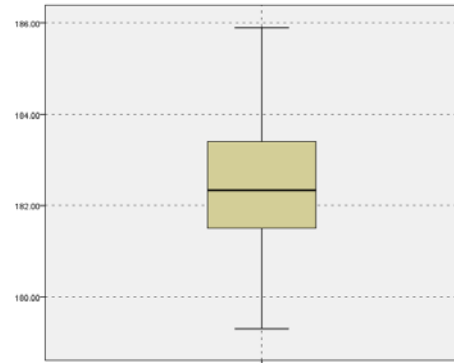
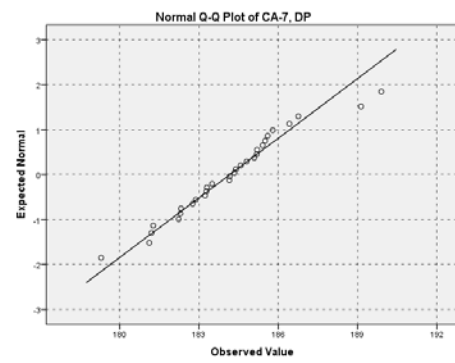
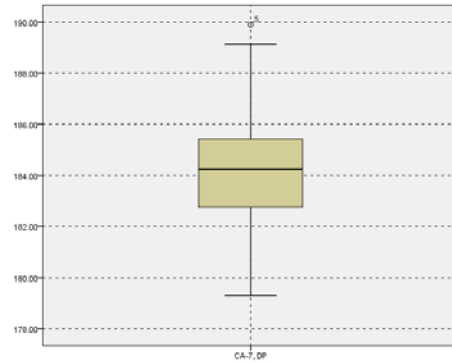
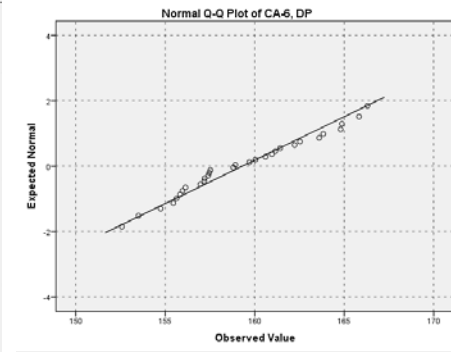
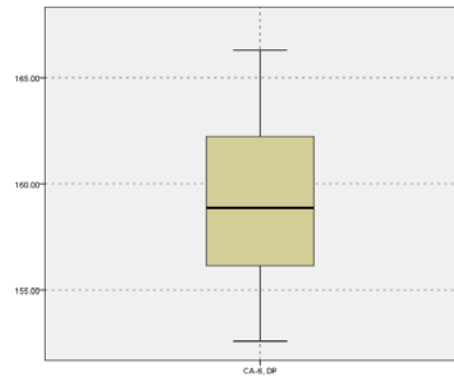
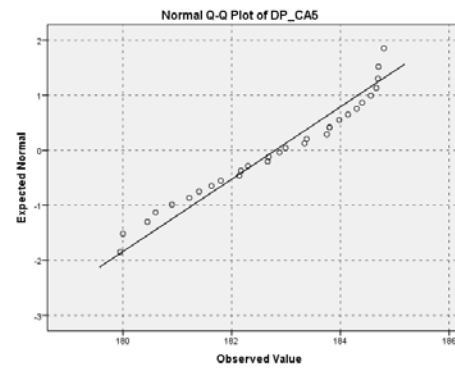
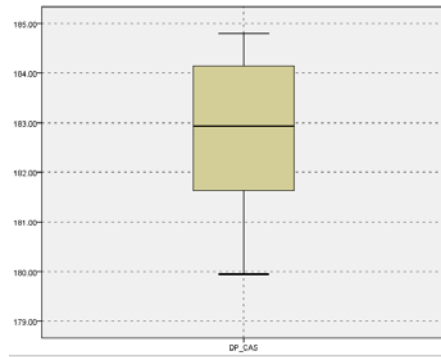
The mean difference between the first and second measurements were not significantly different from each other (p values ranged from 0.070 to 0.975) in all correlated angular parameters. Bland-Altman plots were presented in figure 4.15.

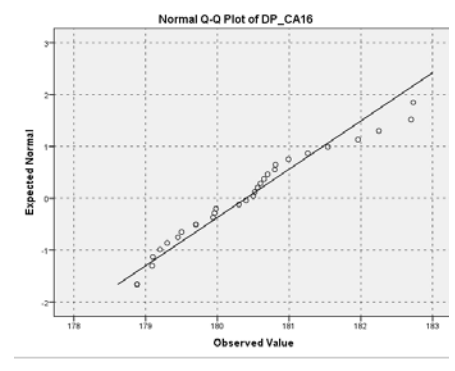
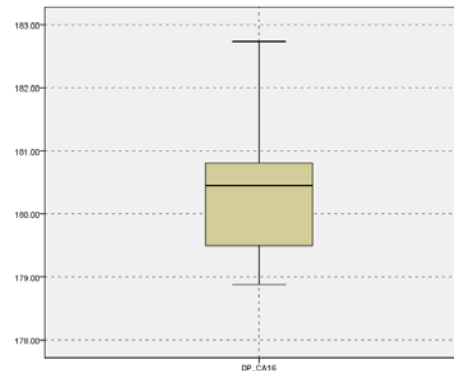
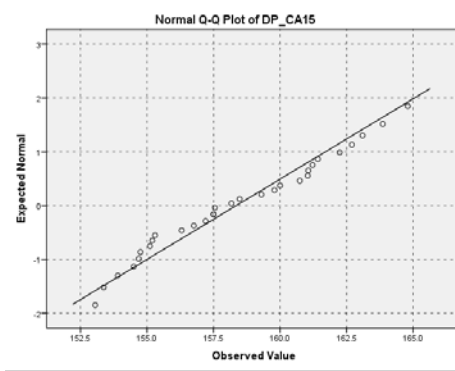
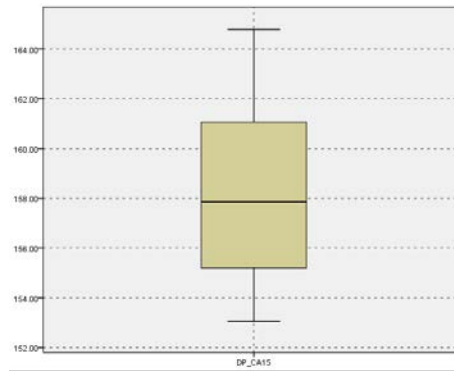
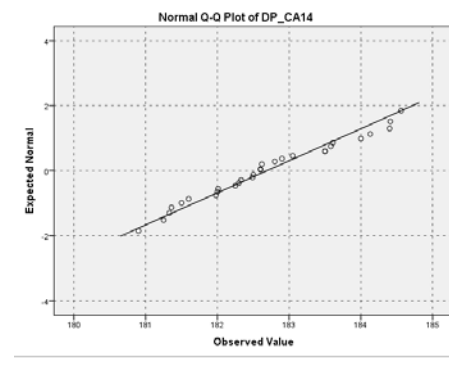
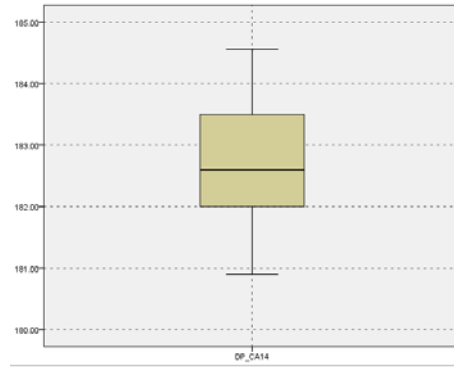
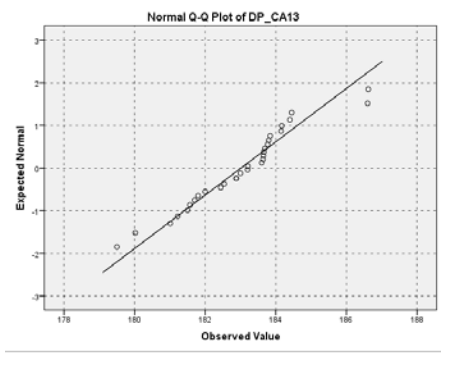
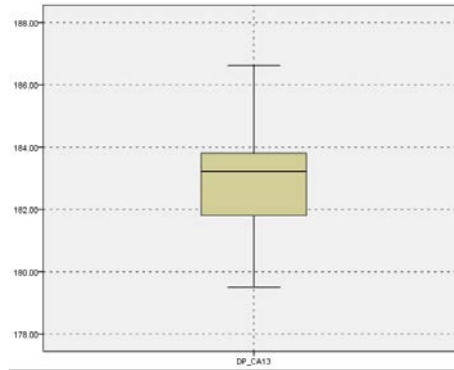
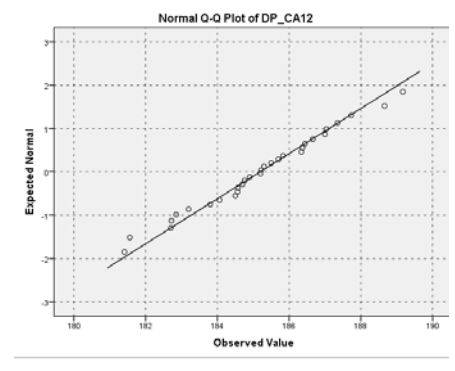
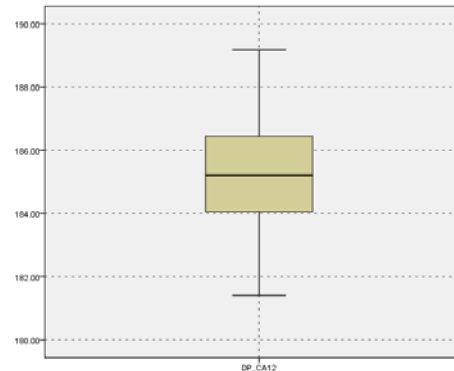
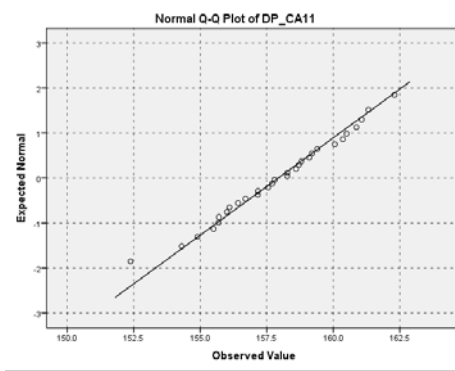
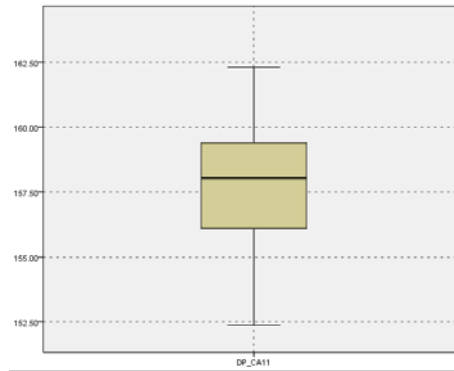
Table 4. 5: Correlated angular parameters measured in 15 samples for equine tarsal conformation

SR. NO.	MEASURABLE PARAMETERS (n=15)	MEAN $\pm$ SD	MEDIAN	95 % CI OF MEAN		SKEWNESS (KURTOSIS)	TEST OF NORMALITY (SHAPIRO-WILK) SIG.
				Lower	Upper		
1	CA-1, PIT-Pcq Joint angle	161.08 $\pm$ 3.49	160.48	159.78	162.38	-0.096 -0.917	0.176
2	CA-2, PIT-Tm Joint angle	182.63 $\pm$ 2.24	182.46	181.79	183.46	-0.593 0.750	0.133
3	CA-3, PIT-Tt Joint angle	180.43 $\pm$ 1.63	180.58	179.83	181.04	-0.035 0.149	0.071
4	CA-4, PC-Tm Joint angle	184.87 $\pm$ 1.99	184.51	184.13	185.62	0.149 -0.669	0.531
5	CA-5, PC-Tt Joint angle	182.80 $\pm$ 1.52	182.94	182.23	183.37	-0.396 -1.036	0.060
6	CA-6, TMT-Pcq Joint angle	159.30 $\pm$ 2.90	158.86	157.90	160.71	0.242 -0.885	0.415
7	CA-7, TMT-Tm Joint angle	184.17 $\pm$ 2.26	184.25	183.33	185.01	0.420 0.913	0.552
8	CA-8, TMT-Tt Joint angle	182.28 $\pm$ 1.56	182.33	181.69	182.86	0.109 -0.048	0.706
9	CA-9, TMT-PIT Joint angle	181.76 $\pm$ 1.19	181.92	181.31	182.21	-0.114 -0.583	0.883
10	CA-10, TMT-PC joint angle	179.40 $\pm$ 1.36	179.35	178.89	179.91	0.697 0.345	0.213

11	CA-11, TMT-DMq joint angle	157.93 ± 2.31	158.04	157.07	158.79	-0.224 -0.200	0.982
12	CA-12, DIT-Tm Joint angle	185.19 ± 1.92	185.21	184.48	185.91	-0.036 -0.233	0.951
13	CA-13, DIT-Tt Joint angle	183.02 ± 1.60	183.21	182.42	183.61	0.071 0.703	0.275
14	CA-14, DIT-PIT joint angle	182.69 ± 1.01	182.60	182.31	183.68	0.241 -0.758	0.357
15	CA-15, DIT-Pcq joint angle	158.35 ± 3.35	157.86	157.10	159.61	0.168 -1.075	0.310
16	CA-16, TMT-PC joint angle	180.40 ± 1.07	180.45	179.99	180.80	0.612 -0.166	0.113
17	CA-17, DIT-TMT Joint angle	179.10 ± 1.50	178.85	178.54	179.66	-0.142 -0.073	0.844
18	CA-18, Pcq-PC Joint angle	158.35 ± 3.85	158.31	156.91	159.79	0.238 -1.162	0.096
19	CA-19, Pcq-DMq Joint angle	178.72 ± 3.78	178.38	177.31	180.13	-0.151 -0.449	0.735

Figure 4. 13: Normality distribution (Normal Q-Q) and mean box plots for correlated angular parameters.





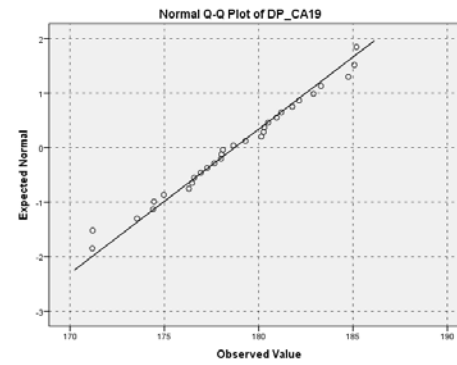
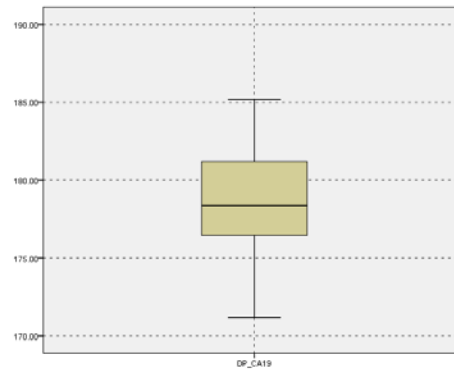
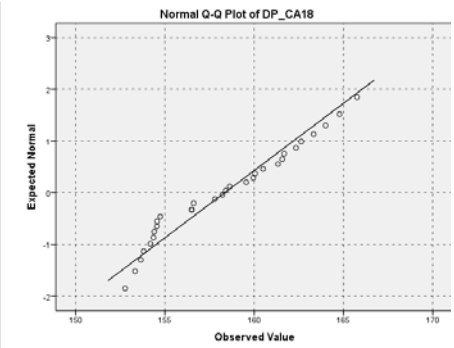
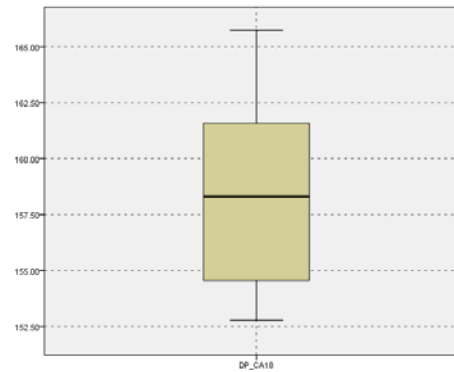
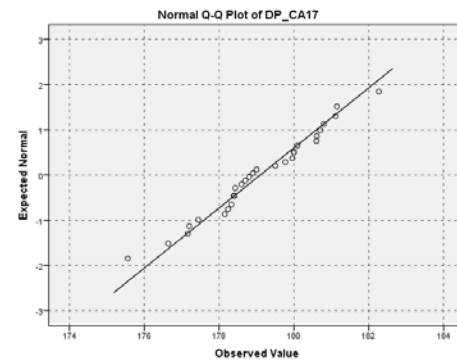
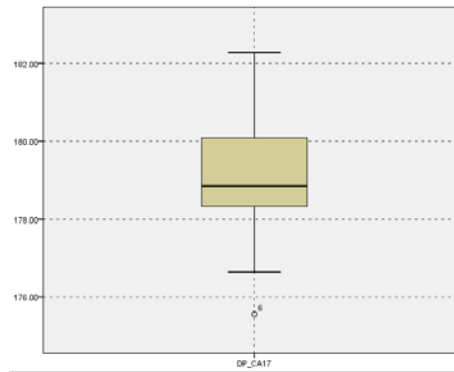


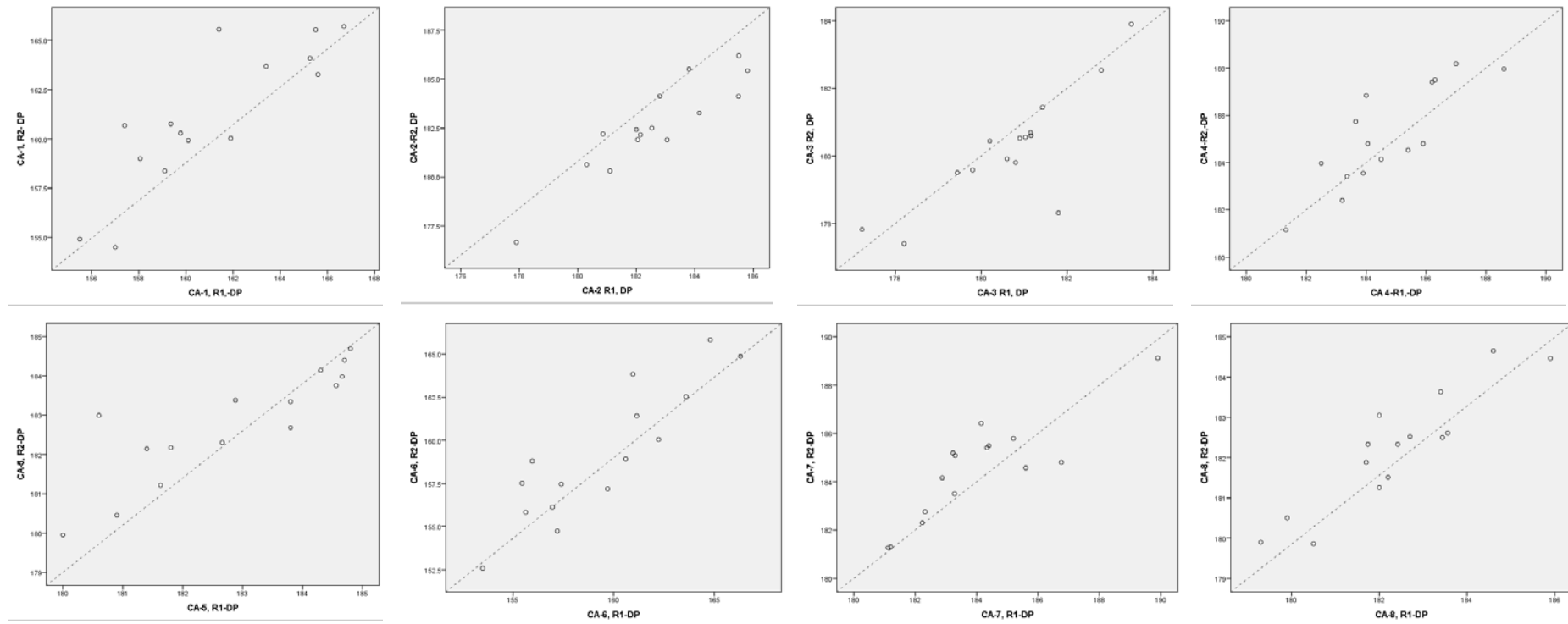
Table 4. 6: Intra-rater repeatability of the correlated angular parameters using ICC and Lin's CCC coefficients in 15 tarsi

Sr. No.	Measurable parameters (n=15)	Measur ement	Mean $\pm$ SD	Intraclass Correlation (ICC)	95 % CI of ICC		Lin's CCC	95 % CI of Lin's CCC	
					Lower	Upper		Lower	Upper
1	CA-1, PIT-Pcq Joint angle	1 st	161.07 $\pm$ 3.54	0.929	0.787	0.976	0.859	0.634	0.950
		2 nd	161.09 $\pm$ 3.57						
2	CA-2, PIT-Tm Joint angle	1 st	182.63 $\pm$ 2.15	0.954	0.862	0.985	0.906	0.753	0.966
		2 nd	182.62 $\pm$ 2.40						
3	CA-3, PIT-Tt Joint angle	1 st	180.67 $\pm$ 1.59	0.894	0.672	0.965	0.797	0.520	0.922
		2 nd	180.20 $\pm$ 1.68						
4	CA-4, PC-Tm Joint angle	1 st	184.66 $\pm$ 1.89	0.898	0.705	0.965	0.804	0.531	0.926
		2 nd	185.09 $\pm$ 2.12						
5	CA-5, PC-Tt Joint angle	1 st	182.83 $\pm$ 1.67	0.926	0.778	0.975	0.853	0.636	0.945
		2 nd	182.77 $\pm$ 1.42						
6	CA-6, TMT-Pcq Joint angle	1 st	159.43 $\pm$ 3.79	0.944	0.834	0.981	0.887	0.699	0.960
		2 nd	159.18 $\pm$ 3.86						
7	CA-7, TMT-Tm Joint angle	1 st	183.99 $\pm$ 2.26	0.915	0.748	0.971	0.834	0.594	0.938
		2 nd	184.48 $\pm$ 2.06						
8	CA-8, TMT-Tt Joint angle	1 st	182.36 $\pm$ 1.72	0.947	0.847	0.982	0.894	0.735	0.960
		2 nd	182.20 $\pm$ 1.45						

9	CA-9, TMT-PIT Joint angle	1 st	181.74 ± 1.30	0.924	0.773	0.975	0.851	0.626	0.945
		2 nd	181.78 ± 1.13						
10	CA-10, TMT-PC joint angle	1 st	179.34 ± 1.64	0.787	0.354	0.929	0.634	0.273	0.838
		2 nd	179.45 ± 1.08						
11	CA-11, TMT-DMq joint angle	1 st	157.98 ± 2.11	0.916	0.749	0.972	0.836	0.653	0.937
		2 nd	157.88 ± 2.56						
12	CA-12, DIT-Tm Joint angle	1 st	184.95 ± 1.95	0.925	0.763	0.975	0.853	0.633	0.945
		2 nd	185.43 ± 1.93						
13	CA-13, DIT-Tt Joint angle	1 st	183.01 ± 1.66	0.970	0.910	0.990	0.938	0.827	0.978
		2 nd	183.02 ± 1.59						
14	CA-14, DIT-PIT joint angle	1 st	182.54 ± 0.89	0.889	0.663	0.963	0.789	0.531	0.914
		2 nd	182.83 ± 1.13						
15	CA-15, DIT-Pcq joint angle	1 st	158.56 ± 3.47	0.936	0.814	0.978	0.872	0.667	0.954
		2 nd	158.15 ± 3.34						
16	CA-16, TMT-PC joint angle	1 st	180.29 ± 1.08	0.890	0.684	0.963	0.791	0.496	0.922
		2 nd	180.51 ± 1.10						
17	CA-17, DIT-TMT Joint angle	1 st	179.00 ± 1.49	0.779	0.340	0.926	0.622	0.189	0.853
		2 nd	179.20 ± 1.56						
18	CA-18, Pcq-PC Joint angle	1 st	158.09 ± 3.88	0.969	0.908	0.990	0.936	0.825	0.977
		2 nd	158.61 ± 3.93						

19	CA-19, Pcq-DMq angle	Joint	1 st	178.78 ± 3.74	0.980	0.940	0.993	0.958	0.881	0.985
			2 nd	178.66 ± 3.94						

Figure 4. 14: Scatter plots of Lin's CCC showing distribution of measurements and level of agreement and reproducibility between first and second measurements of thirteen ratio's parameters (RP); R-2nd, second measurement.



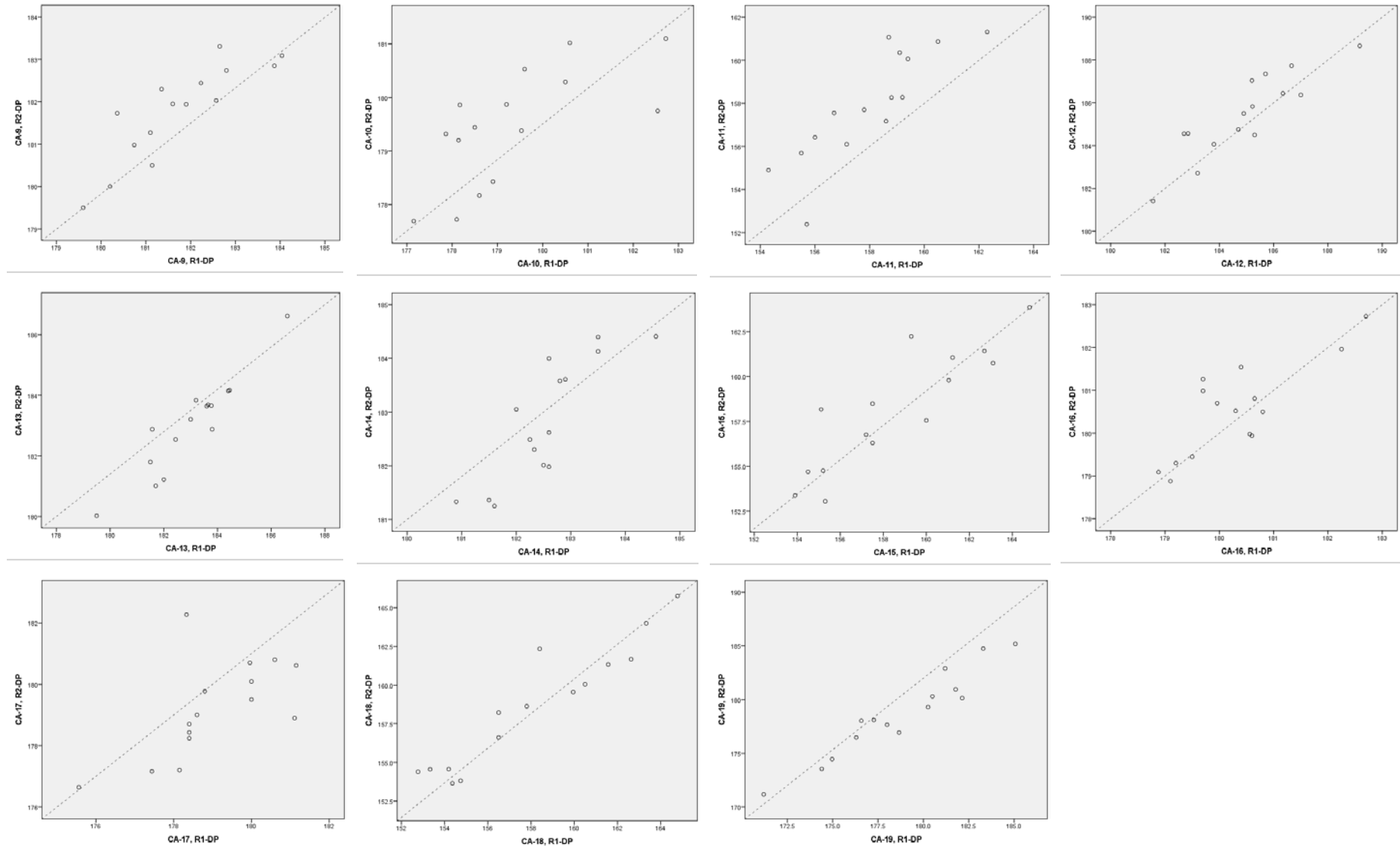


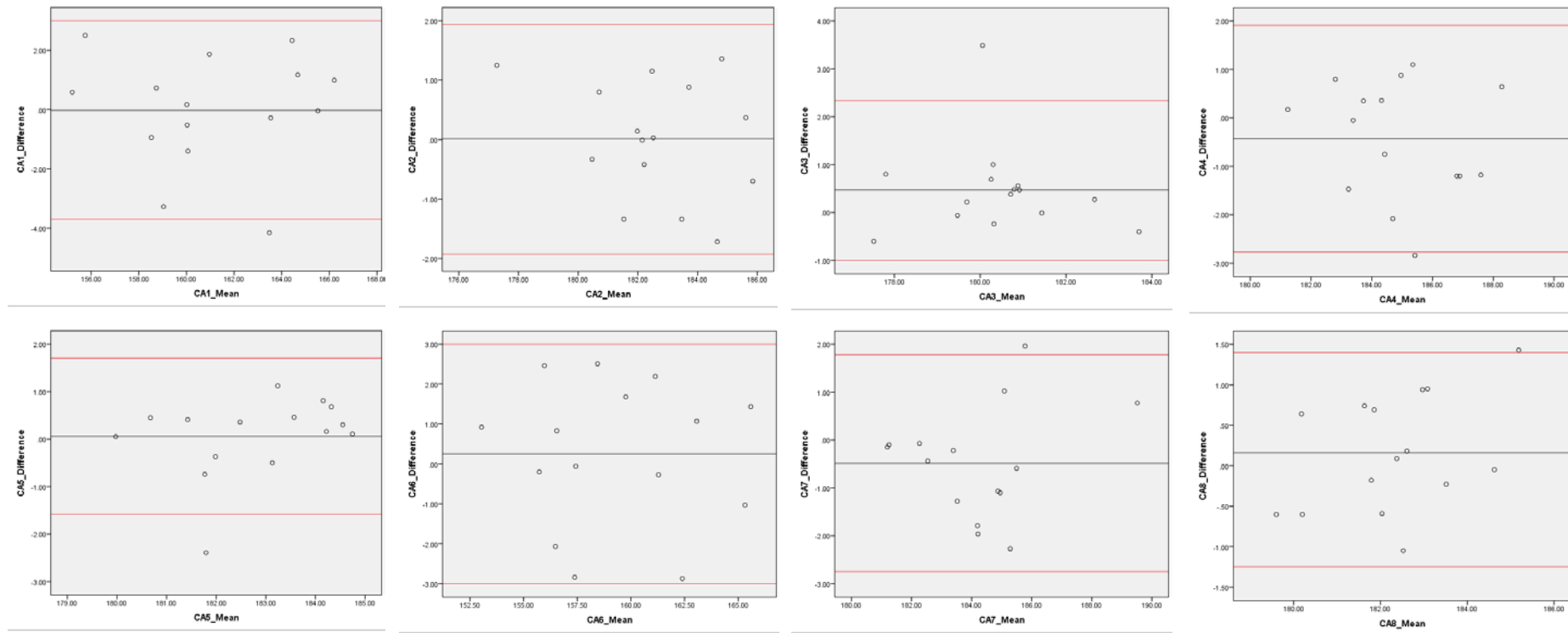
Table 4. 7: Intra-rater/ repeatability of the individual angular parameters in 15 tarsi using mean difference 95% (CI) limit of agreement between repeated measurements for the Bland-Altman Plots.

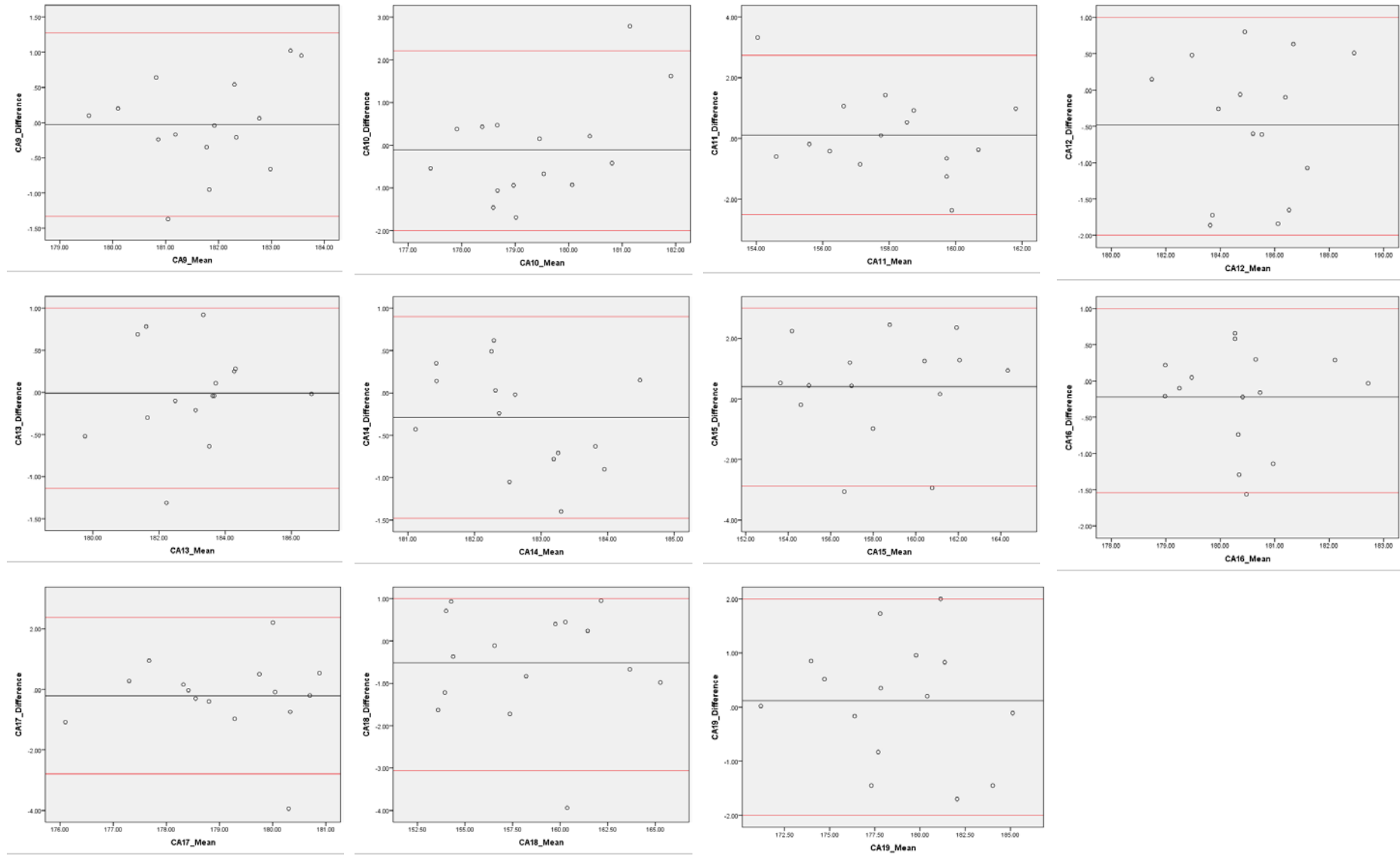
Sr. No.	Measurable parameters (n=15)	Measurement	Mean $\pm$ SD	Mean Difference $\pm$ SD	Sig. level ($p < 0.05$)	Calculation of 95 % Limits of agreement of Bland-Altman plots	
						Lower	Upper
1	CA-1, PIT-Pcq Joint angle	1 st	161.07 $\pm$ 3.54	-0.02 $\pm$ 1.88	0.971	-3.71	3.67
		2 nd	161.09 $\pm$ 3.57				
2	CA-2, PIT-Tm Joint angle	1 st	182.63 $\pm$ 2.15	0.01 $\pm$ 0.99	0.975	-1.93	1.94
		2 nd	182.62 $\pm$ 2.40				
3	CA-3, PIT-Tt Joint angle	1 st	180.67 $\pm$ 1.59	0.47 $\pm$ 0.95	0.076	-1.39	2.33
		2 nd	180.20 $\pm$ 1.68				
4	CA-4, PC-Tm Joint angle	1 st	184.66 $\pm$ 1.89	-0.43 $\pm$ 1.19	0.183	-2.77	1.91
		2 nd	185.09 $\pm$ 2.12				
5	CA-5, PC-Tt Joint angle	1 st	182.83 $\pm$ 1.67	0.06 $\pm$ 0.84	0.783	-1.58	1.70
		2 nd	182.77 $\pm$ 1.42				
6	CA-6, TMT-Pcq Joint angle	1 st	159.43 $\pm$ 3.79	0.25 $\pm$ 1.80	0.600	-3.28	3.78
		2 nd	159.18 $\pm$ 3.86				
7	CA-7, TMT-Tm Joint angle	1 st	183.99 $\pm$ 2.26	-0.49 $\pm$ 1.16	0.126	-2.75	1.78
		2 nd	184.48 $\pm$ 2.06				
	CA-8, TMT-Tt Joint angle	1 st	182.36 $\pm$ 1.72	0.16 $\pm$ 0.72	0.410	-1.25	

8		2 nd	182.20 ± 1.45				1.40
9	CA-9, TMT-PIT Joint angle	1 st	181.74 ± 1.30	-0.03 ± 0.66	0.854	-1.33	1.27
		2 nd	181.78 ± 1.13				
10	CA-10, TMT-PC joint angle	1 st	179.34 ± 1.64	-0.11 ± 1.18	0.723	-2.43	2.21
		2 nd	179.45 ± 1.08				
11	CA-11, TMT-DMq joint angle	1 st	157.98 ± 2.11	0.11 ± 1.34	0.760	-2.52	2.74
		2 nd	157.88 ± 2.56				
12	CA-12, DIT-Tm Joint angle	1 st	184.95 ± 1.95	-0.48 ± 0.95	0.070	-2.34	1.38
		2 nd	185.43 ± 1.93				
13	CA-13, DIT-Tt Joint angle	1 st	183.01 ± 1.66	-0.01 ± 0.57	0.947	-1.14	1.12
		2 nd	183.02 ± 1.59				
14	CA-14, DIT-PIT joint angle	1 st	182.54 ± 0.89	-0.29 ± 0.61	0.083	-1.48	0.90
		2 nd	182.83 ± 1.13				
15	CA-15, DIT-Pcq joint angle	1 st	158.56 ± 3.47	0.41 ± 1.68	0.360	-2.88	3.70
		2 nd	158.15 ± 3.34				
16	CA-16, TMT-PC joint angle	1 st	180.29 ± 1.08	-0.22 ± 0.67	0.219	-1.54	1.09
		2 nd	180.51 ± 1.10				
17	CA-17, DIT-TMT Joint angle	1 st	179.00 ± 1.49	-0.21 ± 1.32	0.552	-2.79	2.37
		2 nd	179.20 ± 1.56				
18	CA-18, Pcq-PC Joint angle	1 st	158.09 ± 3.88	-0.52 ± 1.30	0.143	-3.06	2.02
		2 nd	158.61 ± 3.93				

19	CA-19, angle	Pcq-DMq	Joint	1 st	178.78 ± 3.74	0.12 ± 1.11	0.691	-2.07	2.30
				2 nd	178.66 ± 3.94				

Figure 4. 15: Intra-rater repeatability of the correlated angular parameters in 15 tarsi using mean difference 95% (CI) limit of agreement between repeated measurements for the Bland-Altman Plots.





4.3.2.3 Ratio's parameters:

The ratio's parameters were established by division of some linear measurements of some features of interest from the same bone or within different bones. Some abbreviations need to be clarified, before mentioning the ratio's parameters.

Mh or medial height of tarsus: This was the height which extended from the medial end of the distal surface of the tibia to the dorsomedial end of the proximal surface of the third metatarsal (Mt3) (Figure 4.16/Mh).

Lh or lateral height of tarsus: This was the lateral height which extended from the lateral end of the distal surface of the tibia to the dorsolateral end of the proximal surface of the third metatarsal (Figure 4.16/Lh).

The ratio parameters were:

1. RL/ Dorso-distal lateromedial length of T3 (RL/T3 ratio):

This was the ratio of the length of the reference line (RL) placed as described earlier to the length of the third tarsal bone. The dorso-distal lateromedial length of the third tarsal was extended between the lateral to medial edges of the third tarsal articulation with the third metatarsal (portion of tarsometatarsal joint with third tarsal articulation only) or lateromedial length of the distal articular facet of the third tarsal (Figure 4.16/1 and 2).

2. Lateral/ medial height of central tarsal (Lat. /Med.C.h ratio):

This was the ratio of the lateral to medial height of the central tarsal. The lateral height was measured between the proximo-lateral edge of the central tarsal at its articulation with the distal talus (forming the lateral end of the proximal intertarsal joint). The medial height was measured from disto-medial edge of the central tarsal at its articulation with the proximal surface of the third tarsal forming the distal intertarsal joint (Figure 4.16/3 and 4).

3. Lateral/ medial height of third tarsal (Lat. /Med.h.T3 ratio):

This was the ratio of the lateral to medial height of the third tarsal. The lateral height was measured between the proximo-lateral edge of the third tarsal at its articulation with the central tarsal proximally forming the distal intertarsal joint. The medial height was measured between the disto-lateral edge of the third tarsal at its articulation with the third metatarsal distally (Figure 4.16/5 and 6).

4. Lateral/ medial height of central and third tarsal (Lat. /Med C+T3H ratio):

This was the ratio of the lateral to medial combined heights of the central and third tarsals. The lateral combined height was measured between the proximo-lateral edge of the central tarsal at its articulation with the distal talus to the disto-lateral edge of the third tarsal at its articulation with the proximal third metatarsal. The medial combined height of the central and third tarsal was measured between the proximo-medial edge of the central tarsal at its articulation with the distal talus to the disto-medial edge of the third tarsal at its articulation with the proximal third metatarsal (Figure 4.16/7 and 8).

5. Lateral height of central tarsal / lateral height of Tarsus (Lat.h.C/Lh ratio):

This was the ratio of the lateral height of the central tarsal to the Lh (lateral height of the tarsus). The details of landmarks were described above in Lat/Med.h.C ratio and Lh (Figure 4.16/3 and Lh).

6. Lateral height of third tarsal / lateral height of Tarsus (Lat.h.T3/Lh ratio):

This was the ratio of the lateral height of the third tarsal to the Lh. The details of landmarks were described above in Lat/Med.h.T3 ratio and Lh (Figure 4.16/5 and Lh).

7. Lateral height of fourth tarsal to lateral height of Tarsus (Lat.h.T4/Lh ratio):

This was the ratio of the lateral height of the fourth tarsal to the Lh. The lateral height of the fourth tarsal was extended from the proximo-lateral edge of its articulation with the calcaneus forming calcaneoquartal joint to the disto-lateral edge of its distal articulation with the proximal surface of the third metatarsal (Figure 4.16/9 and Lh).

8. Lateral height of fourth tarsal to medial heights of central and third Tarsals (Lat.h.T4/Med.h.C+T3 ratio):

This was the ratio of the lateral height of the fourth tarsal to the combined medial height of the central and third tarsals. The details of landmarks were described above (Figure 4.16/9 and 8).

9. Lateral combined height of central and third tarsals / lateral height of fourth tarsal (Lat.h.C+T3/ Lat.h.T4 ratio):

This was the ratio of the combined lateral height of both central and third tarsals to the lateral height of the fourth tarsal. The details of landmarks were described above (Figure 4.16/7 and 9).

10. Lateral / medial height of Tarsus (Lh /Mh ratio):

This was the ratio of Lh to the Mh. The details of landmarks were described above (Figure 4.16/Lh and Mh).

11. Medial height of central / medial height of central and third tarsals (Med.h.C/Med.h.C+T3 ratio):

This was the ratio of the medial height of the central to the medial height of the combined central and third tarsals. The details of landmarks were described above (Figure 4.16/4 and 8).

12. Medial height of third tarsal / medial height of central and third tarsals (Med.h.T3/ Med.h.C+T3 ratio):

This was the ratio of the medial height of the third tarsal to the medial height of the combined central and third tarsals. The details of landmarks were described above (Figure 4.16/6 and 8).

13. Medial height of central and third tarsals/ medial height of Tarsus (Med.h.C+T3/Mh ratio):

This was the ratio of the medial height of the combined central and third tarsals to the Mh. The details of landmarks were described above in Med.h.C+T3 and Mh (Figure 4.16/8 and Mh).

14. Middle height of Talus/ medial height of Talus (m.h.T/ Med.h.T ratio):

This was the ratio of the middle height of talus to the medial height of talus. The middle height of talus (m.h.T) was taken from the intersection point between the lines extending along (best fit with) the medial and lateral sides of the groove of the trochlea of talus at the proximal end to the mid-point of (proximal central joint) the distal extremity of talus where it articulates with the proximal surface of the central tarsal.

The medial height of talus (Med.h.T) was taken in parallel from the highest point of the dorsal border of the medial trochlea ridge of talus to the distal extremity of the talus body where it articulates (proximal central joint) with the proximal surface of the central tarsal (Figure 4.16/13 and 11).

15. Width of Talus/ medial height of Talus (W.T/ Med.h.T ratio):

This was the ratio of the lateromedial width of talus to the medial height of talus. The lateromedial width (from lateral to medial borders) of talus was taken from almost the middle of its main body (corpus) at the level of the mid-points of the middle and medial heights of talus. The details of landmarks were described above in Med.h.T (Figure 4.16/12 and 11).

16. Middle height of talus/ Posterior height of calcaneus (m.h.T/ Post.h.Calc ratio):

This was the ratio of the middle height of talus to the posterior height of the calcaneal tarsal (Post.h.Calc). The posterior height of calcaneus was extended from the most proximal point of the dorsal border of the plantar tuber calcaneus to the disto-lateral edge of calcaneus in its articulation with the proximal surface of the fourth tarsal (proximal calcaneoquartal joint). The middle height of talus was described above (Figure 4.16/13 and 10).

17. Width of Talus/ Posterior height of calcaneus (W.T/ Post.h.Calc ratio):

This was the ratio of the width of talus to the posterior height of calcaneus. The details of landmarks were described above (Figure 4.16/12 and 10).

18. Lateral height of tarsus to Posterior height of calcaneus (Lh/ Post.h.Calc ratio):

This was the ratio of the lateral height of tarsus (Lh) to the posterior height of the calcaneal tarsal (Post.h.Calc). The details of landmarks were described above (Figure 4.16/10 and Lh).

19. RL/ Width of talus (RL/ W.T ratio):

This was the ratio of that length of the reference line (RL) to the lateromedial width of talus. The details of landmarks were described above (Figure 4.16/12 and RL).

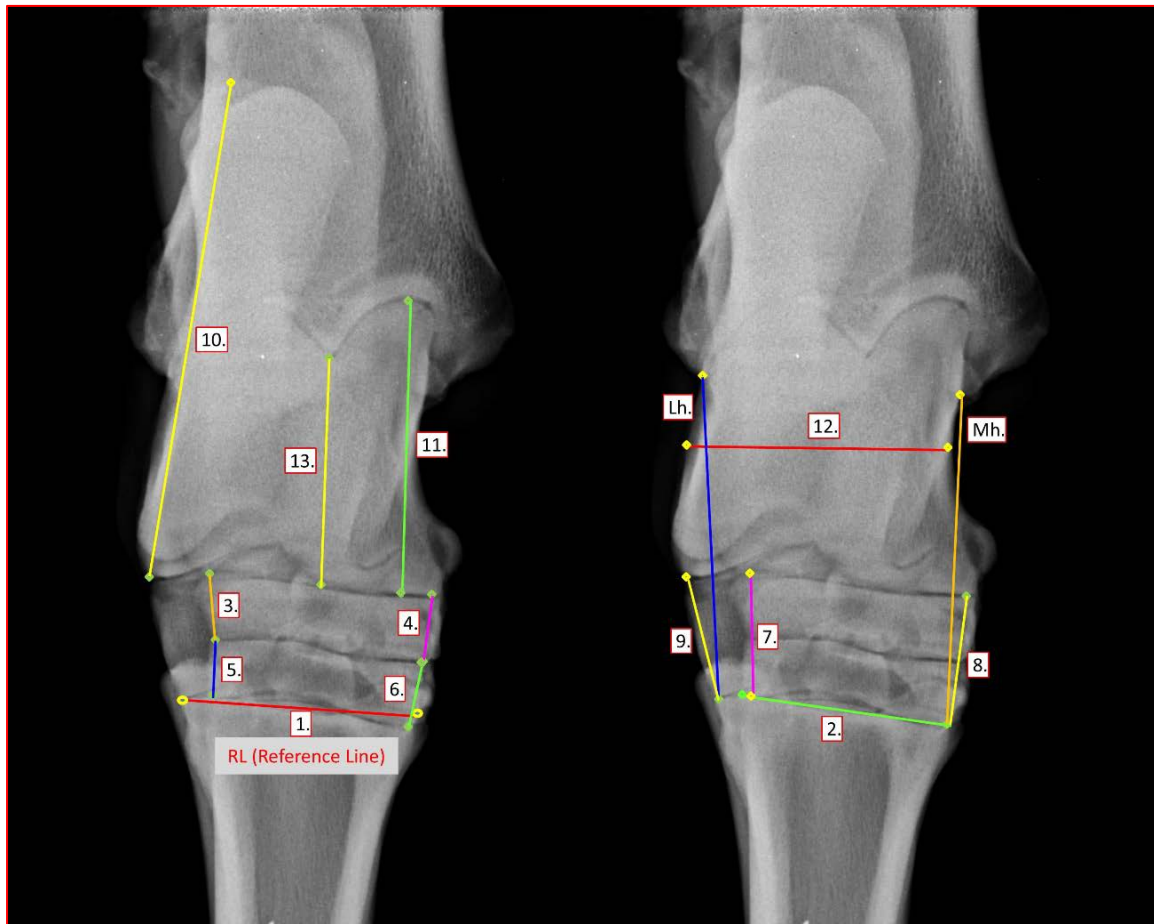


Figure 4. 16: DP radiographs of a right tarsus of a thoroughbred. They show ratio parameters. Reference line was placed on left image (radiograph) only.

Lh., total lateral height of the tarsal joint; Mh., total medial height of the tarsal joint; 1. RL, lateromedial width of lateral and medial anatomical points of reference line as described earlier; 2, dorsodistal width of third tarsal; 3. Proximodistal dorsolateral height of central tarsal; 4. Proximodistal dorsomedial height of central tarsal; 5. Proximodistal dorsolateral height of 3rd tarsal; 6. Proximodistal dorsomedial height of 3rd tarsal (T3); 7, Proximodistal combined dorsal heights of central and 3rd tarsals; 8, Proximodistal combined medial heights of central and 3rd tarsals; 9, Proximodistal dorsolateral height of fourth tarsal; 10. Maximum latero-posterior height of calcaneus; 11. Medial height of talus; 12. Latero-medial width of talus; 13. Proximodistal height of body of talus at the middle point between the lateral and medial trochlea to its distal articular surface with the central tarsal.

4.3.2.3.1 INTRA-RATER REPEATABILITY OF RATIO'S PARAMETERS:

The p values were >0.05 in normality check for 16 of 19 ratio's parameters (Table 4.8). There were only 3 parameters out of 19 which had p-values of <0.05 . These were the Lh /Mh ratio, Med.h.C+T3/Mh ratio and m.h.T/ Med.h.T ratio having p-values slightly below 0.05.

The repeatability statistical analysis was summarized in Tables 4.9 and 4.10. From the tables, it can be realized that generally, the difference between repeated measurements was least. In the ratio's parameters, all the differences between the repeated measurements had higher Intraclass coefficients (0.598 - 0.986). The greatest difference between the repeat measurements was in Med.h.T3/Med.h.C+T3 ratio with an ICC of 0.598 whereas the least difference was in the Lh/ Post.h.Calc ratio with an ICC of 0.986.

Lin's concordance correlation coefficient (CCC) was also generally higher in the ratio's parameters in the comparison between the two repeated measurements. Almost perfect agreement, with a CCC range of 0.81-1.00 was found in 10 out of 19 ratio parameters, these parameters were the Lat.h.C/Lh ratio, Lat.h.T3/Lh ratio, Lh /Mh ratio, Med.h.C+T3/Mh ratio, m.h.T/ Med.h.T ratio, W.T/ Med.h.T ratio, m.h.T/ Post.h.Calc ratio, W.T/ Post.h.Calc ratio, Lh/ Post.h.Calc ratio and RL/ W.T ratio. Substantial agreement (0.61 – 0.80) was found in 7 out of 19 ratio's parameters, these parameters were RL/T3 ratio, Lat. /Med.h.T3 ratio, Lat. /Med C+T3H ratio, Lat.h.T4/Lh ratio, Lat.h.T4/Med.h.C+T3 ratio, Lat.h.C+T3/ Lat.h.T4 ratio and Med.h.C/Med.h.C+T3 ratio. Med.h.T3/Med.h.C+T3 ratio and Lat. /Med.C.h ratio with a Lin's CCC of 0.410 and 0.571 respectively.

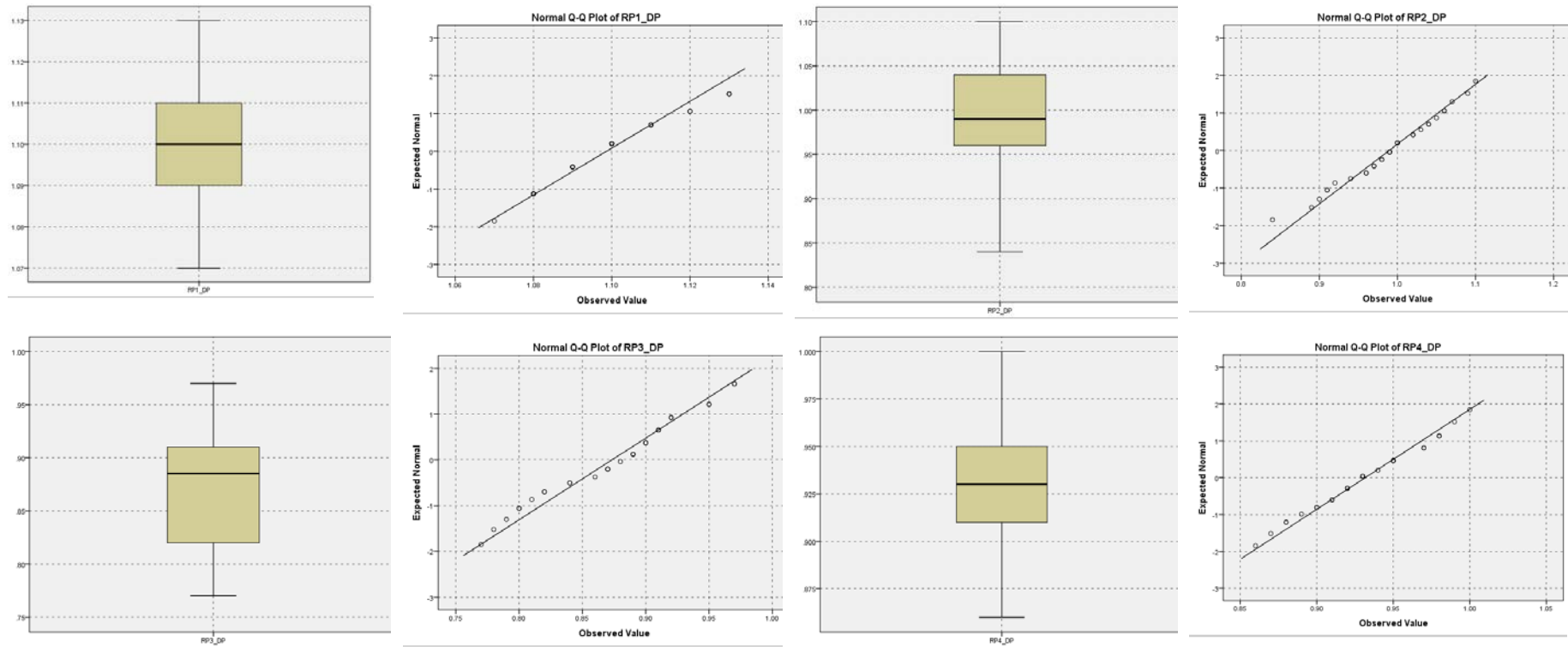
Agreement between the first and second /measurements was quantified by using the differences between observations made using the two repeat measurements that were taken separately after an interval on the same subjects. The mean difference between the first and second measurements were not significantly different from each other (p values

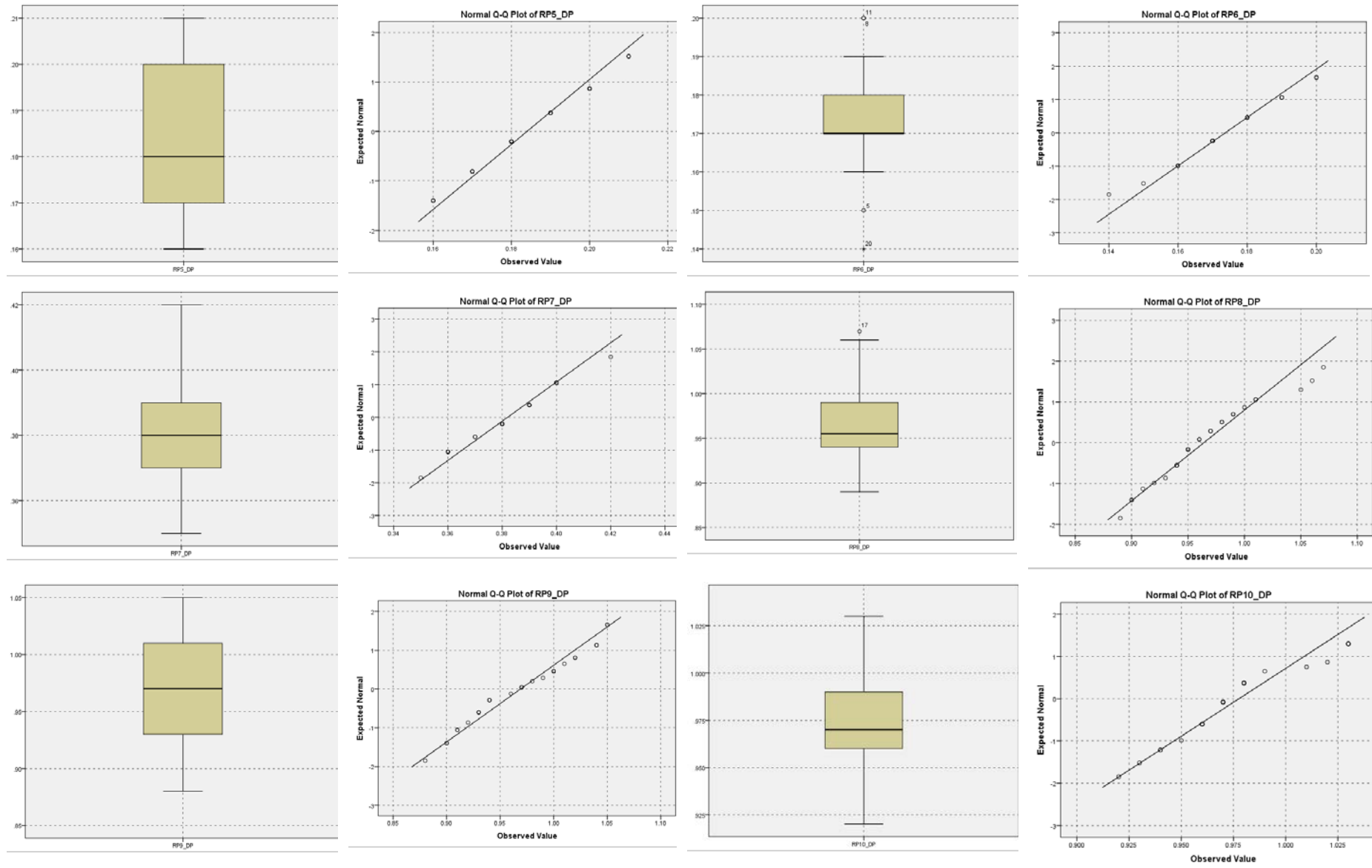
were ranged from 0.088 to 0.744) in 18 out of 19 ratio's parameters. The only ratio that had a significant p value of 0.028 was the Lat.h.C/Lh ratio.

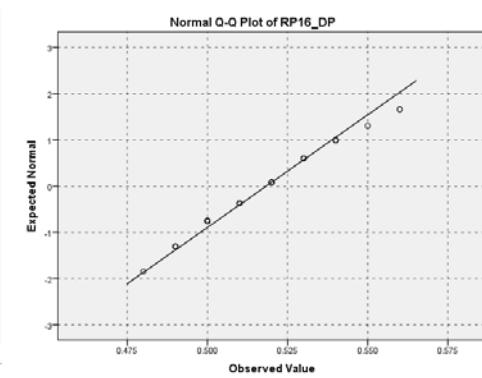
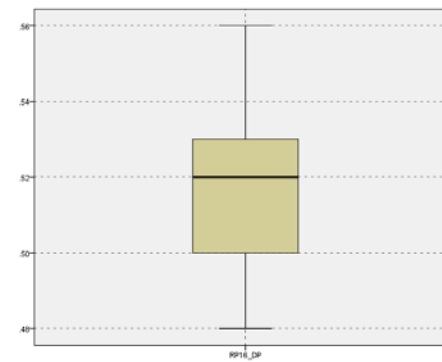
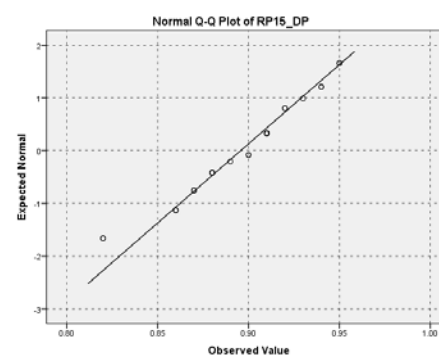
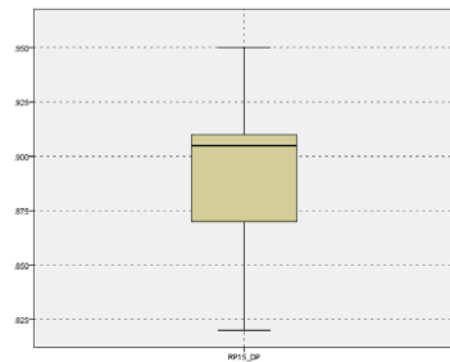
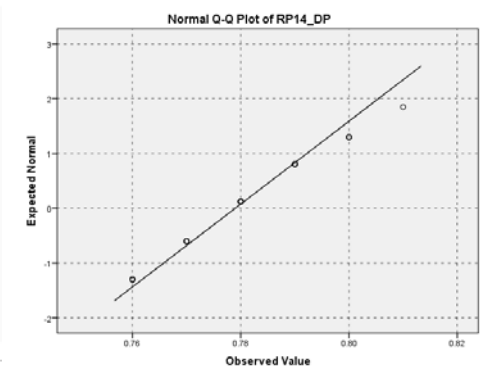
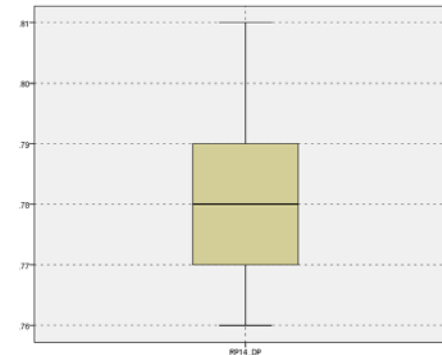
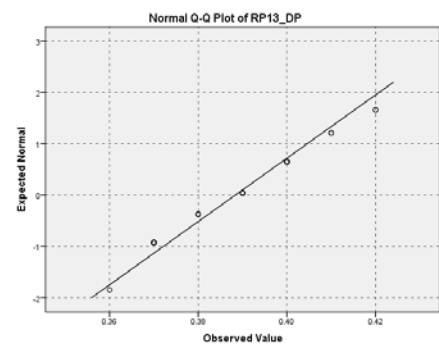
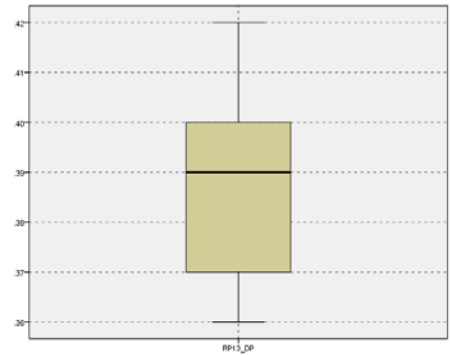
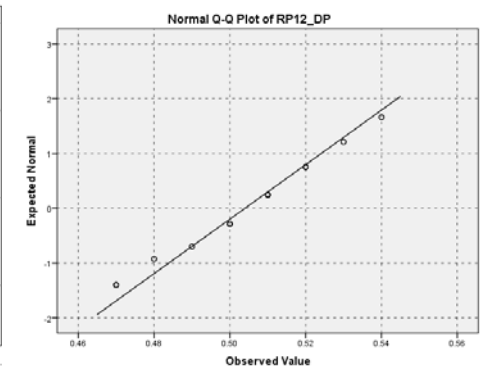
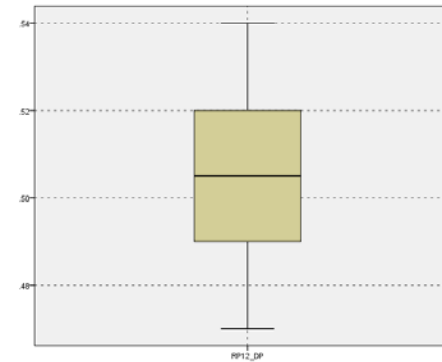
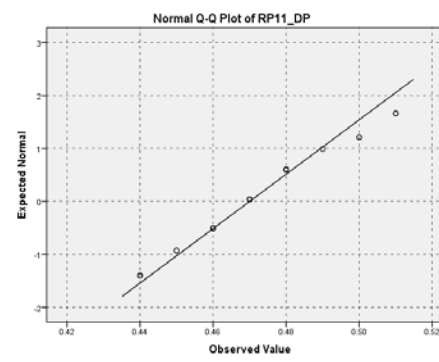
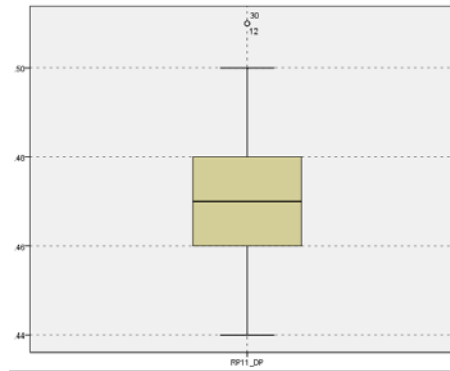
Table 4. 8: Ratios parameters measured in fifteen samples for equine tarsal conformation.

SR. NO.	MEASURABLE PARAMETERS (n=15) N=30	MEAN $\pm$ SD	MEDIAN	95 % CI OF MEAN		SKEWNESS (KURTOSIS)	TEST OF NORMALITY (SHAPIRO-WILK) SIG.
				Lower	Upper		
1	RP-1, RL/T3 ratio	1.10 $\pm$ 0.02	1.10	1.09	1.10	0.495 -0.394	0.052
2	RP-2, Lat. /Med.C.h ratio	0.99 $\pm$ 0.06	0.99	0.97	1.01	-0.347 -0.206	0.824
3	RP-3, Lat. /Med.h.T3 ratio	0.87 $\pm$ 0.06	0.89	0.85	0.89	-0.165 -0.794	0.334
4	RP-4, Lat. /Med C+T3H ratio	0.93 $\pm$ 0.04	0.93	0.92	0.95	-0.031 -0.690	0.670
5	RP-5, Lat.h.C/Lh ratio	0.18 $\pm$ 0.02	0.18	0.18	0.19	0.078 -0.819	0.056
6	RP-6, Lat.h.T3/Lh ratio	0.17 $\pm$ 0.01	0.17	0.17	0.18	-0.127 0.243	0.165
7	RP-7, Lat.h.T4/Lh ratio	0.38 $\pm$ 0.02	0.38	0.38	0.39	-0.053 -0.474	0.072
8	RP-8, Lat.h.T4/Med.h.C+T3 ratio	0.96 $\pm$ 0.05	0.96	0.95	0.98	0.692 0.351	0.153
9	RP-9, Lat.h.C+T3/ Lat.h.T4 ratio	0.97 $\pm$ 0.05	0.97	0.95	0.99	0.106 -1.184	0.141
10	RP-10, Lh /Mh ratio	0.98 $\pm$ 0.03	0.97	0.97	0.99	0.445 -0.486	0.013*
11	RP-11, Med.h.C/Med.h.C+T3 ratio	0.47 $\pm$ 0.02	0.47	0.46	0.48	0.330 -0.218	0.102

12	RP-12, Med.h.T3/Med.h.C+T3 ratio	0.50 ± 0.02	0.51	0.50	0.51	-0.184 -0.559	0.119
13	RP-13, Med.h.C+T3/Mh ratio	0.39 ± 0.02	0.39	0.38	0.39	0.184 -0.789	0.044*
14	RP-14, m.h.T/ Med.h.T ratio	0.78 ± 0.01	0.78	0.77	0.78	0.387 -0.206	0.032*
15	RP-15, W.T/ Med.h.T ratio	0.90 ± 0.03	0.91	0.88	0.91	-0.482 0.101	0.156
16	RP-16, m.h.T/ Post.h.Calc ratio	0.52 ± 0.02	0.52	0.51	0.53	0.240 -0.364	0.355
17	RP-17, W.T/ Post.h.Calc ratio	0.45 ± 0.01	0.45	0.45	0.46	-0.300 -0.584	0.134
18	RP-18, Lh/ Post.h.Calc ratio	0.61 ± 0.04	0.62	0.59	0.62	-0.625 -0.328	0.095
19	RP-19, RL/ W.T ratio	0.91 ± 0.04	0.92	0.89	0.92	-0.336 0.014	0.075

Figure 4. 17: Normality distribution (Normal Q-Q) and mean box plots for ratio parameters.





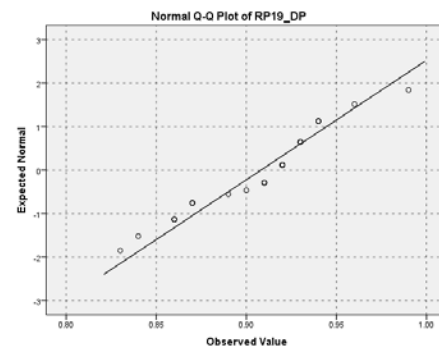
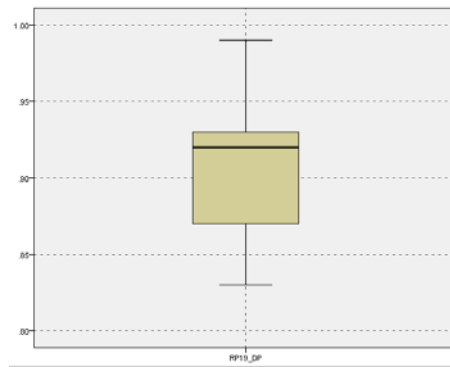
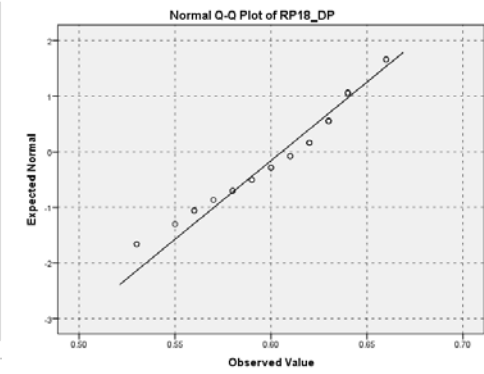
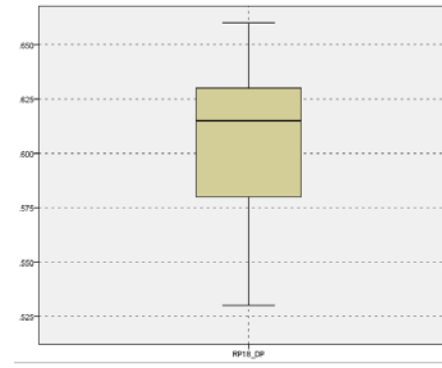
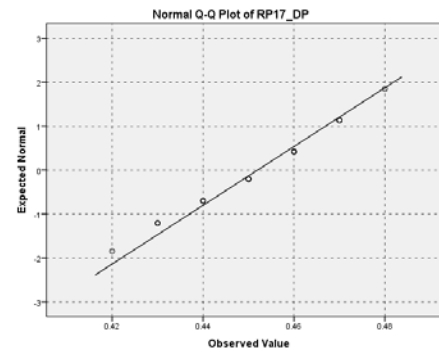
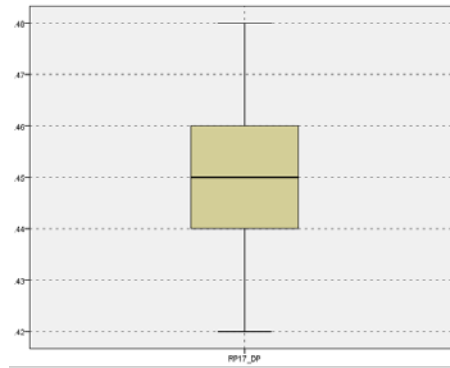
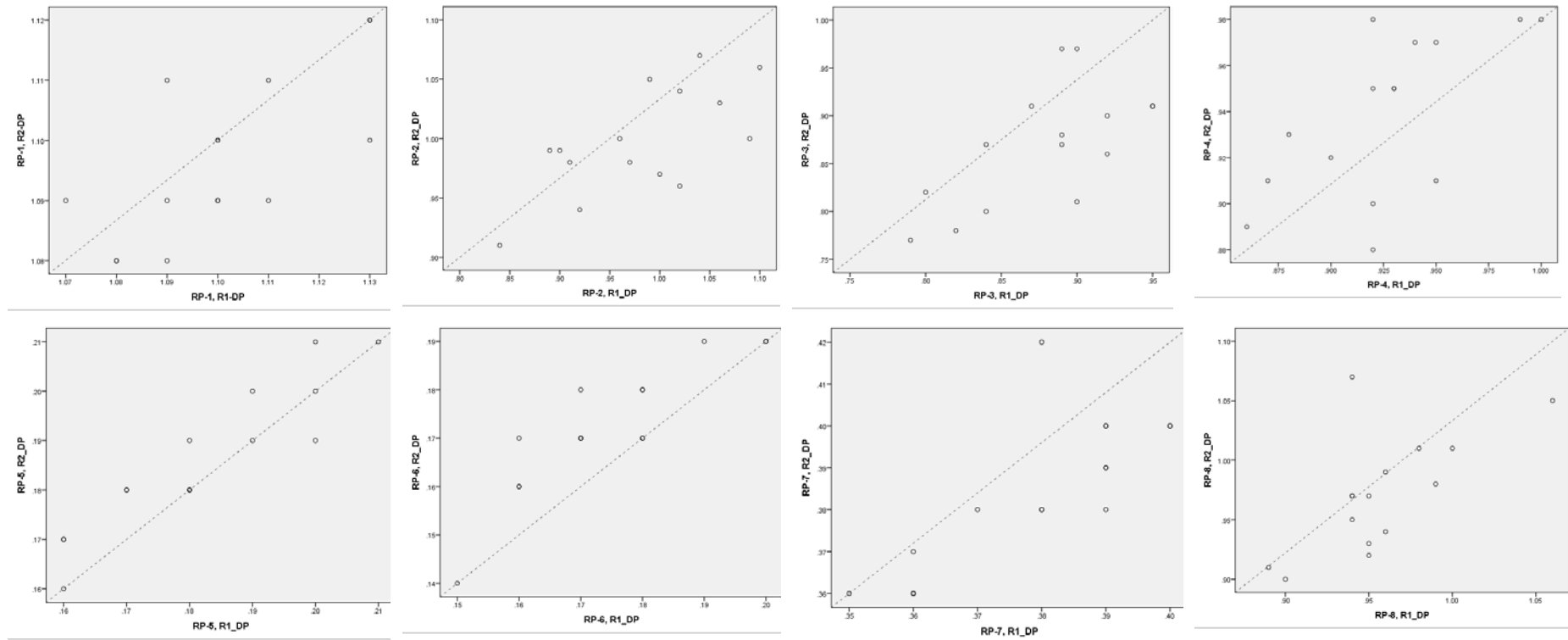


Table 4. 9: Intra-rater repeatability of the ratios parameters using ICC and Lin's CCC coefficients in 15 tarsi.

Sr. No.	Measurable parameters (n=15)	measurements	Mean $\pm$ SD	Intraclass Correlation (ICC)	95 % CI of ICC		Lin's CCC	95 % CI of Lin's CCC	
					Lower	Upper		Lower	Upper
1	RP-1, RL/T3 ratio	1 st	1.10 $\pm$ 0.02	0.807	0.447	0.934	0.661	0.307	0.854
		2 nd	1.10 $\pm$ 0.01						
2	RP-2, Lat. /Med.C.h ratio	1 st	0.98 $\pm$ 0.08	0.740	0.258	0.911	0.571	0.223	0.789
		2 nd	1.00 $\pm$.05						
3	RP-3, Lat. /Med.h.T3 ratio	1 st	0.88 $\pm$ 0.05	0.788	0.373	0.928	0.634	0.229	0.852
		2 nd	0.87 $\pm$ 0.06						
4	RP-4, Lat. /Med C+T3H ratio	1 st	0.93 $\pm$ 0.04	0.765	0.337	0.920	0.604	0.190	0.835
		2 nd	0.94 $\pm$ 0.03						
5	RP-5, Lat.h.C/Lh ratio	1 st	0.18 $\pm$ 0.02	0.942	0.778	0.982	0.883	0.712	0.955
		2 nd	0.19 $\pm$ 0.02						
6	RP-6, Lat.h.T3/Lh ratio	1 st	0.17 $\pm$ 0.01	0.937	0.817	0.978	0.873	0.719	0.954
		2 nd	0.17 $\pm$ 0.01						
7	RP-7, Lat.h.T4/Lh ratio	1 st	0.38 $\pm$ 0.02	0.856	0.571	0.952	0.735	0.407	0.895
		2 nd	0.38 $\pm$ 0.02						
8	RP-8, Lat.h.T4/Med.h.C+T3 ratio	1 st	0.96 $\pm$ 0.04	0.772	0.354	0.922	0.612	0.209	0.837
		2 nd	0.97 $\pm$ 0.05						
9	RP-9, Lat.h.C+T3/ Lat.h.T4 ratio	1 st	0.97 $\pm$ 0.05	0.852	0.554	0.951	0.729	0.369	0.899
		2 nd	0.97 $\pm$ 0.05						
	RP-10, Lh /Mh ratio	1 st	0.98 $\pm$ 0.03	0.946	0.835	0.982	0.891	0.716	

10		2 nd	0.97 ± 0.03						0.961
11	RP-11, Med.h.C/Med.h.C+T3 ratio	1 st	0.47 ± 0.02	0.828	0.478	0.942	0.691	0.322	0.878
		2 nd	0.47 ± 0.02						
12	RP-12, Med.h.T3/Med.h.C+T3 ratio	1 st	0.50 ± 0.02	0.598	-0.104	0.861	0.410	-0.048	0.725
		2 nd	0.51 ± 0.02						
13	RP-13, Med.h.C+T3/Mh ratio	1 st	0.39 ± 0.02	0.928	0.786	0.976	0.857	0.640	0.947
		2 nd	0.39 ± 0.02						
14	RP-14, m.h.T/ Med.h.T ratio	1 st	0.78 ± 0.01	0.909	0.737	0.969	0.824	0.560	0.936
		2 nd	0.78 ± 0.01						
15	RP-15, W.T/ Med.h.T ratio	1 st	0.89 ± 0.04	0.943	0.832	0.981	0.886	0.707	0.958
		2 nd	0.90 ± 0.03						
16	RP-16, m.h.T/ Post.h.Calc ratio	1 st	0.52 ± 0.02	0.956	0.869	0.985	0.911	0.770	0.967
		2 nd	0.52 ± 0.02						
17	RP-17, W.T/ Post.h.Calc ratio	1 st	0.45 ± 0.02	0.922	0.769	0.974	0.846	0.605	0.945
		2 nd	0.45 ± 0.02						
18	RP-18, Lh/ Post.h.Calc ratio	1 st	0.61 ± 0.04	0.986	0.957	0.995	0.970	0.915	0.990
		2 nd	0.60 ± 0.04						
19	RP-19, RL/ W.T ratio	1 st	0.91 ± 0.04	0.916	0.751	0.972	0.835	0.590	0.939
		2 nd	0.90 ± 0.04						

Figure 4. 18: Scatter plots of Lin's CCC showing distribution of measurements and level of agreement and reproducibility between first and second readings of thirteen ratio's parameters (RP); R-2nd, second measurement.



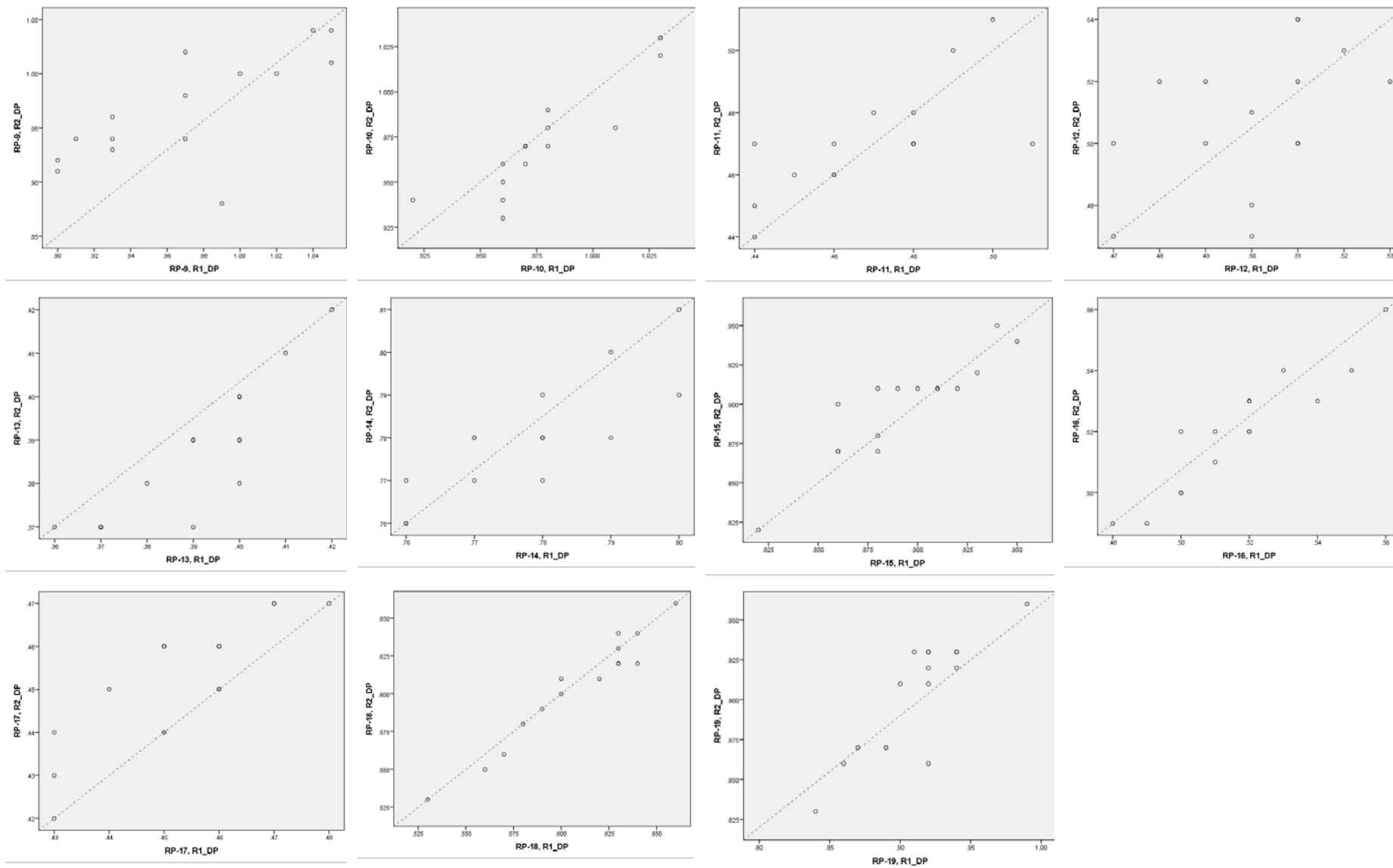
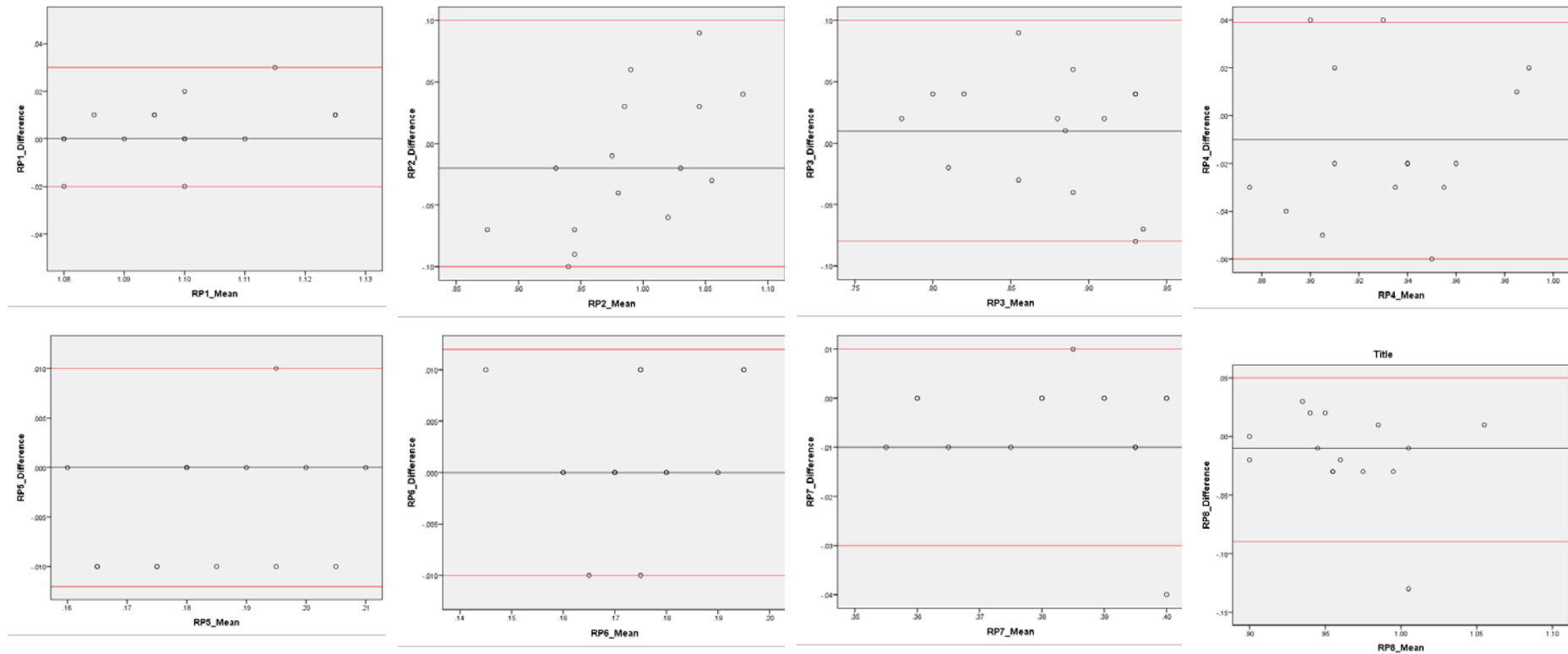


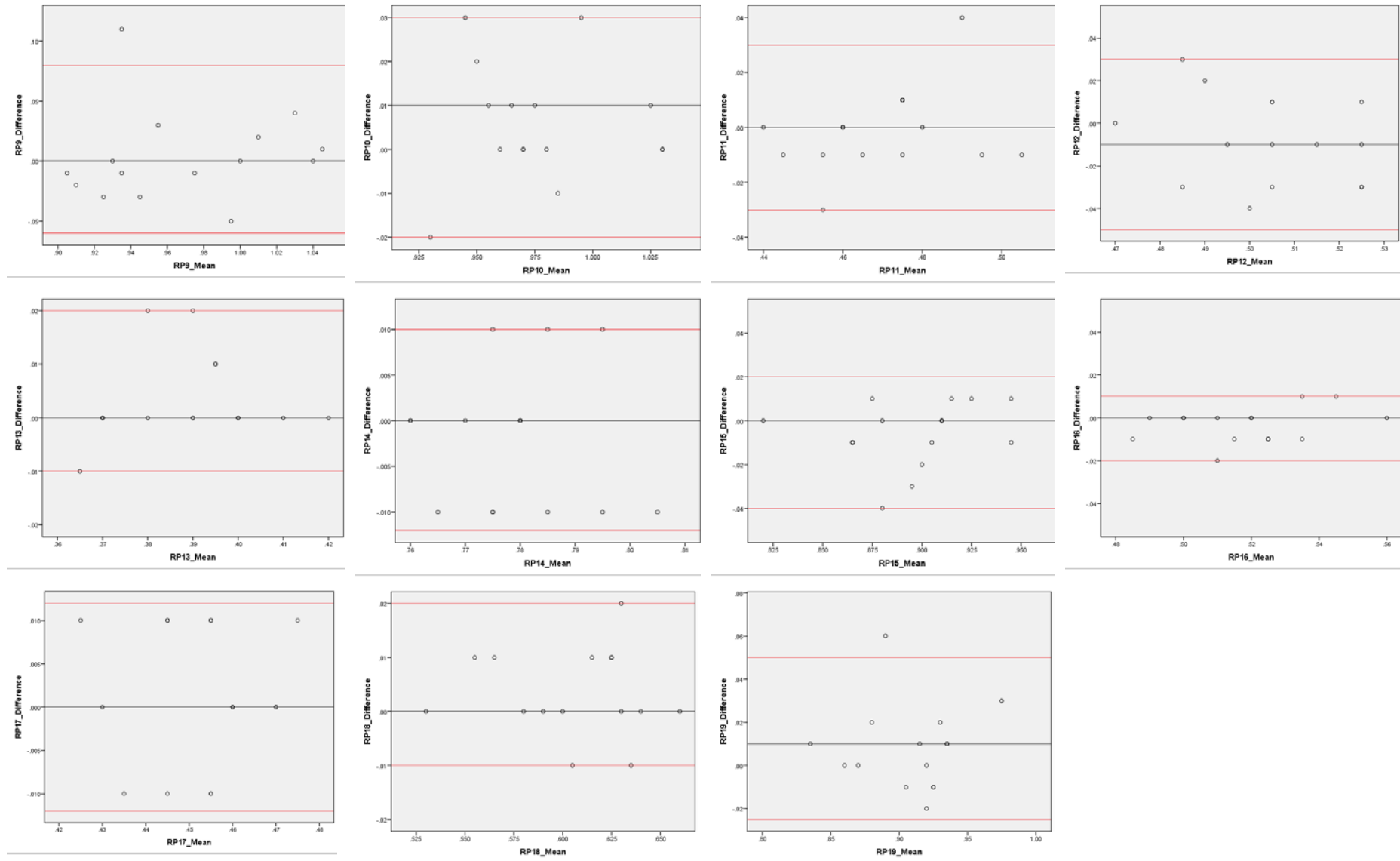
Table 4. 10: Intra-rater repeatability of the ratios parameters in 15 tarsi using mean difference 95% (CI) limit of agreement between repeated measurements for the Bland-Altman Plots.

Sr. No.	Measurable parameters (n=15)	Measurements	Mean $\pm$ SD	Mean Difference $\pm$ SD	Sig. level ($p < 0.05$)	Calculation of 95 % Limits of agreement of Bland-Altman plots	
						Lower	Upper
1	RP-1, RL/T3 ratio	1 st	1.10 $\pm$ 0.02	0.00 $\pm$ 0.01	0.253	-0.02	0.03
		2 nd	1.10 $\pm$ 0.01				
2	RP-2, Lat. /Med.C.h ratio	1 st	0.98 $\pm$ 0.08	-0.02 $\pm$ 0.06	0.259	-0.13	0.10
		2 nd	1.00 $\pm$.05				
3	RP-3, Lat. /Med.h.T3 ratio	1 st	0.88 $\pm$ 0.05	0.01 $\pm$ 0.05	0.464	-0.08	0.10
		2 nd	0.87 $\pm$ 0.06				
4	RP-4, Lat. /Med C+T3H ratio	1 st	0.93 $\pm$ 0.04	-0.01 $\pm$ 0.03	0.139	-0.07	0.05
		2 nd	0.94 $\pm$ 0.03				
5	RP-5, Lat.h.C/Lh ratio	1 st	0.18 $\pm$ 0.02	-0.00 $\pm$ 0.01	0.028	-0.02	0.01
		2 nd	0.19 $\pm$ 0.02				
6	RP-6, Lat.h.T3/Lh ratio	1 st	0.17 $\pm$ 0.01	0.00 $\pm$ 0.01	0.271	-0.01	0.02
		2 nd	0.17 $\pm$ 0.01				
7	RP-7, Lat.h.T4/Lh ratio	1 st	0.38 $\pm$ 0.02	-0.01 $\pm$ 0.01	0.088	-0.03	0.02
		2 nd	0.38 $\pm$ 0.02				
8	RP-8, Lat.h.T4/Med.h.C+T3 ratio	1 st	0.96 $\pm$ 0.04	-0.01 $\pm$ 0.04	0.157	-0.09	0.06
		2 nd	0.97 $\pm$ 0.05				

9	RP-9, Lat.h.C+T3/ Lat.h.T4 ratio	1 st	0.97 ± 0.05	0.00 ± 0.04	0.737	-0.07	0.08
		2 nd	0.97 ± 0.05				
10	RP-10, Lh /Mh ratio	1 st	0.98 ± 0.03	0.01 ± 0.01	0.108	-0.02	0.03
		2 nd	0.97 ± 0.03				
11	RP-11, Med.h.C/Med.h.C+T3 ratio	1 st	0.47 ± 0.02	-0.00 ± 0.02	0.744	-0.03	0.03
		2 nd	0.47 ± 0.02				
12	RP-12, Med.h.T3/Med.h.C+T3 ratio	1 st	0.50 ± 0.02	-0.01 ± 0.02	0.164	-0.05	0.03
		2 nd	0.51 ± 0.02				
13	RP-13, Med.h.C+T3/Mh ratio	1 st	0.39 ± 0.02	0.00 ± 0.01	0.136	-0.01	0.02
		2 nd	0.39 ± 0.02				
14	RP-14, m.h.T/ Med.h.T ratio	1 st	0.78 ± 0.01	-0.00 ± .01	0.334	-0.02	0.01
		2 nd	0.78 ± 0.01				
15	RP-15, W.T/ Med.h.T ratio	1 st	0.89 ± 0.04	-0.01 ± 0.02	0.144	-0.04	0.02
		2 nd	0.90 ± 0.03				
16	RP-16, m.h.T/ Post.h.Calc ratio	1 st	0.52 ± 0.02	-0.00 ± 0.01	0.136	-0.02	0.01
		2 nd	0.52 ± 0.02				
17	RP-17, W.T/ Post.h.Calc ratio	1 st	0.45 ± 0.02	0.00 ± 0.01	0.546	-0.02	0.02
		2 nd	0.45 ± 0.02				
18	RP-18, Lh/ Post.h.Calc ratio	1 st	0.61 ± 0.04	0.00 ± 0.01	0.136	-0.01	0.02
		2 nd	0.60 ± 0.04				
19	RP-19, RL/ W.T ratio	1 st	0.91 ± 0.04	0.01 ± 0.02	0.138	-0.03	0.05
		2 nd	0.90 ± 0.04				

Figure 4. 19: Intra-rater repeatability of the individual angular parameters in 15 tarsi using mean difference 95% (CI) limit of agreement between repeated measurements for the Bland-Altman Plots.





4.4 DISCUSSION:

In the present study, the possibility of identification of reasons for tarsal conformational defects was made more specific and accurate by using the majority of tarsal morphological details and specific landmarks clearly visible on the dorsoplantar radiographs. However, some features such as the dorsal aspect of fourth tarsal (T4) have not been included due to difficulties in locating reliable landmarks consistently on those surfaces. In addition, the first and second tarsal bones have been excluded from the study.

The present study defines the true zero-degree dorsoplantar view (ZDP) in equine tarsus radiography for morphometrical purposes. It takes into account the potential effects of rotations around the vertical and horizontal axes of the tarsus by determining a landmark for each rotation. The achievement of a consistent ZDP is based on some reliable landmarks and their correct identification. The potential effects of rotations around the vertical and horizontal axes of the tarsus need to be considered by determining landmarks for each rotation. These landmarks can be identified in respect to the vertical, sagittal and horizontal axes of the tarsus.

In the vertical evaluation, the true DP was when there was almost complete overlapping of the lateral edges of the distal tibia and calcaneus. The view was considered to be at a good DP in respect to the horizontal rotation when there was a reasonable overlapping of the trochlea of talus and calcaneoquartal joint. This vertical landmark was very sensitive to any minor rotation around the vertical axis of the tarsus. This kind of evaluation has not been addressed in the commonly used dorsoplantar view which has been described in the literature (Butler et al., 2008). Therefore, it could be assumed that the commonly used dorsoplantar view (as described

in the literature) would be more suitable for diagnostic purposes while it might not be as sufficient to overcome the complexity and differentiation involved in the accurate geometrical measurement of tarsus.

Importantly, identification of the view can be considered a fundamental starting point in studying tarsal morphometry. It can be noted that achieving the ZDP may be difficult in field radiography and in-vivo, however, it is possible to radiograph this accurately with some care and with the help of the vertical and horizontal landmarks. This can be achieved easily by changing the position of radiographic machine as compared to limb repositioning to achieve the required landmarks. As in the field radiography, there is minimal repositioning of the limb that can be done so most of the changes must be done with the radiographic machine to achieve the landmarks.

A Reference Line (RL) was developed in which there was a straight line extended between two landmarks on the lateral and medial side of the distal tarsus. The lateral landmark was the point where the dorso-lateral edge of the distal articular surface of the fourth tarsal and the dorso-lateral edge of the articular facet on the proximal surface of the third metatarsal for articulation with the fourth tarsal came together. The medial landmark was the point where the dorso-medial edge of the distal articular surface of the second tarsal and the dorso-medial edge of the articular facet on the proximal surface of the third metatarsal for articulation with second tarsal came together. The proximal extremity of the third metatarsus was defined and its suitability was considered as a reference or reference line.

The consistency and suitability of determining the medial and lateral landmarks of the reference line were assessed. The statistical results showed a significant difference ($p\text{-value} = 0.00$) between the heights of the two landmarks. This result has two meanings. Firstly, the lateral landmark, was consistent in comparison to the medial one. Secondly and interestingly, it could be suggested from the result that the RL was not parallel with the ground because lateral height greater than the medial height. In addition to this, difference in lateral and medial heights from the distal articular surface of the tibia would be affected due to the morphometrical variation between the two condyles. In a previous study, the height of the equine third metacarpal was found to be significantly greater from the proximal articular surface to the lateral condyle than from the proximal articular surface to the medial condyle of the distal articular surface (Alrtib et al., 2013). Most previous geometric studies examined the external conformation of hock using the external landmarks including the long tibial and metatarsal bones, while no specific study to the author's knowledge explored the complete internal geometry of the tarsus including alignment of the small tarsal bones in different loading conditions. In addition, the available geometric studies are few and not addressing the other factors like landmarks and other rotational factors at the same time

The measurable parameters were designed as angles and ratios in order to remove the effects of variations in body size between individual horses. There were 49 parameters divided into 11 individual angular, 19 correlated angular and 19 ratio parameters. The tarsometatarsal- tibial metaphyseal/malleolar joint and tarsometatarsal-tibial tibio-talus joint angle parameters were designed to identify the general conformation of the tarsal region. It could measure tarsal angular deformities such as valgus and varus. The distal extremity of the tibia and the proximal

extremity of Mt3 were measured in previous studies. Hence these parameters would ideally be equivalent to measuring the tarsal angle which is commonly reported in the literature and used in clinics for measuring varus and valgus deformities of the tarsus (Caron, 1988; Tyson, Graham, Colahan, & Berry, 2004; Vanderperren, Raes, Van Bree, et al., 2009).

In similar studies, the tarsal angle was measured in geometric evaluation of the tarsus by drawing bisecting lines along the tibia and the third metatarsal (MtIII) bones on the dorsoplantar view, where the two lines intersect/cross was called the “pivot point” to localize the site of abnormal development (Caron, 1988) and change in tarsal angle (Witte & Hunt, 2009). The severity of the angular deviation can be determined by measuring the angle at the same “point of intersection” (J. Auer, 1991; Caron, 1988). But in the subsequent studies, limb position and projection were the same, as inconsistency in either will substantially change the calculated angle (Caron, 1988). In general, a pivot point in the proximal epiphysis or metaphyseal growth plate (physis) of a long bone indicated a more favourable prognosis as compared to a pivot point within a joint (Caron, 1988). However, the lines for the pivot point have not been standardized in any radiographic diagnostic protocol or evaluation study because the deformities are difficult to accurately define on radiographs as the tibia and the third metatarsal cannot be arranged in the same frontal plane, so the lateromedial midpoints of these bones cannot be joined by a straight line (Clegg, 2003). This might reflect the difficulties such as joining the lateromedial midpoints by a straight line while using superimposition of bony structures to define its orientation in the same frontal plane on representative DP views.

Based on this way of measurement described by these authors, there might be some degree of unreliability in such measurements. For instance, the absence of certain landmarks to identify the axes of the tibia and Mt3 might increase errors in radiographic measurements due to subjectivity in drawing the axes over the radiographs. Furthermore, the long axis is dependent on the third metatarsus long bone axis in these studies, so the normal conformation of the third metatarsal should be assured before any further evaluation or before taking geometrical measurements of the tarsus. The accuracy of these measurements would also be affected due to the normal variations among individuals, age/stage of development and breeds of equines. In addition, no evaluation has been reported to examine the effects of different orientations in dorsoplantar radiographs on the radiographic measurements of the tarsal angle. In studying the equine third metacarpal shape of the midshaft, the accuracy of its radiographic measurements was affected by their orientation of the mid shafts (Walter & Davies, 2001).

In most of the diagnostic methods as described earlier, there was a lack of clarity in the identification of landmarks, and they did not ensure normal conformation of the tibia, Mt3, nor they included any tarsal bones conformational/morphological details or detection of any deviations in the tarsal bones which could impact such measurements. In addition, no evaluation of the effects of the orientation on radiographic measurements has been reported.

The neonatal angular deformities such as supporting soft tissues laxity, hypoplasia or incomplete ossification of the central tarsal and third tarsal bones, or configurational abnormalities of the epiphysis and metaphysis in foals can be detected but needs more specific techniques (J. Auer et al., 1982; Caron, 1988; Leitch, 1985). The Proximal intertarsal- tibial metaphyseal/malleolar

joint and/or Proximal intertarsal- tibio-talus joint parameters were aimed to identify any medial or lateral conformational deviation confined to the distal extremity of the tibia. In addition, the tarsometatarsal- proximal intertarsal joint and the distal intertarsal-tarsometatarsal joint parameters were aimed to identify medial or lateral conformational deviations confined to the proximal extremity of the third metatarsal. While in the evaluation of the cuboidal bones of the tarsus, using the dorsoplantar projection to find any imbalance in the tarsal bones, the Mh to Lh ratio parameter could locate any hypoplasia or displacement within the bones medially or laterally. Then by using the ratios for each tarsal bone such as Lat.h.C/Lh ratio, Lat.h.T3/Lh ratio, Lat./Med C+T3H ratio parameters, the affected bone could be identified.

The repeatability of the parameters was generally higher for the individual and correlated angular parameters as compared to ratio parameters. This seems to be due to their small absolute values. Hence, considering their repeatability should probably depend more on the Lin's CCC. Most of individual angular parameters had an intraclass correlation coefficient (ICC) of higher than 0.90 in 6 out of 11 parameters. Four individual angular parameters had values slightly below 0.90 except the tarsometatarsal (TMT) angle which had an ICC of 0.661. The Lin's CCC values also indicated the same pattern as six individual angular parameters showed almost perfect agreement (0.81–1.00). Such results clearly indicate that the individual angular parameters were measured consistently.

Bland-Altman plots were developed for individual angles to better visualize the level of agreements between repeat measurements. However, the ICC for the correlated angular parameters were higher (> 0.9) in most of the correlated angles (13 out of 19 correlated angles)

and according to the Lin's CCC showed almost perfect agreement (0.81-1.00). A lower ICC (<0.9) was observed in six parameters indicating that there was substantial agreement (0.61-0.80). The reasons for such differences in the repeatability between the individual and correlated angular parameters might be the actual properties of the landmarks after removing the effect of the reference line in some parameters, which reveal the importance of landmarks and radiographic differentiation of superimposed structures. Hence, it might be suggested that the repeatability of correlated angles could be more related to the identification and precision of landmarks.

The current study has also established specific parameters to investigate reasons for the high incidence of some common lesions and fractures in the tarsus. Radiographic abnormalities with osteochondrosis (OCD) includes fragments, an irregular, flattened contour of the subchondral bone surfaces, with or without radiolucencies of the subchondral and/or sclerosis, and sometimes secondary osteoarthritis/degenerative joint disease (DJD) (Butler et al., 2000; Trotter & McIlwraith, 1981). OCD of the medial malleolus of the tibia can be best visualized with a dorsoplantar (DP) or dorsolateral-plantaromedial oblique (DL-PIMO) projection and must be differentiated from avulsion fractures (McIlwraith, Foerner, & Davis, 1991; Nixon, 2004). Tibial metaphyseal/malleolar and Tibio-talus joint individual angles were designed to identify the relationship between the morphometry of the tibial malleolus and the distal articular surfaces of the tibia and any avulsion of the medial malleolus of the tibia on the radiographic dorsoplantar view.

Tarsal luxations occur commonly as a result of severe traumatic disruptions, which typically involve the proximal intertarsal and the tarsometatarsal joints. The tarsocrural joint is less often

involved. Luxation of the distal intertarsal joint is extremely rare due to its relationship with the fourth tarsal bone height which equals the combined heights of the central and third tarsal bones and therefore, bridges and stabilizes the distal intertarsal joint (Moll et al., 1987) and to a lesser extent by the fused first and second tarsal bones (Abuja, Bubeck, Quinteros, & García-López, 2013; Bolt et al., 2003; AJ Nixon, 1996).

Tarsal bone collapse and incomplete ossification of the smaller tarsal bones occurs in premature foals, twins or underdeveloped newborn foals most commonly (Baird, D. K. 2002; G. Baxter & Turner, 2002; J. A. Butler, C. M. Colles, S. J. Dyson, S. E. Kold, & P. W. Poulos, 2000). These immature bones cannot bear excessive loading and may result in valgus deformities and/or pathological fractures associated with collapse of the third tarsal and/or central tarsal bones (Bolt et al., 2003). The radiographic findings include a compressed or wedge-shaped third and central tarsal bones, fragmentation/fracture of the dorsal aspect of the tarsal bones, narrowed joint spaces and osteoarthritis/secondary degenerative joint disease (DJD) (Butler et al., 2000; Trotter & McIlwraith, 1981).

Luxation of the tarsometatarsal joint is associated with ligamentous disruption and tarsal and metatarsal fractures (Bolt et al., 2003; Moll et al., 1987). No radiographic morphometrical study has been reported concerning any relationship between conformation and the likelihood of luxation. The tibial metaphyseal/malleolar angle, tibio-talus joint angle, third tarsal-metatarsal joint angular parameters were developed to find such conformational pathologies in this study.

The third tarsal-metatarsal joint angle, Lat.h.C/Lh ratio, Lat.h.T3/Lh ratio, Lat./Med C+T3H ratio, Med.h.C/Med.h.C+T3 ratio, Med.h.T3/Med.h.C+T3 ratio and Med.h.C+T3/Mh ratio parameters could measure any misalignment or shape deformations in the tarsal joints of such foals.

The landmarks which were not easily located included the most medial edges of the central and third tarsals at the levels of the proximal, distal and tarsometatarsal joints where dorsal and plantar borders were joined in ZDP radiographs. The landmarks were hard to identify in these areas because of the curvature of these tarsal bones, while dorsal ridges on the dorsal aspects of these bones also make the differentiation of particular landmarks difficult to identify. The landmarks for latero-medial width of talus lacked specific bony landmarks and relies on the orientation of talus on the radiographs where a slight difference in orientation might change these measurements. Therefore, the reason for such variations was not clear and hence there might be some undetectable factors that led to the disagreement between the statistical results and the actual work in the identification of the landmarks.

In the ratio parameters, the results of the repeatability were quite variable, although the ICC values were relatively higher (> 0.9) for 10/19 ratios, which indicated absolute agreement (0.81-1.00) according to Lin's CCC values. The other ratio parameters had ICC values < 0.9 and demonstrated substantial agreement according to Lin's CCC values (0.61-0.80) except the Med.h.T3/Med.h.C+T3 ratio which had a ICC value of 0.6 which showed only a moderate level of agreement (Lin's CCC value 0.41). This considerable variation in ratios seems to be due to their small absolute values as compared to other parameters. The reason for low agreement in ratios must be considered as these measurements were lacking specific landmarks.

The consistency between the repeat measurements depended on easily located tarsal landmarks which can consequently lower the chances of errors in landmark identification. In addition, if all the measurements were taken by one rater that would be an extra way to minimize the variations between the repeat measurements (Kublashvili et al., 2004).

It can be concluded that the present study has provided a base for the measurement of tarsal conformation using radiography and established a foundation for the measurements of the tarsus bones in particular on DP radiographs which can be used to understand the relationships between bone morphometry, tarsus conformation and pathology. Furthermore, to use these radiographic morphometric parameters in clinical dorsoplantar radiographs, they should be evaluated on radiographs oriented around the tarsal axes. This included determining DP at a zero view and identifying most of the measurable parameters in these radiographs. In addition, further studies will be required to evaluate the effects of possible rotations during radiography on these tarsal measurements. When morphometrical data is available, a relationship might be discovered between mechanical and conformational information to elucidate the reasons for the high incidence of damage in a particular region.

Chapter 5: Effect of possible limb or radiography machine rotations on the radiographic measurements of tarsal parameters in a lateromedial view of the tarsus

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5.1 Abstract

Radiography is an important diagnostic tool to evaluate limb deformities but is sensitive to rotations such as changes in orientation of object, cassette or radiography machine. The effect of rotations of radiography machine in field radiography has vital importance in diagnosis of tarsal pathologies and differentiation of any changes caused by such rotations. The rotations can happen either by limb rotation or rotation of the radiography machine in vertical and horizontal planes. There is minimal repositioning of the limb that can be done during field or clinical radiography, so most of the time the rotations can be happen with the radiographic machine positional changes along with changes in the angle of the digital cassette. Influences of such rotations on radiographic measurements of lateromedial tarsal radiographs have not been addressed in spite of their significant effects. This study measured effects of possible radiographic machine rotations during tarsal radiography around vertical and horizontal axes on 10 tarsal radiographic parameters and identified an acceptable range of rotation where those parameters experienced a minimum change. Fifteen hindlimbs from 12 adult equine cadavers (that died for

reasons not related to this study) were transected at the distal third of the tibia, fixed vertically, and then digitally radiographed 13 times. The first radiograph was aligned to a specific set of vertical and horizontal landmarks called the zero-degree lateromedial (ZLM) view and defined by overlapping of the lateral and medial trochlea of the talus and a contact point between the dorsal edges of the lateral and medial borders of the central tarsal bone. Subsequent radiographs were rotated through -15° to $+15^{\circ}$ in both axes with 5° intervals. Parameters were measured on the 195 radiographs and analysed using a paired t-test to compare each angle of rotation (-15° , -10° , -5° , $+5^{\circ}$, $+10^{\circ}$ and $+15^{\circ}$) with the zero angle of rotation for each parameter. This was done for both the vertical and the horizontal rotations. Most of the parameters showed significant alterations during vertical and horizontal rotations. Using rotational angles of Vertical $\pm 5^{\circ}$ and Horizontal $\pm 5^{\circ}$ in only one axis as the maximum acceptable range of rotation from ZLM would offer the best potential to avoid significant effects of rotation on these tarsal radiographic measurements. No ethical approval was required for this study.

5.2 INTRODUCTION

In tarsus radiography, despite the use of different methods to achieve a true lateromedial radiographic view, archival radiographs demonstrate a wide range of apparent variations, particularly in the alignment and/or morphological aspects of the tarsus conformation. This wide range of morphological variations between tarsal radiographs in clinical archives might be a result of conformational variations among animals even within the same species. Radiography is still a readily available noninvasive and important diagnostic tool which is widely used both inside clinics and in the field. This technique is highly sensitive to any changes in the orientation of the radiographing subject, cassette or radiography machine.

There could be misalignments of tarsus, cassette and the x-ray machine during radiography. Therefore, such variations might lead to misunderstandings in diagnosis between normal tarsal morphometry because of a wide range of various shapes among normal tarsus and pathological tarsals particularly in cases of conformational defects. The rotations or inconsistency in taking proper radiographic views can also play a vital role in this regard as misalignments of radiographic machine, cassette or object can affect the accuracy (Han & Hurd, 2005; Walter & Davies, 2001; Yoon et al., 2001). This wide range of variations in morphology between tarsi and even same tarsus if radiographed twice with some interval seemed to be a hindrance to a clear distinction between normal tarsal morphometry and tarsal pathological conditions and/or any defects in conformations of the tarsus.

In equine tarsal radiography, a few parameters were established using LM views by a number of authors such as (Caron, 1988; Clegg, 2003; Dahn & Ueltschi, 1989). The acquisition of a

radiographic view reflecting the correct dimensions of an object which can reliably compared between individuals was a major problem in the field conditions and in the clinics. The rotations around the sagittal axis only changed the position of the object on the radiographic image (Ahlqvist, Eliasson, & Welander, 1983; Y. J. Yoon et al., 2002) because the axis of rotation was parallel to the center of the x-ray beam (Major et al., 1996).

Some investigators like Lonner et al., (1996) and Radtke et al., (2010) determined the effects of limb rotation on the radiographic alignments of total knee arthroplasties on synthetic femurs and tibias in humans to identify an acceptable range of object rotation through which the parameters did not demonstrate significant errors (Lonner, Laird, & Stuchin, 1996; Radtke, Becher, Noll, & Ostermeier, 2010). Furthermore, some of the studies identified equations to recalculate measurements on the rotated radiographs to reduce the requirement to repeat radiographs (Radtke et al., 2010). Although all the parameters were aimed to identify tarsal deformities, the influence of limb rotation on the resultant radiographic measurements was not reported in any of these studies. Hence there is scant information addressing such factors in the literature which might cause errors in the interpretation of these geometric measurements.

In the current study therefore, it was hypothesized that radiography machine rotation during the acquisition of LM radiographs would affect the developed radiographic parameters identified by the author (Akbar et al. 2019). The following study aimed to identify the effect of limb rotation around the vertical and the horizontal axes of the tarsus on the radiographic parameters, and to determine a range of rotation through which the parameters experienced a minimum amount of change.

5.3 MATERIALS AND METHODS:

5.3.1 Animals

Fifteen hind limbs from twelve horse of various breeds including adult Standardbred and Thoroughbred were used in this experiment. All the hind limb (cadavers) in this experiment were collected from the Pathology department of the faculty of veterinary and animal sciences (FVAS), The University of Melbourne. The horses were euthanized or died for reasons that were not associated with the locomotor system and this study. The hind limbs were transected at the distal third of the tibia and were prepared with the marked point placed as described earlier in chapter 3.

5.3.2 Combination of the transparent radiographic plate, base, and radiography machine together

The radiography machine was best fit in the transparent radiography machine holding device fixed over a radiographic base scale (Figure 5.1A). The major X line is a line perpendicular to the Y major lines, while all other minor lines were 5 degrees apart from the Y major line. The dotted line was placed as an extra line for matching the adjustment of the radiography holding device with the base. The 0° alignment represented the exact overlapping of major lines at the frontal and caudal ends and both Y lines with the central x-ray beam and the head of the radiography machine for the zero-degree lateromedial view (ZLM). The transparent radiographic holding device was rotated around its vertical axis over the radiographic base scale for required vertical rotations of the radiography machine in this experiment as indicated in figure 5.1C. The digital cassette was placed in cassette holder. This cassette holder had two parts, upper cassette

holding part with the base having scale (similar to radiographic base) (Figure 5.1B) to rotate digital cassette at same angle according to the head of radiographic machine (5.2).

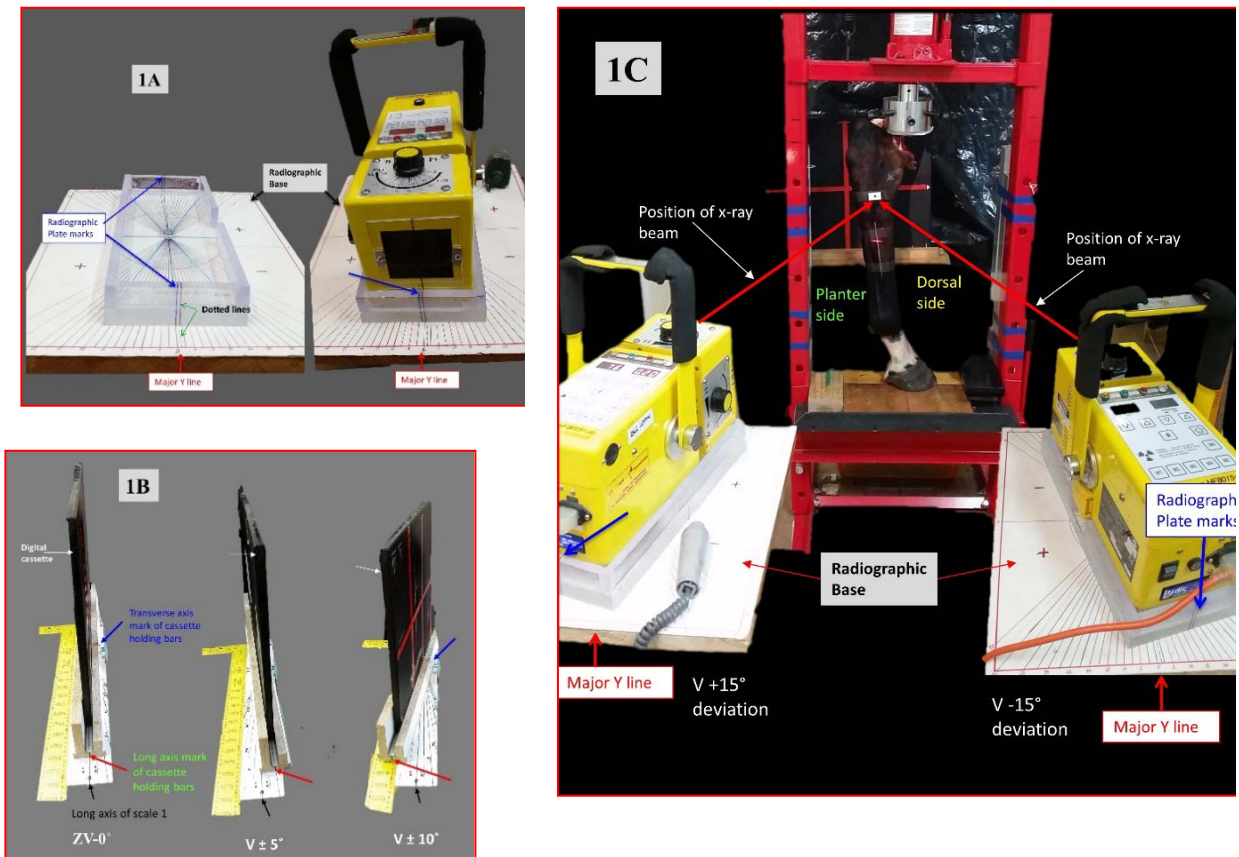


Figure 5. 1: The radiography apparatus including radiographic holding device, base, and radiography machine used for assessment of the vertical axis of the radiography machine with the help of alignment marks, (dotted lines on the radiographic plate) with the Major Y line of the radiography base during each rotation.

This 0° (zero degree) alignment was used for ZLM. **1B**, Rotations of digital cassette holder used in this study for positioning the digital cassette according to vertical rotation of the head of the radiography machine. **1C**, Radiographing a right tarsus (for LM views) of a Thoroughbred horse at $V+15^\circ$ and $V-15^\circ$ according to rotation of radiographic plate.

5.3.3 Digital cassette and radiographic processor:

Digital cassettes were used. The radiographs were produced automatically using a digital radiographic scanner machine. Radiograph plates were scanned using scanner (FireCR+ Veterinary 80 CR reader) and imported into DicomPACS®DX-R X-Ray acquisition software. All the radiographs were saved on a laptop directly to above mentioned special program. The radiographs were then collected after being filed and labeled according to which specimen they came from.

5.3.4 Positioning wedges:

For the horizontal rotation of the radiographic machine, the positioning wedges were made of wood. They were designed to fit between the two transparent perspex radiographic sheets as indicated and explained in figure 5.4. There were four different wedges used to make rotations of 5° and 10° around the horizontal axis of the radiographic machine.

Method

5.3.5 Preparation of limb for radiography:

1. The limbs were prepared, and the first radiograph was taken for each limb according to the procedures for taking the LM view at zero degrees. The lateral and medial trochlea of talus overlapped completely, and the proximal and distal talo-calcaneal joints were clearly visible. However, some radiographs had insufficient superimposition of the lateral and medial trochlea of the talus, then those limbs were re-manipulated dorsoplantarly using the limb base and re-

radiographed until the desired alignment was achieved for the ZLM radiographs. These landmarks were used to maintain the correct position of the vertical axis while the horizontal axis was found.

2. At the level of the distal inter-tarsal joint on the radiographs, the aim was to have a contact point between the dorsal edge of the lateral and medial borders of the distal end of the central tarsal and the overlapping of the dorsal borders of the second and fourth metatarsus. The radiographs that lacked this feature, were re-manipulated lateromedially and re-radiographed until the contact point between the borders was achieved as described earlier in chapter 3, figure 3.3.

5.3.6 Rotations around the vertical and horizontal axes

5.3.6.1 Radiographing the tarsus in rotation around the vertical axis of radiography machine:

After achieving ZLM, the alignment of the radiographic holding device with radiographic base was designated as the 0° alignment (Figure 5.1A) and monitored by the dorsal and plantar marks along with additional dotted lines. Two other marks, the medial and lateral, were also used on the radiographic holding device to maintain alignment with the major X line (this line was perpendicular to major Y line) of the radiographic base. The radiographic holding device and digital cassette were then rotated $\pm 5^\circ$ during each rotation over the radiographic base while this radiography machine was relocated (Figure 5.2) accordingly (within 100cm distance) on both side of limb (for external and internal rotations). The radiographic beam was positioned at the same marked that was placed on the prepared limb during each rotation and this was

used for beam alignment.

During vertical rotation of the radiography machine, the radiography machine and the apparatus holding the radiographic plate were rotated consistently. This rotation resulted in changing the marks of the radiographic plate from being on the major lines, the Y and the X for the 0° or ZLM, to be on one of the minor lines such as the, 5°, 10°, 15° as per the scale of the radiographic base. The radiographic beam was directed at the same marked point on the fixed prepared limb (as for the ZLM). This position was achieved by relocation of the radiographic apparatus dorsoplantarly according to beam position (Figure 5.1C). Simultaneously, there was no change in the position of the prepared hind limb while the cassette holder was also rotated according to the rotation of radiography machine in such a way that the surface which faced the radiography machine, was exactly perpendicular to the head of the radiography machine (Figure 5.1B and C). When the rotation of the radiographic machine was laterally to the body or plantar side of the prepared limb or ZLM projection, this rotation was called the external rotation (V+). It included three moves. Each move was made through an angle of 5° of rotation around the vertical axis of the radiography machine. The three movements were labelled as, V+5°, V+10° and V+15° along with the limb and horse identity.

When the radiography machine rotation was medially to the body or dorsal side of limb for ZLM projection, it was called the internal rotation (-V). It was performed in three moves as well. Each move formed an angle of 5° rotation around the vertical axis. The radiographs were labelled as, V-5°, V-10° and V-15° accordingly. The height of radiography machine from the floor remained constant in each step, so the radiographic beam remained at the same height during each step of vertical rotation. Therefore, in the vertical rotation each limb was radiographed

an additional 6 times after the first 0° radiograph. Three times were in the external rotation and the other three for the internal rotation (Figure 5.3). The total range of vertical rotation in each limb was 30°.

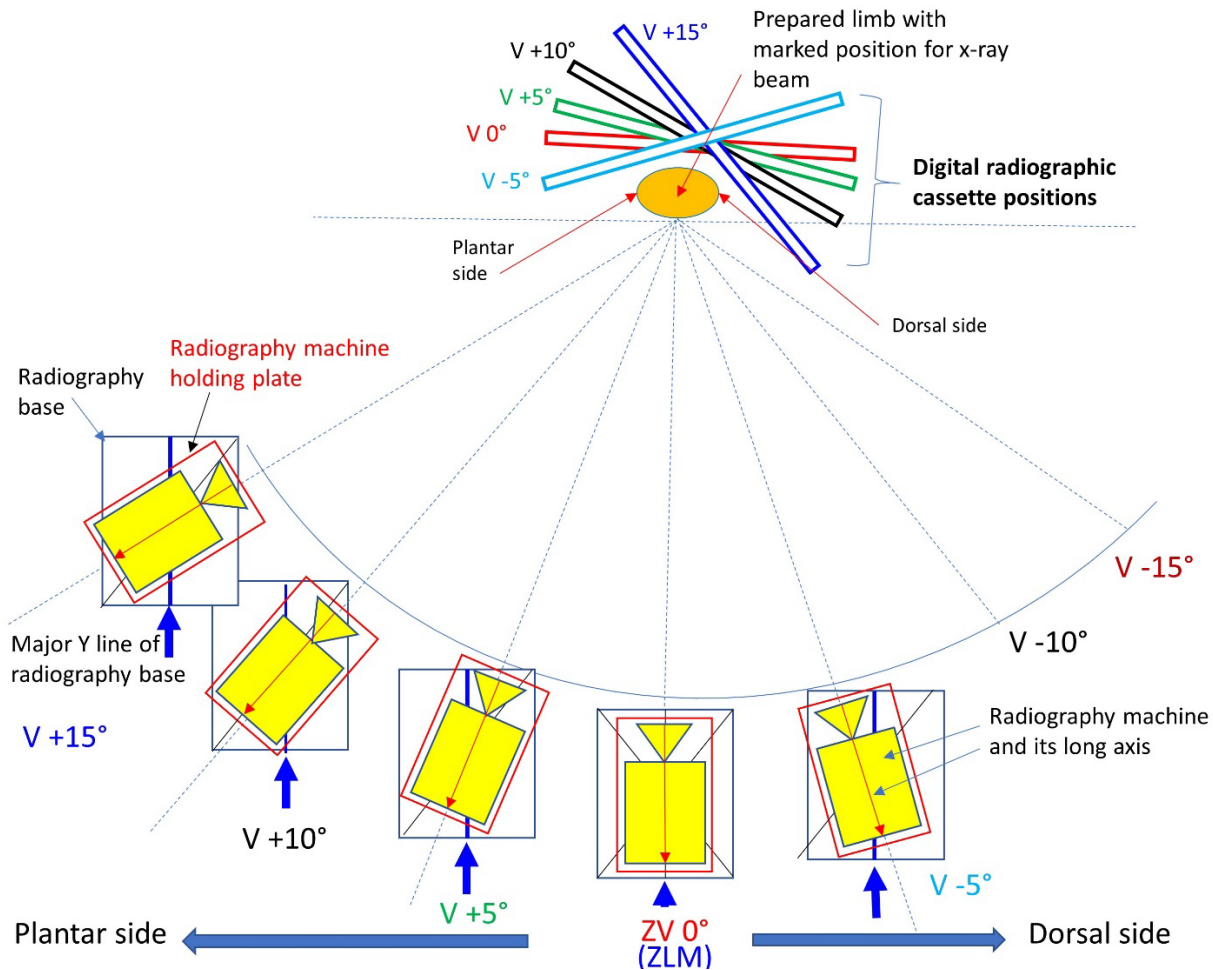


Figure 5. 2: The diagrammatic representation of rotational radiography of a right limb (tarsus) lateral surface with marked position of x-ray beam from the radiography machine. The rotations around the vertical axis of the radiography machine and positioning of the digital cassette during each vertical deviation of the radiography machine.

ZV 0°, zero vertical position represented the ZLM according to their specific landmarks and with perfect alignment (superimposition) of both long axes of the radiography machine and radiography base (Major Y lines). V +5°, V +10°, and V +15°, indicate rotations of the radiography apparatus around 5° towards the plantar side for each rotation and towards the dorsal side (for V -5°, V -10°, and V -15°). The digital cassette was positioned perpendicular accordingly in each deviation.

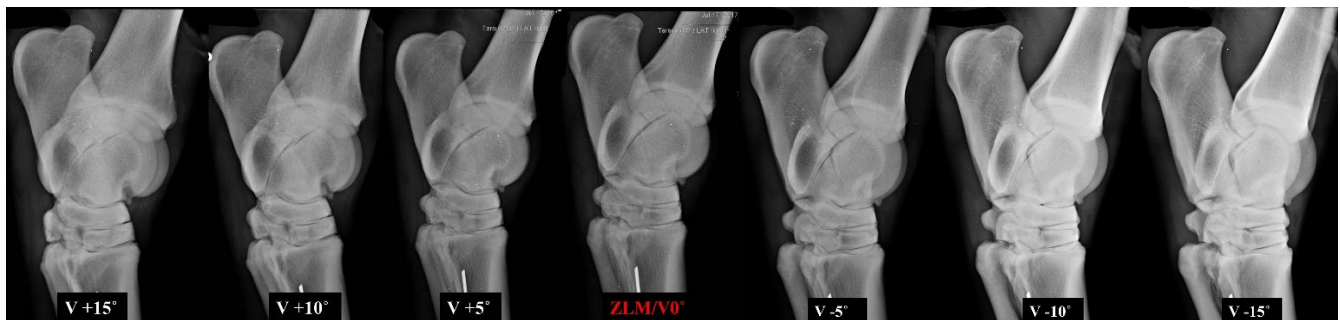


Figure 5. 3: Lateromedial radiographs of an equine right tarsus during vertical rotations from the ZLM. The three (left) radiographs showed the resultant distortions in the tarsal morphology due to the external rotation (moving around the plantar aspect) by +5°, +10° and +15° from the ZLM. The last three (right) radiographs showed the resultant distortions in the tarsal morphology due to the internal rotation (moving around the dorsal aspect) by -5°, -10° and -15°.

5.3.6.2 Radiographing the tarsus in rotation around the horizontal axis

In contrast to the radiography in the vertical rotation, the two transparent radiographic plates with a wooden wedge in between were (Figure 5.4) used for horizontal rotation of the radiography machine. All the movements were performed on radiographic plates by using the positioning wedges (Figure 5.4A and B). There were two directions of rotation, an upper and a lower horizontal rotation in respect to tarsus. In the upper horizontal rotation (H+), the two radiographic plates were without any positioning wedge but at the same height with the floor as when radiographing the ZLM.

The radiographic plate 1 was elevated from its posterior end using the positioning block (Figure 5.4A). This elevation was made in two steps. Each step made an angle of 5° of rotation around the horizontal axis of the radiography machine from 2 different blocks, one being 5 degrees and other one 10 degrees. The two steps were labelled as H+5° and H+10° along with the limb and horse ID. The height of the radiography machine was adjusted until the radiographic beam was positioned at the marked point in each step, so the radiographic beam remained at a constant position to the marked point on limb during each step of the horizontal rotation by moving the large stool up and down by using smaller stool. The small adjustments of height were done with help of wooden boxes. The distance between the cassette and the radiography machine head was between approximately under 100 cm during these horizontal rotations (Figure 5.5). In the lower horizontal rotation (H-), the radiographic sheet/plate 1 was elevated from its anterior end using the positioning block (Figure 5.4B). This elevation was made in two steps. Each step made an angle of 5° of rotation around the horizontal axis of the radiography machine. The radiographs were labelled as H-5° and H-10°.

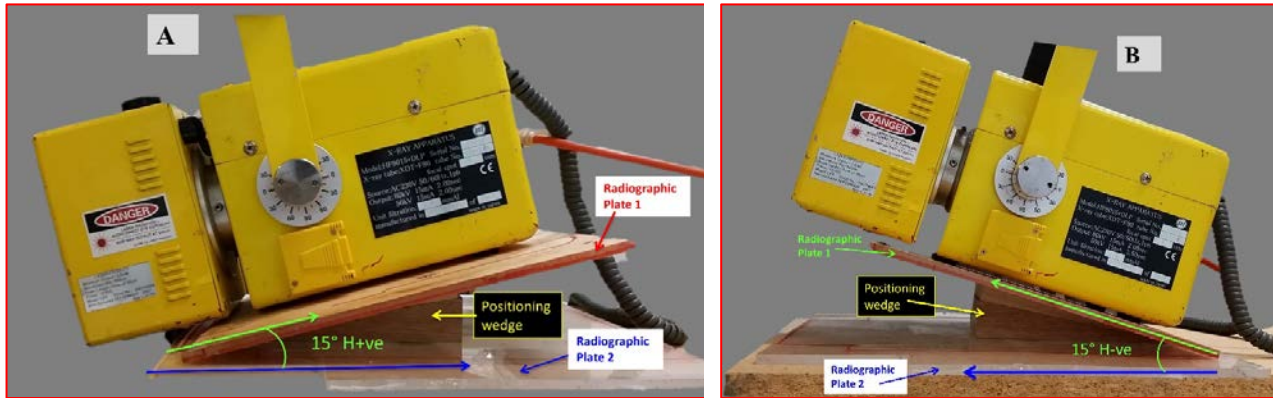


Figure 5. 4. Horizontal rotation of the radiography machine at H +15° and H -15° just for presentation purpose while H $\pm$ 10° rotations were used in current experiment (B) by using two radiographic plates with positioning wedges and height of this radiography apparatus was adjusted to keep the x-ray beam at a constant position in relation to the marked point on the limb.

The cassette holder was adjusted or tilted according to the resultant oblique position of the radiography machine created during this rotation in such a way that the surface which faced the radiography machine, was exactly perpendicular to the head of the radiography machine. The height of radiography machine was adjusted in each step, until the radiographic beam was positioned at the marked point during each step of horizontal rotation (Figure 5.5). The distance between the cassette and the radiography machine head was between approximately 90 cm to 100 cm during these horizontal rotations.

In the horizontal rotation each limb was radiographed 4 times after the initial ZLM radiograph (Figure 5.6). Two radiographs were in the upper rotation and the other two in the lower rotation. The total range of the horizontal rotation in each limb was 20° around the horizontal axis of the radiography machine.

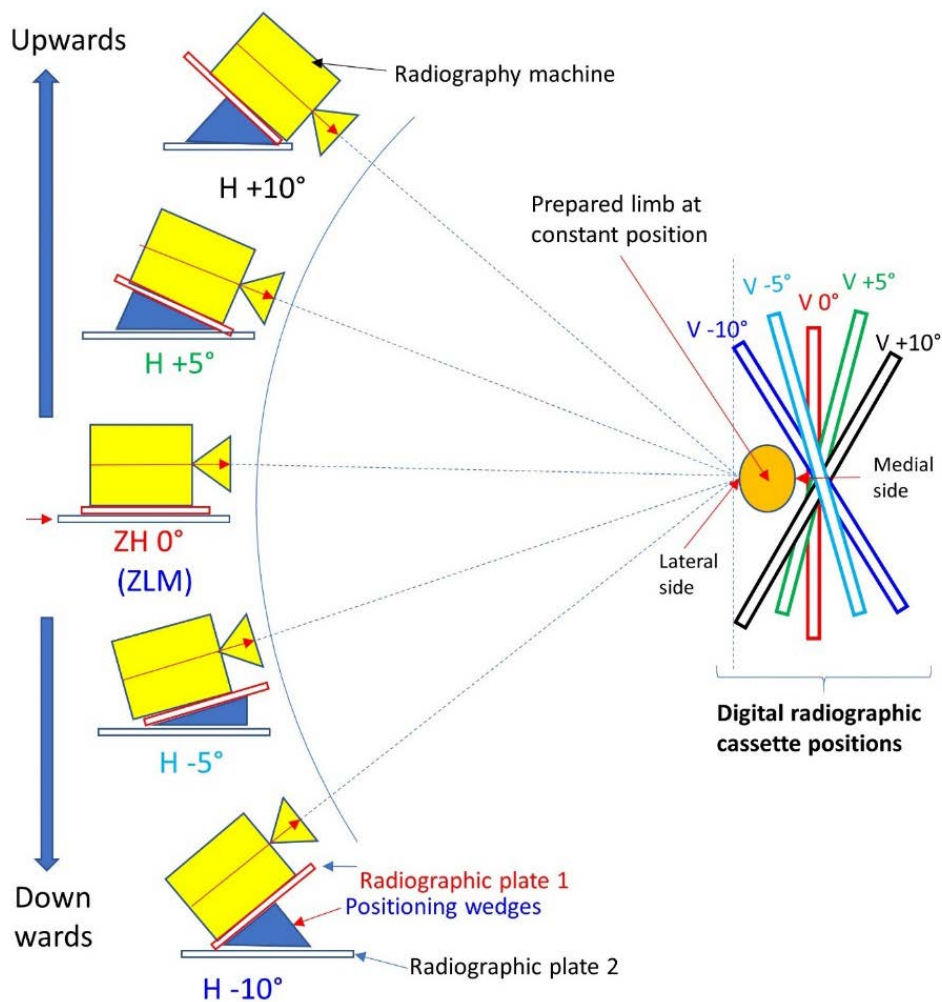


Figure 5. 5: Diagrammatic representation of rotations around the horizontal axis of the radiography machine and positioning of the digital cassette during each horizontal deviation of the radiography machine. $ZH 0^\circ$, (zero horizontal position) represented the ZLM according to specific landmarks and while keeping a perfect alignment (parallel) of both long axes of the radiography plate 2 and radiography machine.

$H +5^\circ$ and $H +10^\circ$, indicate rotations of the long axis of the radiography machine and plate 1 incrementally 5° upwards. Downwards (for $H -5^\circ$ and $H -10^\circ$) was achieved by positioning wedges in each deviation while the digital cassette was positioned perpendicular to the head of the radiography machine accordingly.



Figure 5. 6: Lateromedial radiographs of an equine right tarsus during horizontal rotations from the ZLM. The two (left) radiographs showed the resultant distortions in the tarsal morphology due to the upper rotation by +5° and +10° from the ZLM. The last two (right) radiographs showed the resultant distortions in the tarsal morphology due to the lower rotation by -5° and -10°.

5.3.6.2.1 Collecting and measuring the radiographs:

All cassettes were processed digitally, and all 12 radiographs of each tarsus were saved automatically to the DicomPACS®DX-R X-Ray acquisition software which was directly connected with the digital scanner of the digital radiography apparatus. All radiographs were labelled and saved. Adjustment of the contrast of radiographs was conducted whenever it was required. The software (mentioned above) was used to conduct all measurements. To decrease the risk of errors associated with fatigue, no more than 8 radiographs were measured in a day and all measurements of parameters were recorded on indexed paper sheets then typed into Microsoft Excel. All measurements in this experiment were conducted by the first author.

Tarsal Parameters

As described earlier in chapter 3 and published (Akbar, Al-Juhaishi, Olusa, & Davies, 2019)

1. *Distal intertarsal-Metatarsus joint angle/Individual angular parameter # 1 (IAP-1)*: this angle was measured at the intersection point between a line extending along the distal intertarsal joint and the reference line (RL, Figure 5.7A and B) which is the long axis of the third metatarsus (Mt3). The long axis of Mt3 was defined by taking the midpoints of its dorsoplantar diameter at 5 to 6 and 10 to 12 cm distal to the tarsometatarsal joint, then joining those two points with a straight line to represent the long axis of the third metatarsus (Figure 5.7A/1).
2. *Proximal intertarsal-Metatarsus joint angle/Individual angular parameter # 2 (IAP-2)*: this angle was at the intersection point between the RL and a line extending along the proximal intertarsal joint (Figure 5.7A/2).
3. *Dorso-distal edge of calcaneus- Talocalcaneal angle/ Individual angular parameter # 3 (IAP-3)*: this angle was at the intersection point between a line extending along the dorso-distal edge/incisura calcanei of the calcaneus and the proximal and distal end points of the curved talocalcaneal joint (Figure 5.7A/3).
4. *Sustentaculum tali of calcaneus-Metatarsus joint angle/ Individual angular parameter # 4 (IAP-4)*: this angle was at the intersection point between the RL and the long axis of sustentaculum tali of the calcaneus (St-Calc) which was found by joining two midpoints between the dorsal and plantaro-distal edges of St-Calc (Figure 5.7A/4).

5. *Distal intertarsal- tarsometatarsal joint/ Correlated angular parameter # 5 (CAP-5):*
this was the angle between a line perpendicular to the distal intertarsal joint and a line perpendicular to the best fit line of the tarsometatarsal joint (Figure 5.7B/5).
6. *Proximal intertarsal-distal intertarsal joint/ Correlated angular parameter # 6 (CAP-6):*
This was the angle between a line perpendicular to the proximal intertarsal joint and a line perpendicular to the distal surface of the central tarsal or the distal intertarsal joint (Figure 5.7B/6).
7. *Calcaneoquartal-Proximal intertarsal tarsal joint/ Correlated angular parameter # 7 (CAP-7):* This was the angle between a line perpendicular to the calcaneo-quartal joint and a line perpendicular to the distal surface of the talus or the proximal intertarsal joint (Figure 5.7B/7).
8. *Dorsal height of central tarsal/ dorsal height of 3rd tarsal ratio parameter (Ratio parameter # 8 (RP-8)):* The dorsal height of the central (Figure 5.7C/dHC) and third (Figure 5.7C/dH3rd) tarsal bones were extended from the most dorsal proximal extremity to their distal extremities.
9. *Length of sustentaculum tali/width of talus ratio (Ratio parameter # 9 (RP-9)):* The craniocaudal length of St-Calc was taken by extending the line joining the midpoints of the proximo-distal diameter measured near to the cranial and caudal ends of St- Calc (Figure 5.7C/L-stCalc). The width of the talus (Figure 4C/WT) was extended from the upper end of the proximal talocalcaneal joint at level of dorsodistal edge of calcaneus to the proximal end of the most dorsal part of the dorsal border of the lateral trochlea of talus.

10. *Width of calcaneus/ width of talus ratio (Ratio parameter # 10 (RP-10))*: The width of the calcaneus was taken from almost middle of anterior height and posterior heights extended from dorsal border to palmar border (Figure 5.7C/WCalc) to the dorsopalmar width of the talus as described above (Figure 5.7C/ WT).

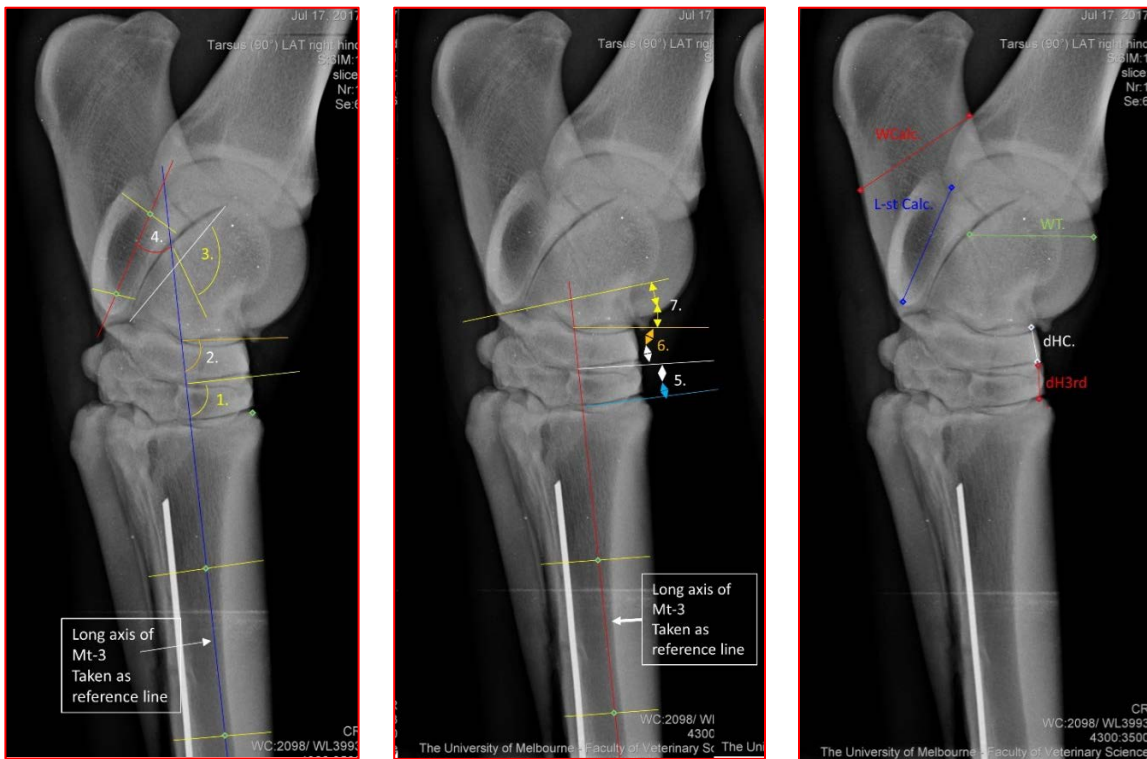


Figure 5. 7. LM radiographs of a right tarsus showing the lines used for ratios and angular parameters. RL: long axis of metatarsus (Mt-3) taken as the RL reference line.

1. Distal intertarsal-Metatarsus joint angle, 2. Proximal intertarsal-Metatarsus joint angle, 3. Dorso-distal TaloCalcaneal junction angle, 4. Sustentaculum tali (St.)-Metatarsus angle, 5. Distal intertarsal-Tarsometatarsus (DI-TMT) joint angle, 6. Proximal intertarsal-Distal intertarsal (PI-DI) joint angle, 7. Calcaneoquartal-Proximal intertarsal (CalQ-PI) tarsal joint, WCalc. Width of calcaneus, WT. Width of talus, L-St-Calc. Length of sustentaculum tali of calcaneus, dHC. Dorsal height of central tarsal, dH3rd. Dorsal height of third tarsal.

5.3.6.2.2 Statistical analysis:

The Normality of the differences between comparing angles was assessed by the Shapiro-Wilk test for Normality. A paired t-test was used to compare each angle of rotation (-15°, -10°, -5°, +5°, +10° and +15°) with the zero angle of vertical rotation for each parameter. This was done for both the vertical and the horizontal rotation (-10°, -5°, +5°, +10°). A two-tailed P-value < 0.05 was considered statistically significant. The IBM SPSS Statistics version 24 was used for analyses. A generalized linear mixed model, with random effects of horse and limb, and a fixed effect of vertical or horizontal angle of rotation, was used to model the outcome parameters. Angles of rotation had values of -15°, -10°, -5°, 0°, +5°, +10° and +15°. The significance of the slope was used to assess the linear association between the outcome and the angle. A 95% confidence interval was used and values where $P < 0.05$ was considered to be statistically significant. Left and right tarsi were regarded as independent observations and combined for the paired t-tests.

5.3.7 Acceptable range of rotation:

Identifying the acceptable range of rotation around the vertical and horizontal axes was based on the statistical results. The means of the amount of change of all angular, individual and correlated angles, and ratio parameters were measured in each rotational angle. The acceptable range of rotation at which these parameters expressed the lowest amount of change was identified.

5.4 RESULTS

The statistical analysis generally showed that most of the tarsal parameters were affected by vertical and horizontal rotations (Tables 5.1 and 5.2).

5.4.1 Effect of Vertical Rotation:

The majority of the angles were considerably affected by the vertical rotation (Table 5.1). However, the vertical rotation was not significantly affected in any rotational angle in the caudo-distal facet of calcaneo-4th tarsal-metatarsus joint angle, dorso-distal calc-metatarsus angle and the sustentaculum calcanei-metatarsus joint angle. The smallest changes were found in the caudo-distal facet of calcaneo-4th tarsal-metatarsus joint angle where the amount of change at the V+5° and V+15° was only -0.30 ± 1.86 and 0.64 ± 1.90 respectively. Whereas the parameter which experienced the largest changes in every rotational angle was the proximal intertarsal-metatarsus joint angle and dorso-distal calc-talocalcane junction angle. These changed from 90.71° at ZLM to reach a maximum change of 85.67° at V-15° and from 117.20° at ZLM to reach a maximum change to 125.82° at V-15° respectively (Figure 5.8). In respect to the increase or decrease of the angular values based on the direction of rotation, it can be seen generally that the values of most of the angles increased during the external rotation and decreased during the internal rotation.

5.4.2 Effect of Horizontal Rotation

The effect of horizontal rotation on the angles was variable among the parameters (Table 5.2). Although the rotation resulted in significant changes in most of the parameters, the changes in proximal intertarsal-metatarsus joint angle, dorso-distal calc-talocalcane junction angle, sustentaculum calcanei-metatarsus joint angle and calcq-pi joint angle were not significant in any rotational angle with a predicted change of just 0.02 ± 0.07 per 5 degrees. Whereas the ratio parameters such as l-st calc/wt and wcalc/wt ratio were not significant in any rotational angle with a predicted change of only -0.01 ± 0.07 per 5 degrees.

The most affected parameter in the study were the Proximal intertarsal-distal intertarsal joint angle with a maximum alteration of $3.71^\circ \pm 2.57$ resulting from a change from 175.33° at the ZLM to 171.61° at H10°. In contrast to the vertical rotation in which most of the parameters increased in one direction and decreased in the other direction of rotation, the parameters were influenced differently in the horizontal rotation. The parameters which showed no significant changes in all or some of the rotational angles in the upper and lower rotation, presented some consistency in their pattern of change (Figure 5.9). In all parameters the upper (+ve) rotations produced non-significant changes as compared to lower (-ve) rotations.

The quality of radiographs was progressively diminished as the radiographic positions were further away from the ZLM view. Changes in radiography machine positions, digital cassette rotations and horizontal beam rotations (HBR) had no effect on the quality of the radiographs. These rotational variations merely produced changes in the location of the tarsal image on the radiographic plate, minimal magnification of images and no distortion of images. Vertical beam

rotation (VBR) of the projection angle of the primary X-ray beam (-15° , -10° , -5° , $+5^{\circ}$, $+10^{\circ}$, $+15^{\circ}$) produced most of the significant differences between the measurement at ZLM and other rotational positions.

Table 5. 1: Comparison between parameter values in all upper (+) and lower (-) Vertical rotations in 15 tarsi. All angular measurements were in degrees.

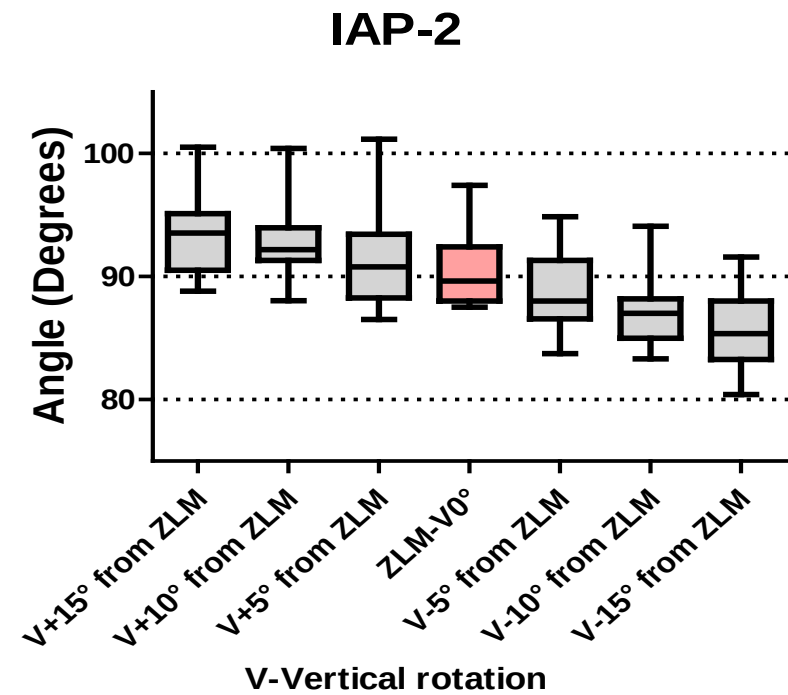
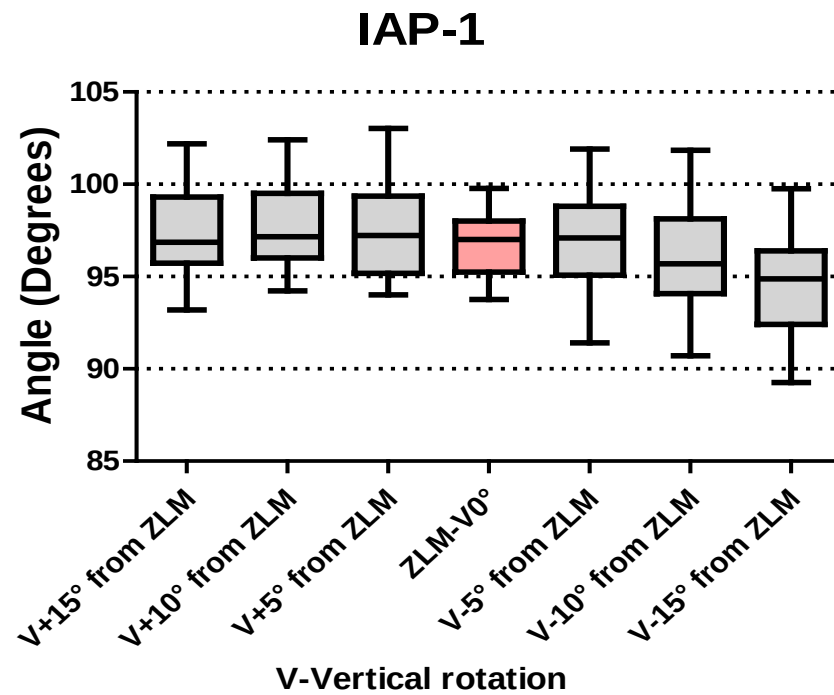
PARAMETERS	ROTATIONAL POSITIONS	PAIRED T-TEST						
		Means $\pm$ SD	Paired Mean Difference (ZLM-V0°-others) $\pm$ SD	Std. Error of Mean Difference	95 % CI of the Difference		Paired sample correlations (ZLM-others)	Sig. (2-tailed) p<0.05
					Lower	Upper		
IAP-1, LM. DISTAL INTERTARSAL-METATARSUS JOINT ANGLE	V+15° from ZLM	97.22 $\pm$ 2.58	-0.44 $\pm$ 1.66	0.43	-1.36	0.48	0.765	0.326
	V+10° from ZLM	97.80 $\pm$ 2.37	-1.02 $\pm$ 1.12	0.29	-1.59	-0.17	0.885	0.003*
	V+5° from ZLM	97.66 $\pm$ 2.68	-0.88 $\pm$ 1.28	0.33	-1.59	-0.17	0.898	0.019*
	ZLM-V0°	96.79 $\pm$ 1.91	0	0	0	0	0	0
	V-5° from ZLM	96.66 $\pm$ 3.05	0.13 $\pm$ 1.29	0.33	-0.59	0.84	0.968	0.709
	V-10° from ZLM	95.77 $\pm$ 3.10	1.01 $\pm$ 1.77	0.46	0.03	1.99	0.856	0.044*
	V-15° from ZLM	94.55 $\pm$ 2.93	2.24 $\pm$ 1.71	0.44	1.29	3.18	0.832	0.000**
IAP-2, LM. PROXIMAL INTERTARSAL-METATARSUS JOINT ANGLE	V+15° from ZLM	93.58 $\pm$ 3.57	-2.86 $\pm$ 1.98	0.51	-3.96	-1.77	0.845	0.000**
	V+10° from ZLM	93.05 $\pm$ 3.30	-2.34 $\pm$ 1.18	0.30	-2.99	-1.68	0.943	0.000**
	V+5° from ZLM	91.38 $\pm$ 4.13	-0.67 $\pm$ 1.79	0.46	-1.66	0.32	0.903	0.170
	ZLM-V0°	90.44 $\pm$ 3.95	0	0	0	0	0	0
	V-5° from ZLM	88.70 $\pm$ 3.39	2.01 $\pm$ 1.66	0.43	1.09	2.93	0.887	0.000**
	V-10° from ZLM	87.06 $\pm$ 3.07	3.65 $\pm$ 1.87	0.48	2.61	4.69	0.849	0.000**
	V-15° from ZLM	85.67 $\pm$ 3.31	5.04 $\pm$ 1.79	0.46	4.05	6.03	0.867	0.000**
IAP-3, LM.	V+15° from ZLM	111.72 $\pm$ 2.29	5.48 $\pm$ 3.15	0.81	3.74	7.23	0.774	0.000**

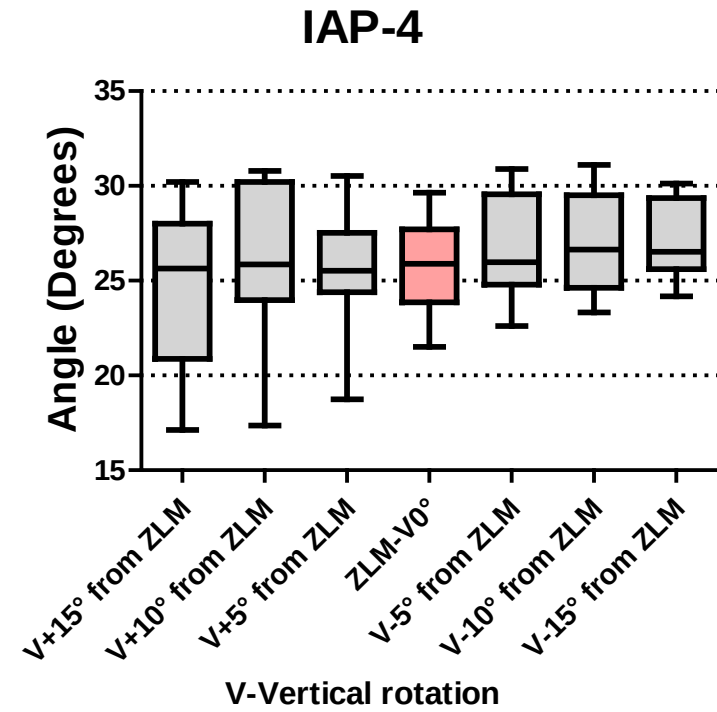
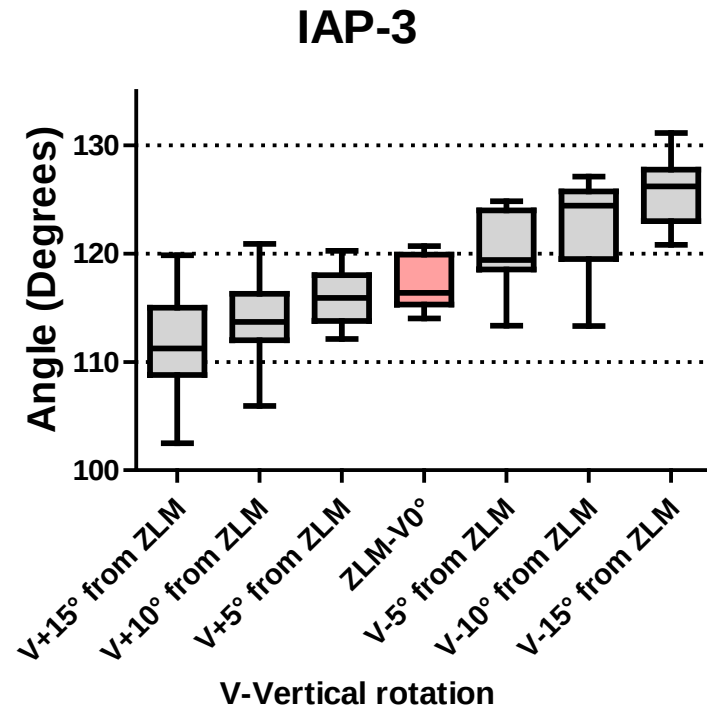
DORSO-DISTAL CALC- TALOCALCANE JUNCTION ANGLE	V+10° from ZLM	114.08 ± 3.40	3.12 ± 2.62	0.68	1.67	4.57	0.640	0.000**
	V+5° from ZLM	115.97 ± 2.39	1.23 ± 2.21	0.57	0.1	2.45	0.555	0.049*
	ZLM-V0°	117.20 ± 2.50	0	0	0	0	0	0
	V-5° from ZLM	120.21 ± 3.29	-3.01 ± 2.99	0.77	-4.67	-1.36	0.472	0.002*
	V-10° from ZLM	122.77 ± 4.08	-5.57 ± 4.19	1.08	-7.89	-3.25	0.235	0.000**
	V-15° from ZLM	125.82 ± 3.02	-8.61 ± 3.43	0.89	-10.52	-6.71	0.185	0.000**
IAP-4, LM. SUSTENTACULUM CALACANI- METATARSUS JOINT ANGLE	V+15° from ZLM	24.80 ± 4.24	1.04 ± 2.98	0.77	-0.61	2.70	0.726	0.196
	V+10° from ZLM	25.78 ± 3.94	0.06 ± 2.33	0.60	-1.23	1.35	0.830	0.920
	V+5° from ZLM	25.66 ± 3.28	0.18 ± 1.77	0.46	-0.80	1.17	0.845	0.694
	ZLM-V0°	25.85 ± 2.42	0	0	0	0	0	0
	V-5° from ZLM	26.49 ± 2.48	-0.64 ± 2.27	0.59	-1.90	0.62	0.583	0.294
	V-10° from ZLM	27.14 ± 2.64	-1.29 ± 1.92	0.49	-2.36	-0.23	0.719	0.021*
	V-15° from ZLM	27.18 ± 2.07	-1.33 ± 2.66	0.69	-2.81	0.14	0.334	0.073
DI-TMT JOINT ANGLE (CAP-5)	V+15° from ZLM	181.12 ± 2.02	-3.31 ± 1.89	0.49	-4.36	-2.27	0.600	0.000**
	V+10° from ZLM	179.82 ± 2.43	-2.01 ± 2.83	0.62	-3.32	-0.69	0.471	0.006*
	V+5° from ZLM	178.81 ± 1.95	-1.01 ± 1.55	0.40	-1.87	-0.15	0.723	0.025
	ZLM-V0°	177.81 ± 2.18	0	0	0	0	0	0
	V-5° from ZLM	177.41 ± 2.54	0.39 ± 1.33	0.34	-0.34	1.14	0.850	0.270
	V-10° from ZLM	176.83 ± 2.84	0.98 ± 1.86	0.48	-0.06	2.01	0.756	0.062
	V-15° from ZLM	176.30 ± 3.25	1.51 ± 2.89	0.75	-0.09	3.11	0.492	0.062
PI-DI JOINT ANGLE (CAP-6)	V+15° from ZLM	176.09 ± 1.73	-0.76 ± 1.61	0.42	-1.66	0.13	0.687	0.088
	V+10° from ZLM	175.91 ± 1.68	-0.58 ± 1.51	0.39	-1.41	0.25	0.730	0.157

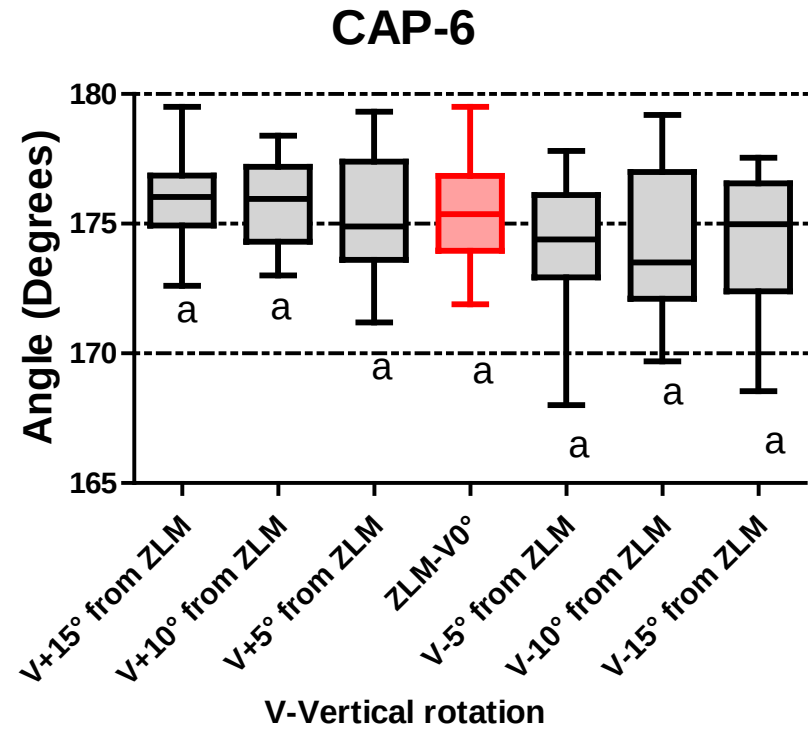
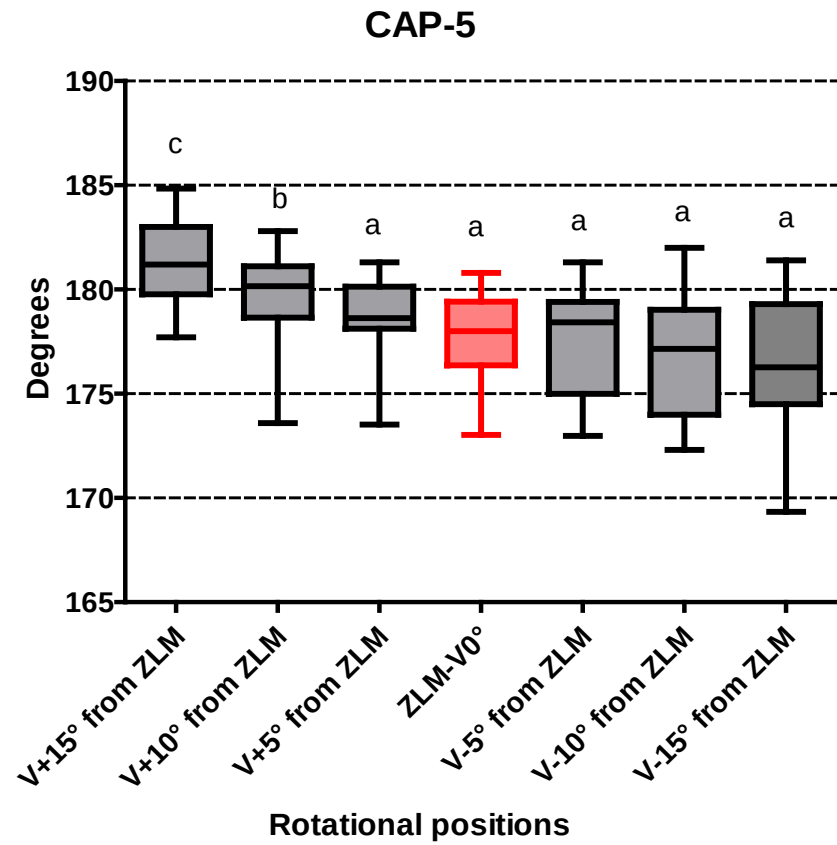
	V+5° from ZLM	175.24 ± 2.20	0.08 ± 1.30	0.34	-0.64	0.80	0.826	0.806
	ZLM-V0°	175.33 ± 2.20	0	0	0	0	0	0
	V-5° from ZLM	174.21 ± 2.61	1.12 ± 1.58	0.41	0.25	2.00	0.796	0.016
	V-10° from ZLM	174.09 ± 2.99	1.24 ± 1.74	0.45	0.27	2.20	0.817	0.015
	V-15° from ZLM	174.43 ± 2.59	0.89 ± 2.42	0.62	-0.45	2.23	0.500	0.174
CALCQ-PI JOINT ANGLE (CAP-7)	V+15° from ZLM	184.27 ± 9.14	1.66 ± 5.63	1.45	-1.46	4.78	0.807	0.273
	V+10° from ZLM	182.45 ± 9.33	3.48 ± 6.19	1.59	0.05	6.91	0.762	0.047
	V+5° from ZLM	184.05 ± 8.03	1.88 ± 4.63	1.19	-0.68	4.44	0.825	0.137
	ZLM-V0°	185.60 ± 5.43	0	0	0	0	0	0
	V-5° from ZLM	186.53 ± 6.33	-0.60 ± 3.99	1.03	-2.82	1.61	0.785	0.569
	V-10° from ZLM	189.13 ± 7.14	-3.19 ± 6.88	1.78	-7.01	0.61	0.445	0.094
	V-15° from ZLM	189.13 ± 6.77	-3.19 ± 4.49	1.16	-5.68	-0.70	0.753	0.016
DHC/DH3RD RATIO (RP-8)	V+15° from ZLM	0.93 ± 0.08	0.02 ± 0.10	0.03	-0.03	0.08	0.494	0.435
	V+10° from ZLM	0.93 ± 0.10	0.01 ± 0.09	0.02	-0.04	0.06	0.604	0.630
	V+5° from ZLM	0.93 ± 0.10	0.02 ± 0.08	0.02	-0.03	0.06	0.721	0.467
	ZLM-V0°	0.94 ± 0.11	0	0	0	0	0	0
	V-5° from ZLM	0.96 ± 0.12	-0.01 ± 0.08	0.02	-0.05	0.03	0.795	0.594
	V-10° from ZLM	1.01 ± 0.13	-0.06 ± 0.10	0.03	-0.12	-0.01	0.635	0.032
	V-15° from ZLM	1.04 ± 0.11	-0.10 ± 0.11	0.03	-0.16	-0.03	0.508	0.005*
L-ST CALC/WT (RP-9)	V+15° from ZLM	1.10 ± 0.06	-0.14 ± 0.05	0.01	-0.17	-0.11	0.564	0.000**
	V+10° from ZLM	1.03 ± 0.05	-0.08 ± 0.4	0.01	-0.10	-0.05	0.639	0.000**
	V+5° from ZLM	0.98 ± 0.04	-0.03 ± 0.03	0.01	-0.04	-0.01	0.757	0.007*

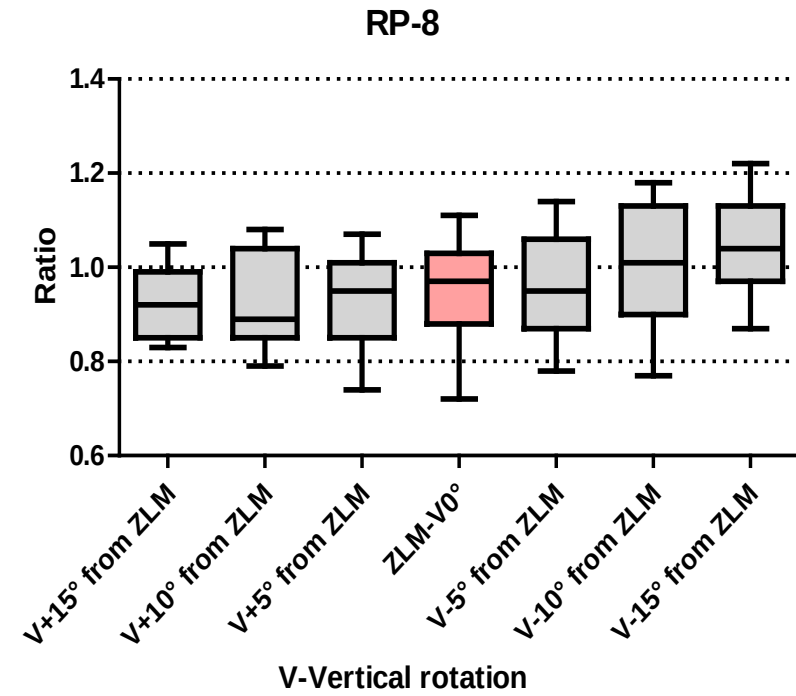
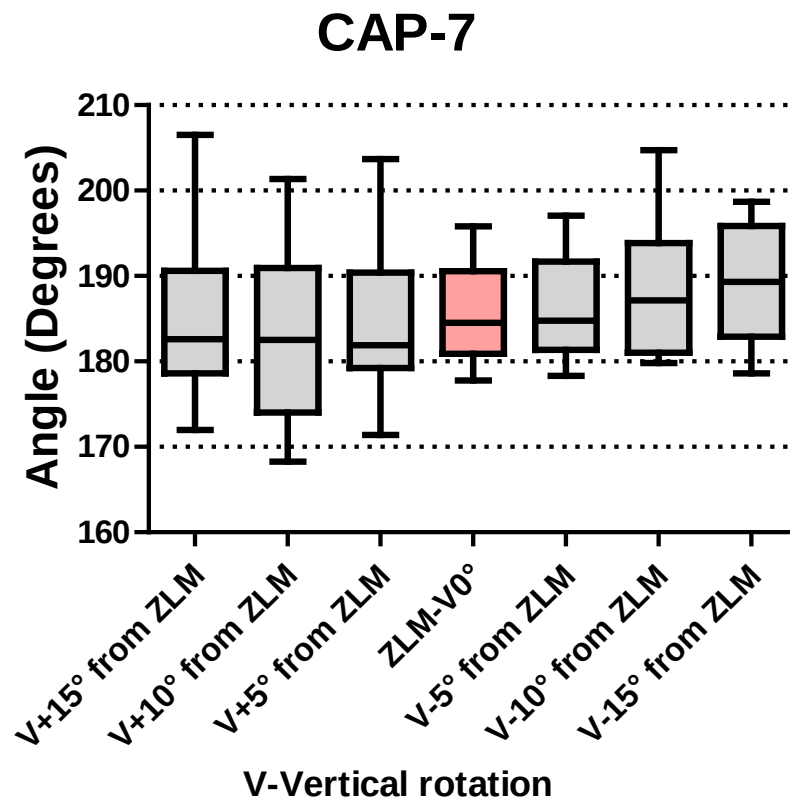
	ZLM-V0°	0.96 ± 0.05	0	0	0	0	0	0
	V-5° from ZLM	0.95 ± 0.04	0.01 ± 0.02	0.01	-0.01	0.02	0.841	0.319
	V-10° from ZLM	0.93 ± 0.03	0.02 ± 0.04	0.01	0.00	0.04	0.639	0.018
	V-15° from ZLM	0.93 ± 0.04	0.03 ± 0.04	0.01	0.01	0.05	0.605	0.010
WCALC/WT RATIO (RP-10)	V+15° from ZLM	1.13 ± 0.07	-0.11 ± 0.04	0.01	-0.13	-0.09	0.784	0.000**
	V+10° from ZLM	1.08 ± 0.06	-0.05 ± 0.03	0.01	-0.07	-0.04	0.854	0.000**
	V+5° from ZLM	1.04 ± 0.05	-0.01 ± 0.02	0.01	-0.03	0.00	0.883	0.053
	ZLM-V0°	1.02 ± 0.05	0	0	0	0	0	0
	V-5° from ZLM	1.01 ± 0.04	0.01 ± 0.03	0.01	-0.01	0.03	0.781	0.177
	V-10° from ZLM	1.00 ± 0.04	0.02 ± 0.03	0.01	0.00	0.04	0.804	0.016
	V-15° from ZLM	0.99 ± 0.04	0.04 ± 0.03	0.01	0.02	0.06	0.707	0.001*

Figure 5. 8: Mean box plots of selected parameters for vertical rotations.









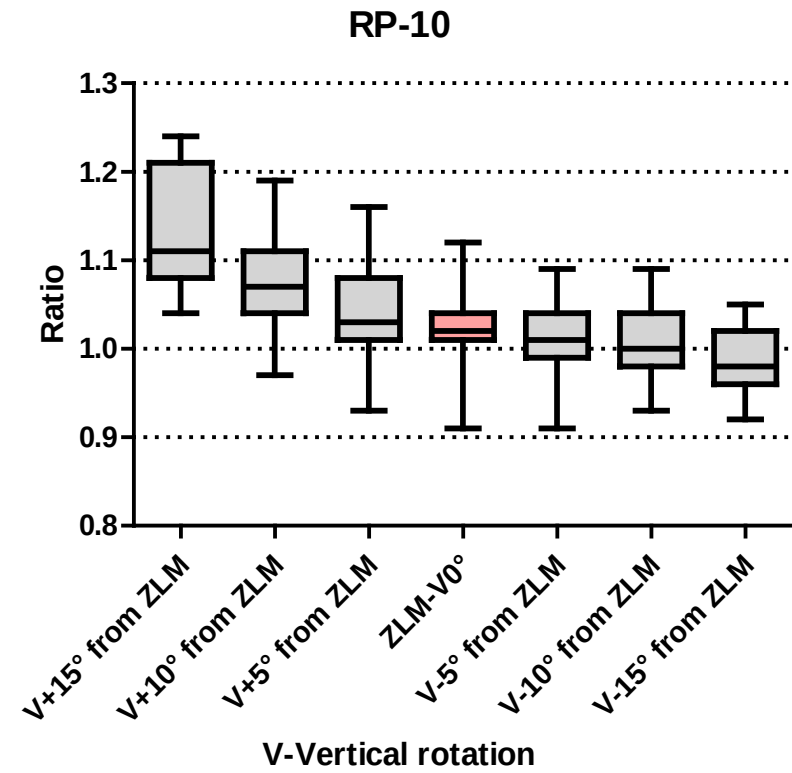
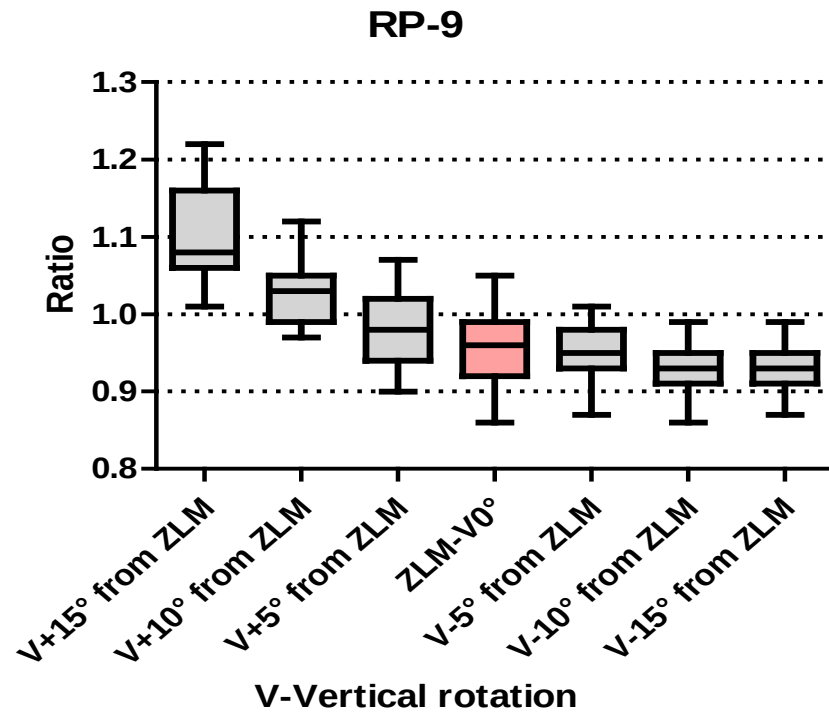


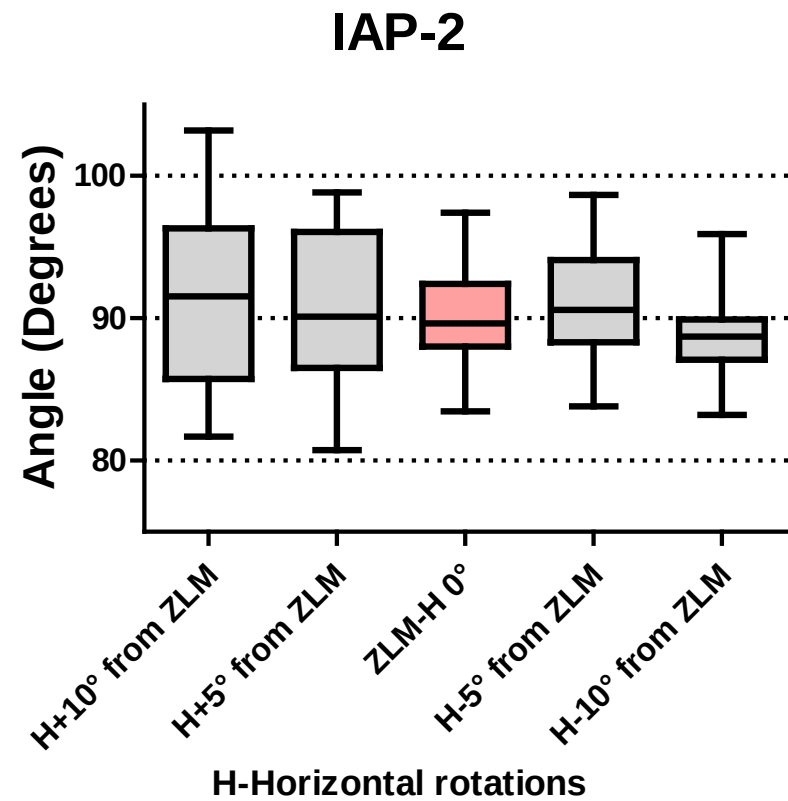
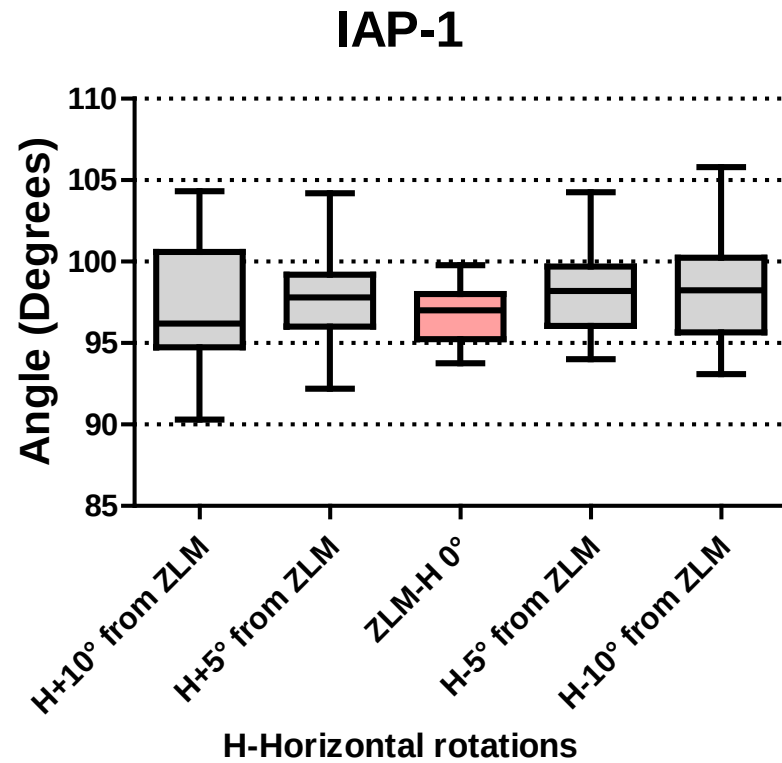
Table 5. 2: Comparison between parameter values in all upper (+) and lower (-) horizontal rotations in 15 tarsi. All angular measurements were in degrees.

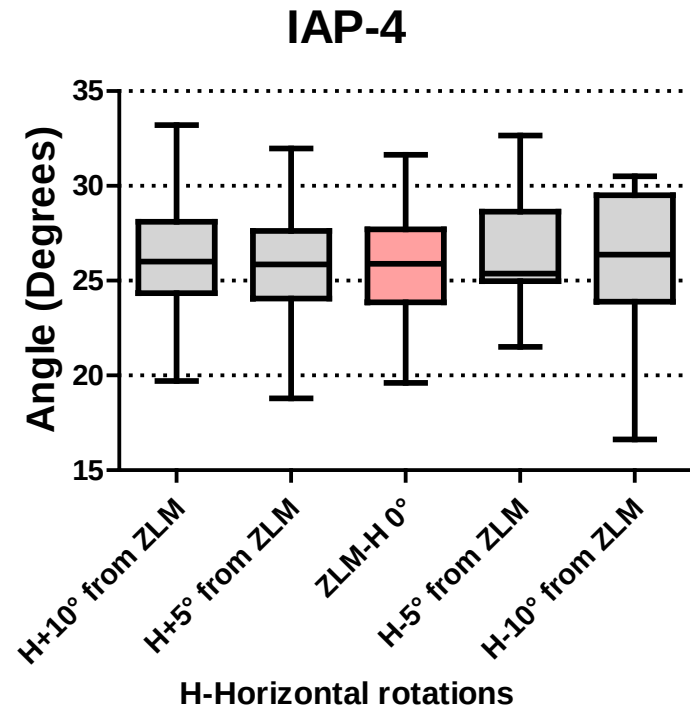
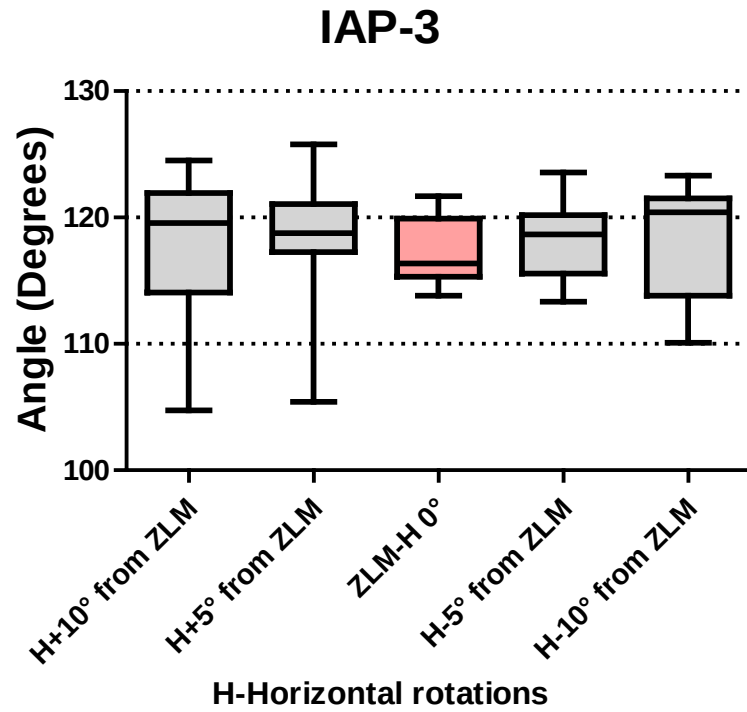
PARAMETERS	ROTATIONAL POSITIONS	PAIRED T-TEST						
		Means $\pm$ SD	Paired Mean Difference (ZLM-H0°-others) $\pm$ SD	Std. Error of Mean Difference	95 % CI of the Difference		Paired sample correlations (ZLM-others)	Sig. (2-tailed) p<0.05
					Lower	Upper		
IAP-1, LM. DISTAL INTERTARSAL-METATARSUS JOINT ANGLE	H+10° from ZLM	96.88 $\pm$ 4.17	-0.09 $\pm$ 3.06	0.79	-1.79	1.60	0.734	0.907
	H+5° from ZLM	97.55 $\pm$ 2.90	-0.76 $\pm$ 1.59	0.41	-1.64	0.11	0.862	0.084
	ZLM-H0°	96.79 $\pm$ 1.90	0	0	0	0	0	0
	H-5° from ZLM	98.13 $\pm$ 2.82	-1.34 $\pm$ 1.57	0.41	-2.21	-0.47	0.847	0.005*
	H-10° from ZLM	98.59 $\pm$ 3.51	-1.81 $\pm$ 2.15	0.55	-2.99	-0.62	0.847	0.006*
IAP-2, LM. PROXIMAL INTERTARSAL-METATARSUS JOINT ANGLE	H+10° from ZLM	91.02 $\pm$ 6.12	-0.58 $\pm$ 3.55	0.92	-2.55	1.38	0.837	0.535
	H+5° from ZLM	91.13 $\pm$ 5.37	-0.69 $\pm$ 2.39	0.62	-2.02	0.64	0.912	0.284
	ZLM-H0°	90.44 $\pm$ 3.95	0	0	0	0	0	0
	H-5° from ZLM	90.89 $\pm$ 4.20	-0.45 $\pm$ 2.35	0.61	-1.75	0.85	0.835	0.472
	H-10° from ZLM	89.03 $\pm$ 3.43	1.41 $\pm$ 2.77	0.71	-0.12	2.94	0.727	0.069
IAP-3, LM. DORSO-DISTAL CALC-TALOCALCANE JUNCTION ANGLE	H+10° from ZLM	118.25 $\pm$ 5.15	-1.05 $\pm$ 4.92	1.28	-3.79	1.69	0.322	0.425
	H+5° from ZLM	117.86 $\pm$ 5.35	-0.66 $\pm$ 4.49	1.16	-3.15	1.83	0.548	0.578
	ZLM-H0°	117.20 $\pm$ 2.50	0	0	0	0	0	0
	H-5° from ZLM	118.21 $\pm$ 3.13	-1.01 $\pm$ 3.87	0.99	-3.15	1.13	0.069	0.328

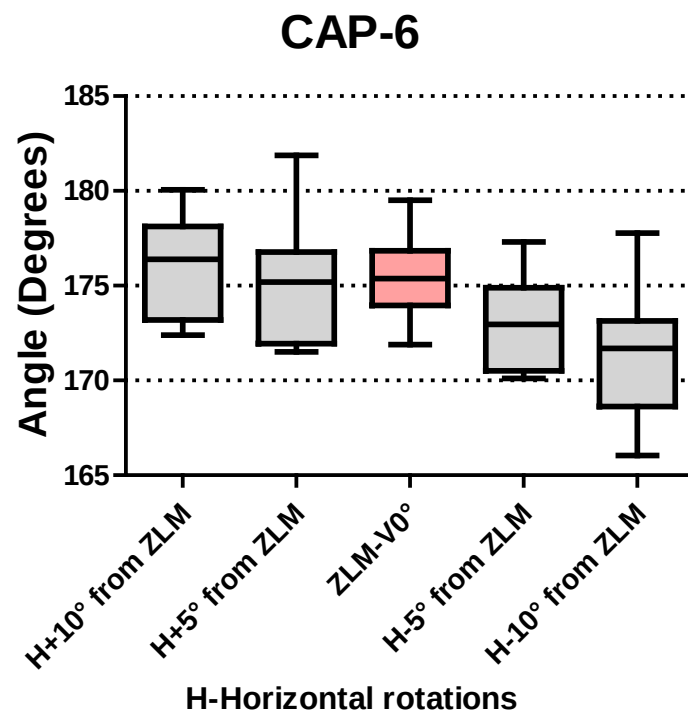
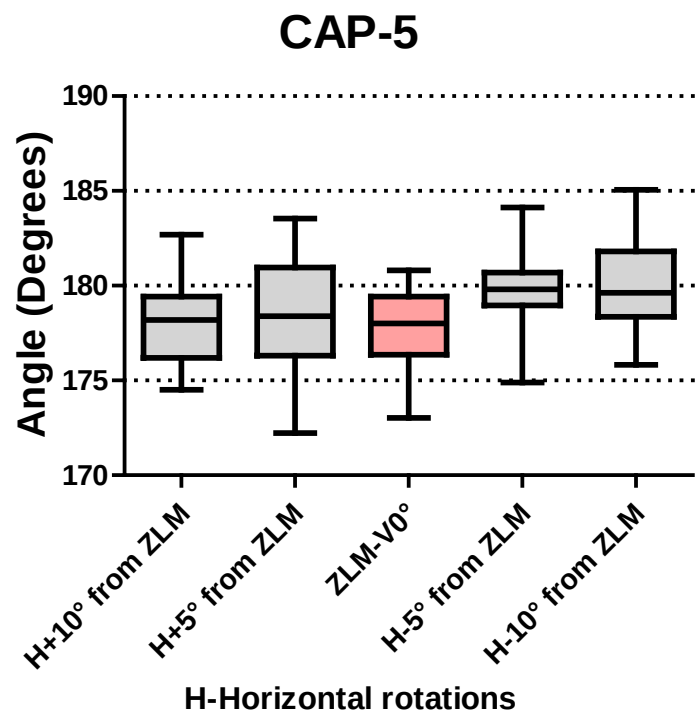
	H-10° from ZLM	117.81 ± 4.73	-0.61 ± 5.30	1.37	-3.55	2.33	0.021	0.663
IAP-4, LM. SUSTENTACULUM CALACANI- METATARSUS JOINT ANGLE	H+10° from ZLM	26.56 ± 3.54	-0.72 ± 2.34	0.60	-2.01	0.58	0.757	0.256
	H+5° from ZLM	25.66 ± 3.42	0.19 ± 1.44	0.37	-0.61	0.98	0.908	0.617
	ZLM-H0°	25.85 ± 3.42	0	0	0	0	0	0
	H-5° from ZLM	26.63 ± 2.79	-0.78 ± 1.91	0.49	-1.84	0.27	0.786	0.135
	H-10° from ZLM	26.02 ± 3.63	-0.17 ± 3.39	0.87	-2.05	1.70	0.490	0.845
DI-TMT JOINT ANGLE (CAP-5)	H+10° from ZLM	178.15 ± 2.39	-0.35 ± 1.66	0.42	-1.27	0.57	0.741	0.427
	H+5° from ZLM	177.94 ± 3.39	-0.13 ± 2.23	0.57	-1.37	1.11	0.761	0.825
	ZLM-H0°	177.80 ± 2.18	0	0	0	0	0	0
	H-5° from ZLM	179.64 ± 2.50	-1.83 ± 2.26	0.58	-3.09	-0.58	0.541	0.007*
	H-10° from ZLM	179.98 ± 2.71	-2.17 ± 1.89	0.49	-1.12	-1.12	0.719	0.001**
PI-DI JOINT ANGLE (CAP-6)	H+10° from ZLM	175.96 ± 2.41	-0.63 ± 1.54	0.39	-1.47	0.22	0.781	0.137
	H+5° from ZLM	175.11 ± 3.14	0.21 ± 2.38	0.61	-1.11	1.53	0.653	0.736
	ZLM-H0°	175.33 ± 2.20	0	0	0	0	0	0
	H-5° from ZLM	172.84 ± 84	2.49 ± 2.07	0.53	1.34	3.63	0.580	0.000**
	H-10° from ZLM	171.61 ± 3.24	3.71 ± 2.57	0.66	2.28	5.13	0.612	0.000**
CALCQ-PI JOINT ANGLE (CAP-7)	H+10° from ZLM	184.40 ± 10.03	1.19 ± 6.39	1.65	-2.34	4.73	0.819	0.481
	H+5° from ZLM	184.09 ± 7.09	1.51 ± 4.56	1.18	-1.02	4.03	0.766	0.222
	ZLM-H0°	185.60 ± 5.43	0	0	0	0	0	0

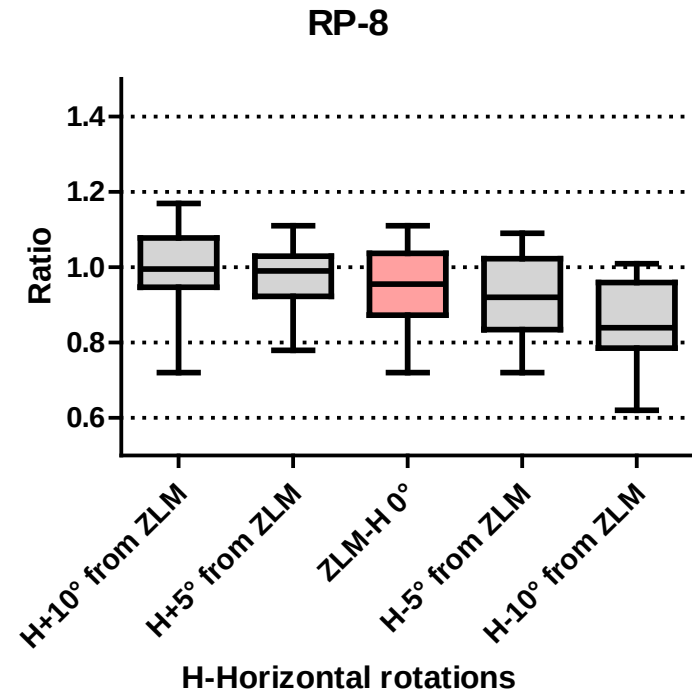
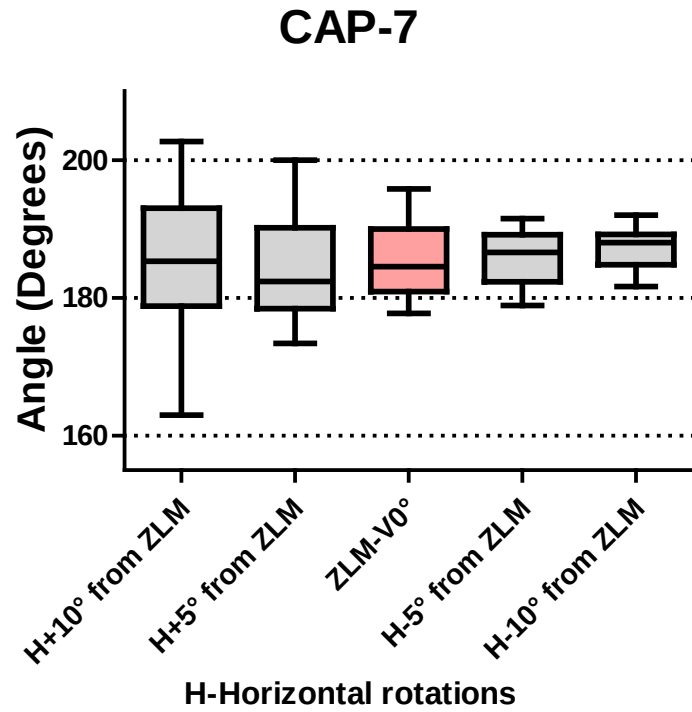
DHC/DH3RD RATIO (RP-8)	H-5° from ZLM	185.90 ± 3.96	-0.31 ± 3.50	0.91	-2.24	1.64	0.764	0.740
	H-10° from ZLM	187.28 ± 3.30	-1.68 ± 4.50	1.16	-4.17	0.81	0.561	0.170
	H+10° from ZLM	0.99 ± 0.12	-0.05 ± 0.09	0.02	-0.10	0.01	0.681	0.074
	H+5° from ZLM	0.97 ± 0.09	-0.03 ± 0.05	.01	-0.06	0.00	0.889	0.083
	ZLM-H0°	0.94 ± 0.11	0	0	0	0	0	0
	H-5° from ZLM	0.92 ± 0.12	0.03 ± 0.51	0.01	-0.00	0.05	0.906	0.067
	H-10° from ZLM	0.84 ± 0.11	0.10 ± 0.06	0.02	0.06	0.13	0.836	0.000**
L-ST CALC/WT (RP-9)	H+10° from ZLM	0.93 ± 0.04	0.03 ± 0.06	0.02	-0.01	0.06	0.028	0.115
	H+5° from ZLM	0.94 ± 0.03	0.02 ± 0.06	0.02	-0.02	0.05	-0.046	0.251
	ZLM-H0°	0.96 ± 0.05	0	0	0	0	0	0
	H-5° from ZLM	0.97 ± 0.03	-0.01 ± 0.06	0.02	-0.05	0.02	-0.193	0.533
	H-10° from ZLM	0.97 ± 0.03	-0.01 ± 0.06	0.02	-0.04	0.02	0.035	0.496
WCALC/WT RATIO (RP-10)	H+10° from ZLM	1.00 ± 0.06	0.02 ± 0.08	0.02	-0.02	0.07	-0.071	0.268
	H+5° from ZLM	1.00 ± 0.05	0.02 ± 0.07	0.02	-0.02	0.06	-0.041	0.281
	ZLM-H0°	1.02 ± 0.05	0	0	0	0	0	0
	H-5° from ZLM	1.02 ± 0.05	0.00 ± 0.07	0.02	-0.04	0.05	-0.140	0.866
	H-10° from ZLM	1.02 ± 0.05	0.00 ± 0.07	0.02	-0.04	0.04	0.062	0.969

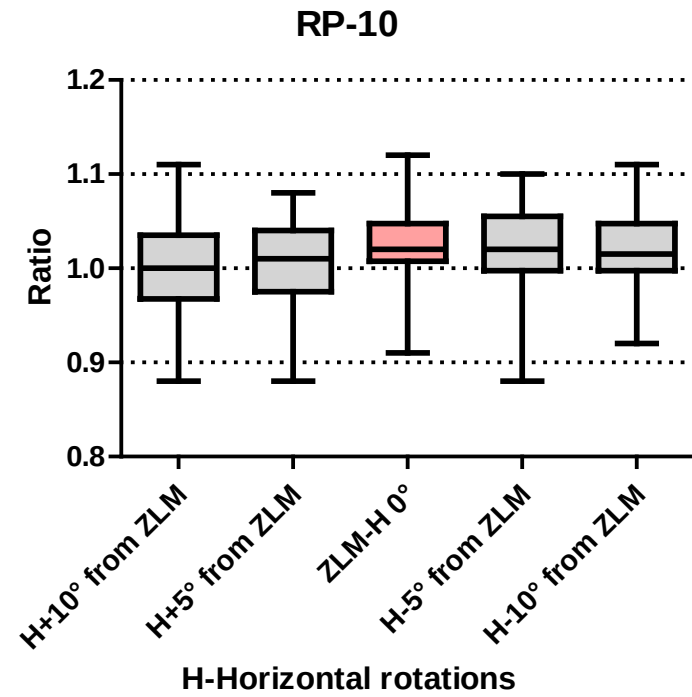
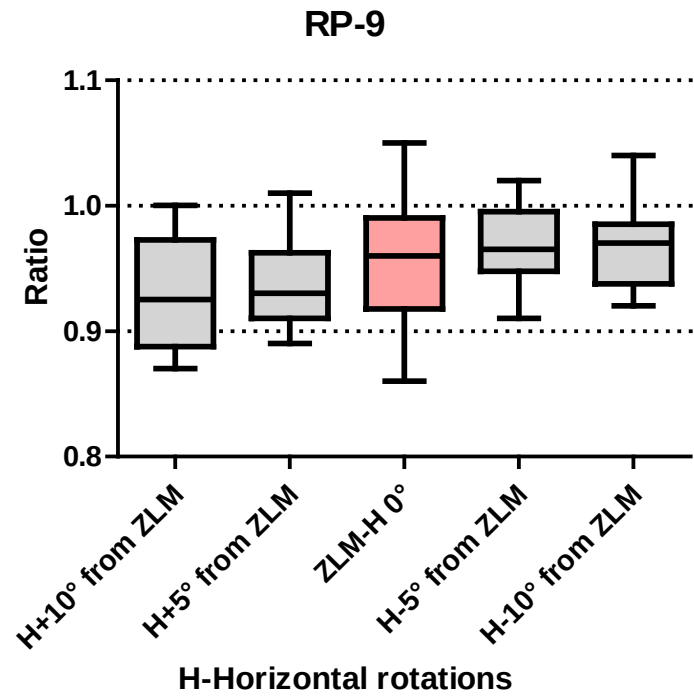
Figure 5. 9: Mean box plots of selected parameters for horizontal rotations.











5.4.2.1 Acceptable Range of Rotation around the Vertical and Horizontal Axes

According to the statistical analysis of the parameters in each rotational angle of the vertical and horizontal rotation, the tarsal parameters were not more affected by the horizontal rotation than by the vertical rotation. In the vertical rotation, there was difference between the changes associated with the external and the internal rotations. The overall limb rotation at rotational angles of $V\pm5^\circ$, $V\pm10^\circ$ and $V\pm15^\circ$ resulted in approximately 1.94° change in the parameters.

The limb rotation at rotational angles of $V\pm5^\circ$ resulted in approximately 0.9° change in the parameters. This was the smallest change in comparison with the rotational angles of $V\pm10^\circ$ and $V\pm15^\circ$ which presented changes of approximately 2.1° and 2.8° respectively. The lower horizontal rotation resulted in bigger changes in all the rotational angles than in the upper horizontal rotation. The rotational angles of $H+5^\circ$ and $H-5^\circ$ presented the minimum changes with changes of 0.77° . Whereas the other rotational angles showed a relatively large amount of change especially at $H-10^\circ$.

5.5 DISCUSSION

Effects of inconsistency in radiographing the equine tarsus in the lateromedial view have not been reported in detail. The present study was not only designed to evaluate the effect of limb or radiography machine rotation during radiography on previously published tarsal parameters (Akbar et al., 2019) but also to find the acceptable range of rotation where these tarsal measurements might be relied on. The statistical analysis in the study found that all the parameters were significantly affected at all or some of the rotational angles of the vertical and/or horizontal rotations. The landmarks of tarsal parameters were identified on the digital radiographs and each pair of landmarks was connected dorsoplantarly to represent horizontal anatomical postures of their articular surfaces or bone surfaces.

In most of cephalometric studies, it has been shown that rotation around the vertical axis can affect the horizontal parameters while rotation around the horizontal axis affected the vertical parameters/measurements (Malkoc et al., 2005). The relationship between landmarks were found to be altered during the rotation of a subject which can cause changes in their represented parameters (Yoon et al., 2002). In the current study however, both vertical and horizontal rotations resulted in a significant effect on most of the angles. Therefore, it is necessary to consider a number of possible factors which might cause any disagreement. The results did not reveal a clear relationship between the value of angles and the amount of change. In the vertical rotation for example the caudo-distal facet of calcaneo-4th tarsal-metatarsus joint angle had a mean of 84.07° and changed by an average of -0.46 ± 2.63 per 5° of rotation. Whereas the calcaneo-metatarsus joint angle which had a different mean of 165.65° , changed by an average of $-0.5^\circ \pm 0.83$ per 5° of rotation. In these

examples the two angles had very different means but their response to the rotation seems to be identical.

In another example, the amount of change appears to be dependent on the inclination of the correlated surfaces regardless of the values of the angles. Importantly, it was noted that when an angle was formed by articular surfaces oriented almost parallel to the rotational axis, it would produce the minimum distortion as a result of rotation. For instance, the PI-DI Joint angle was formed between the distal articular surface of the talus and the proximal articular surface of the third tarsal bone that were both almost parallel to the tarsal axis. This angle had a mean of 175.33° and showed the lowest amount of change in the study with an average of $0.77^\circ \pm 1.69$ per 5° of vertical rotation and similarly the Calcq-PI joint angle changed minimally in the horizontal rotation. Hence, if angles which were closer to 180° at ZLM in the tarsal parameters had less change suggest being cautious regarding their applicable suitability unless their articular surfaces are anatomically parallel to the rotational axis.

The vertical and horizontal rotations can affect the values of each parameter in respect to increase and decrease during each rotation and there were some variations between the parameters. Some parameters always increased or decreased throughout the rotation, while others increased and decreased in only one direction of rotation and were not affected in the opposite direction. In the vertical rotations, most of the measurements of the parameters showed a positive slope towards outwards/external rotation V+. The results also highlight the question of why there were both positive and negative slopes among the radiographic parameters. It was reported in a cephalometric study that the landmarks located anterior to the axis of rotation were moved with the direction of skull and

consequently resulted in a positive slope. The landmarks which were found to be located posterior to the axis of rotation were moved in a opposite direction to the skull and hence formed a negative slope (Yoon et al. 2002). This indicated that the resultant positive and negative slopes of the landmarks during each rotation depended on their positions in relation to the axis of rotation.

In the horizontal rotation, there were more regular patterns of changes which may illustrate a relationship between orientation and location of the surfaces. When the angle was formed by two differently inclined surfaces in which the upper surface was inclined distodorsally and the lower surface inclined distopantarly as in Proximal intertarsal-Distal intertarsal (PI-DI) joint angle, the rotation caused a positive slope. Whereas, when the upper surface was oriented distopantarly and the lower surface inclined distodorsally, as in the Calcaneoquartal-Proximal intertarsal (CalQ-PI) tarsal joint, there would be a slight negative slope. Furthermore, the other parameters which changed significantly in all or most of the rotational angles, presented a variable pattern of slopes in relation to the rotation of x-ray machine.

The caudo-distal facet of the calcaneo-4th tarsal-metatarsus joint angle showed a negative slope in every rotational angle. The talo-metatarsus joint angle and dorso-distal calc-talocalcane junction angle demonstrated a negative slope towards the external rotation. Perhaps these angles had some specific characters that were not in the others, such as the caudo-distal facet of calcaneo-4th tarsal-metatarsus joint angle which was an angle formed by two inclined articular surfaces. It might be assumed that the change or increase or decrease in the values of measurements was not only based on their landmark's locations in relation to the axis of rotation, but also was affected by other factors such as inclination of

the articular surfaces. Such fluctuations indicate the difficulty in determining any real minimum changes in these parameters. The amount of distortion was small and most likely did not influence major alterations if these alterations were due to some errors in identification of the landmarks. In addition, the distances between radiography machine, object and cassette included two major factors which had to be considered; firstly, the distance between the x-ray source (focal spot) and film, the “focus-film distance” which was kept almost constant during each of rotations in this experiment. The second factor was the distance between object and film, the “object-film distance”. Increasing or decreasing distances in the two factors would affect the image quality. Decreasing focus-film distance and/or increasing the object-film distance could increase magnification which caused an enlargement of the object on the image to more than its real dimensions (Curry, T. S. Dowdey, & Murry, 1990; Oheida et al., 2017a; Walter & Davies, 2001).

Although in this experiment, the tarsi were always almost in contact with the digital radiography cassette, in the rotational angles of H5° and H10° there was some distance between them. This distance was difficult to eliminate due to the oblique disto-palmar position of Mt3 during radiography. The distance was approximately 1.25 cm and 3.00 cm in H5° and H10° respectively. As the subject was not in contact with the cassette during radiography, there might be slight magnification in the images and consequently in the measurements (Curry et al., 1990). However, the distances between digital radiography cassettes and hind limbs in H5° and H10° appeared to have only a minimal effect on the parameters. Thus, the measurements of the radiographic parameters did not show any unusual pattern of change in results. Instead, there was an obvious gradual increase starting from H-5° which was radiographed with a very slight distance between the tarsus and the

digital radiographic cassette. In other similar studies authors found no effects of distances between cassettes and carpus/third metacarpus on radiographic measurements and suggested that the magnification was proportional (Oheida et al., 2017a; Walter & Davies, 2001).

The rotational angle of 5° resulted in less than 1° of change in the parameters and was identified as an acceptable range of limb rotation at which the parameters expressed a minimal amount of change. While in the other two rotational angles the changes were greater and relatively larger in the lower horizontal rotation. These findings agreed with cephalometric and other radiographic studies which found that the increasing rotational angle resulted in a higher angle distortion (Ahlqvist, Eliasson, & Welanders, 1988; Oheida et al., 2017a). However, measuring the parameters which were most affected and changed by rotations required to be more cautious and need careful considerations of specimen alignment and landmarks identifications.

In conclusion, it can be suggested that the effect of radiography machine rotation was significant for most of the tarsal radiographic parameters considering both vertical and horizontal rotations. Horizontal rotation generally had more effect on the parameters than vertical rotation. Some factors such as inclination of the articular surfaces and morphology were significant to determine the response of a parameter to a particular rotation. Using the rotational angles of $V \pm 5^\circ$ and $H \pm 5^\circ$ in only one axis as an acceptable range of rotation from ZLM would offer the best potential to minimize the effect of rotation.

Chapter 6: Inter-Rater reliability

6.1 INTRODUCTION

The interrater reliability assessment is often necessary to select the most reliable parameters to use any measurable parameters for clinical or research purposes, where the parameters are measured by trained or untrained observers. This estimation provides a way to quantify the degree or level of agreement between two or more independent observers who conduct similar measurements about some features or landmarks of interest (Hallgren, 2012). The assessment of the inter-rater reliability could easily be affected by the experience of the observers as insufficient training and/or knowledge in the use of measurement techniques would increase the chances of inter-rater variation. This increase of variation can cause errors in the evaluation of the validity and reproducibility of newly developed parameters (Rudolph, Sinclair, & Coggins, 1998). There are number of studies to develop methods to repeat measurements or recalculate the deviated radiographs from the ideal view (Barr, 1994; Oheida et al., 2017a; Radtke et al., 2010). Hence observers should have appropriate training and understanding of the anatomy and radiology of the tarsus to minimize these errors and obtain an accurate evaluation. The current experiment included only the parameters that exhibited significant reliability. Examining the reproducibility of selected parameters by other raters promoted the experiments described in this chapter. In this experiment, it was hypothesized that there would be significant variations in selected measurements between the different raters. So, the main objective was to Validate the inter-rater's repeatability of some of the most reliable parameters.

6.2 MATERIALS AND METHODS

The inter-rater reliability was conducted by three raters, the author and two other raters (other lab members having an adequate knowledge base to complete this task) who were not working in this project. Furthermore, the author ran a training session with them. Twenty digital radiographs (ten radiographs for each of LM and DP aspects) were selected randomly from the horses (Table 6.1) by the author. All the horses were Thoroughbreds. The raters were trained for a short time by the author to measure the radiographs using the DicomPACS®DX-R X-Ray acquisition software program.

Table 6. 1: Details of the 10 horses measured in the inter-rater reliability study.

No.	Horse breed	Used limb/s	Age (in years)	Gender
1	Thoroughbred	Right	14	Female
2	Thoroughbred	Right	3	Female
3	Thoroughbred	Left	5	Male
4 & 5	Thoroughbred	Right and Left	3	Female
6 & 7	Thoroughbred	Right and Left	7	Male
8	Thoroughbred	Left	9	Female
9	Thoroughbred	Right	4	Female
10	Thoroughbred	Left	3	Female

6.2.1 Selected parameters

Their measurements were performed separately. Six parameters (3 parameters for each of LM and DP aspects) were chosen from the total of 86 (37 parameters of LM view + 49 parameters of DP view) parameters. Selection of these parameters was based on their significant statistical results in previous studies, the location of their landmarks and their ability to measure the morphometrical details on tarsal radiographs from both LM and DP views. These parameters included cranial-caudal (anterio-posterior) angle of proximo-caudal angle of Calc, PTCALC-DTCALC joint angle, CALCQ-DTCALC joint angle, HT/AHCALC ratio, HT/PHCALC ratio and AHCALC/PHCALC ratio.

6.2.2 Statistical analysis

Lin's concordance correlation coefficient (Lin, 1989, Lin, 2000) was used to assess inter-rater reliability. Suggestions for interpretation of Lin's concordance correlation coefficient are;

SCALE

0.21–0.40 Fair

0.41–0.60 Moderate

0.61–0.80 Substantial

0.81–1.00 Almost perfect

(Lawrence & Lin, 1989, 2000; Tedeschi, 2006)

The paired t-test and the Bland-Altman 95% limits of agreement were also calculated to compare raters (Bland & Altman, 1999). IBM SPSS Statistics version 24 and Excell 2016 software were used to perform the analyses. A two-sided $P < 0.05$ was considered to be statistically significant. Box plots were developed to compare the differences in their measurements (Figure 6.1). Bland-Altman plots of each radiographic parameter were

developed using 95% limits of agreement between the different raters (R1, R2 and R3) as shown in figure 6.2. The differences between the raters for each parameter were plotted on the Y axis against their means on the X-axis. Significance was set at $p < 0.05$.

6.3 RESULTS:

The assessment of the inter-rater reliability of the six parameters showed slight differences between raters' measurements (Table 6.2). Almost perfect agreement was found in three out of six parameters (Lin's CCC 0.81–1.00). Narrow limits of agreement were found between rater 1 and 3 (Lin's CCC 0.9) in HT/AHCALC and HT/PHCALC ratio measurements (–0.02 to 0.02 limits of agreement for Bland Altman plots respectively). Similar perfect agreement was found in PtCalc-DtCalc joint angle again between rater 1 and 3 (Lin's CCC 0.84) with substantial agreement with rater 2. From the statistical analyses (Table 6.2), the mean differences in this joint angle were -2.67° , -1.31° and $+1.35^{\circ}$ between rater 1 and 2, 1 and 3, and 2 and 3 respectively.

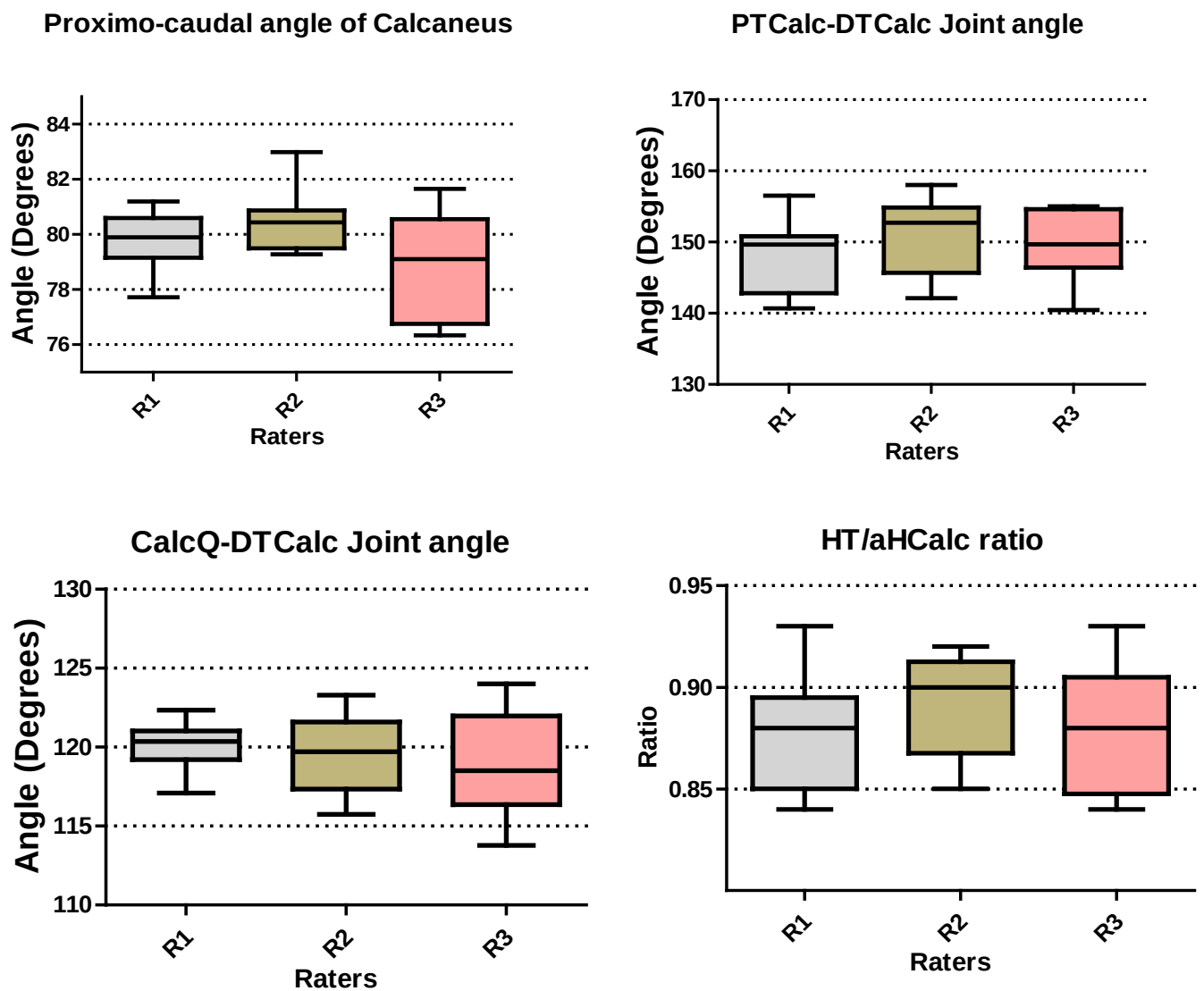
The aHCalc/pHCalc ratio and Calcq-dtcalc joint angle showed a substantial agreement among all three raters with higher Lin's CCC (0.7) along with no significant differences between them. Fair agreement was found in only one out of six parameters, the cranial-caudal (anterio-posterior) angle of proximo-caudal angle of Calcaneus has Lin's CCC of 0.26-0.46 with limit of agreement of -2.47 to 5.57 among all three raters.

Table 6. 2: Comparison of the inter-rater reliability in four parameters as assessed in 10 horses.

PARAMETERS	Raters	MEANS $\pm$ SD	DIFFERENCE OF RATERS	MEAN DIFFERENCE $\pm$ SD	STD. ERROR MEAN	SIG. (2-TAILED) P<0.05	LIN'S CCC	95 % CI OF THE LIN'S CCC		95 % LIMITS OF AGREEMENT OF BLAND-ALTMAN PLOTS	
								Lower	Upper	Lower	Upper
ANTERIO-POSTERIOR ANGLE OF PROXIMO-CAUDAL ANGLE OF CALC	R1	79.76 $\pm$ 1.06	R1 – R2	-0.69 $\pm$ 1.61	0.51	0.211	0.461	-0.069	0.788	-3.85	2.47
	R2	80.45 $\pm$ 1.11	R1 – R3	0.87 $\pm$ 2.51	0.79	0.304	0.454	0.017	0.745	-4.05	5.79
	R3	78.90 $\pm$ 1.94	R2 – R3	1.55 $\pm$ 2.05	0.65	0.041	0.259	-0.034	0.510	-2.47	5.57
PTCALC-DTCALC JOINT ANGLE*	R1	148.21 $\pm$ 5.01	R1 – R2	-2.67 $\pm$ 7.69	2.43	0.302	0.766	0.417	0.918	-17.74	12.40
	R2	150.88 $\pm$ 5.61	R1 – R3	-1.31 $\pm$ 7.00	2.21	0.568	0.839	0.500	0.955	-15.03	12.41
	R3	149.53 $\pm$ 4.83	R2 – R3	1.35 $\pm$ 5.34	1.69	0.444	0.754	0.344	0.923	-9.12	11.82
CALCQ-DTCALC JOINT ANGLE	R1	120.05 $\pm$ 1.68	R1 – R2	0.62 $\pm$ 3.22	1.02	0.555	0.657	0.211	0.876	-5.69	6.93
	R2	119.43 $\pm$ 2.49	R1 – R3	1.12 $\pm$ 3.24	1.03	0.302	0.458	-0.077	0.788	-5.23	7.47
	R3	118.93 $\pm$ 3.19	R2 – R3	0.50 $\pm$ 3.47	1.09	0.660	0.687	0.153	0.911	-6.30	7.30
HT/AHCALC RATIO	R1	0.88 $\pm$ 0.03	R1 – R2	-0.01 $\pm$ 0.02	0.01	0.154	0.649	0.160	0.882	-0.05	0.03
	R2	0.89 $\pm$ 0.03	R1 – R3	0.00 $\pm$ 0.01	0.00	1.000	0.964	0.871	0.990	-0.02	0.02
	R3	0.88 $\pm$ 0.03	R2 – R3	0.01 $\pm$ 0.02	0.01	0.119	0.642	0.157	0.877	-0.03	0.05
	R1	0.65 $\pm$ 0.02	R1 – R2	-0.01 $\pm$ 0.01	0.00	0.052	0.336	-0.053	0.637	-0.03	0.01

HT/PHCALC RATIO	R2	0.66 ± 0.01	R1 – R3	-0.00 ± 0.01	0.00	0.279	0.886	0.640	0.967	-0.02	0.02
	R3	0.66 ± 0.02	R2 – R3	-0.00 ± 0.02	0.01	0.242	0.273	-0.146	0.609	-0.04	0.04
AHCALC/PH CALC RATIO	R1	0.74 ± 0.01	R1 – R2	-0.01 ± 0.01	0.00	0.051	0.750	0.352	0.918	-0.03	0.01
	R2	0.75 ± 0.02	R1 – R3	-0.00 ± 0.01	0.00	0.394	0.766	0.393	0.923	-0.02	0.02
	R3	0.74 ± 0.02	R2 – R3	0.00 ± 0.01	0.00	0.496	0.669	0.151	0.899	-0.02	0.02

Figure 6. 1: Mean box plots for individual angular parameters. Solid lines in the box indicate median. Abbreviation; R1. Rater 1, R2. Rater 2; R3. Rater 3.



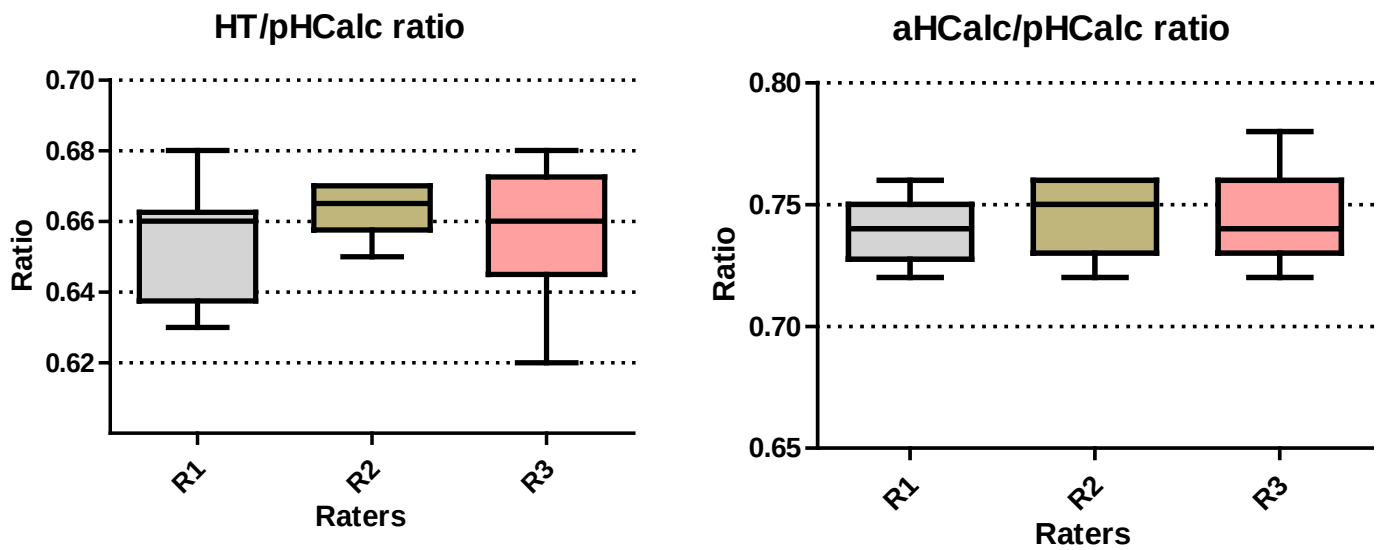
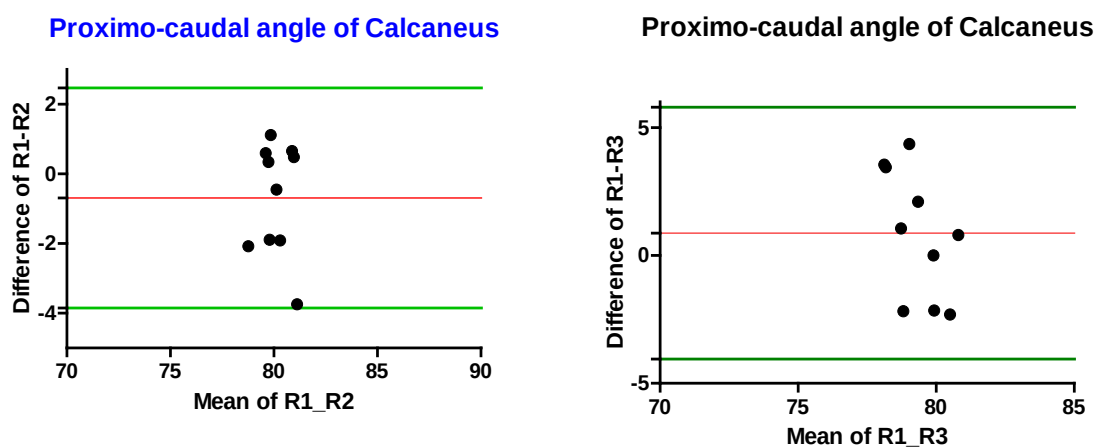
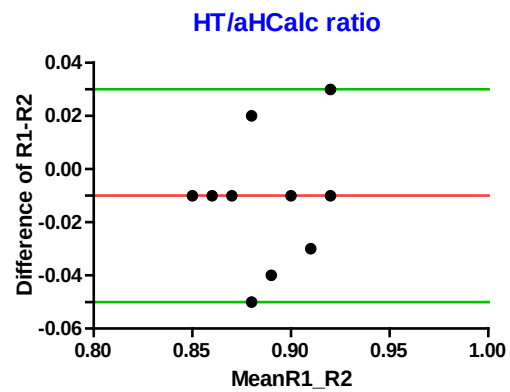
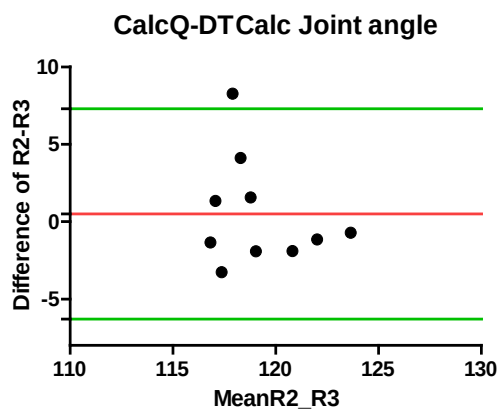
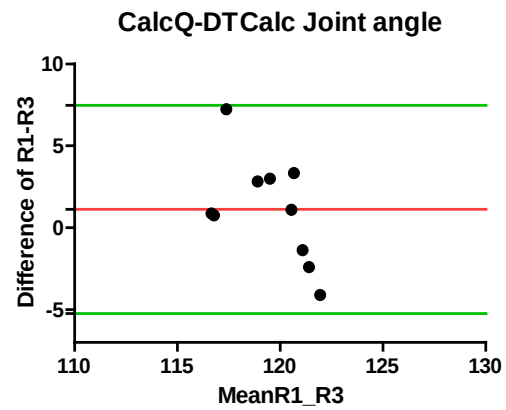
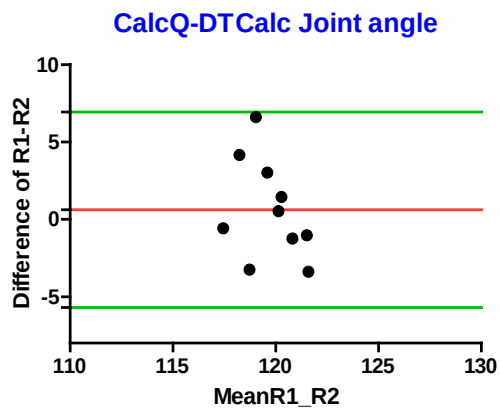
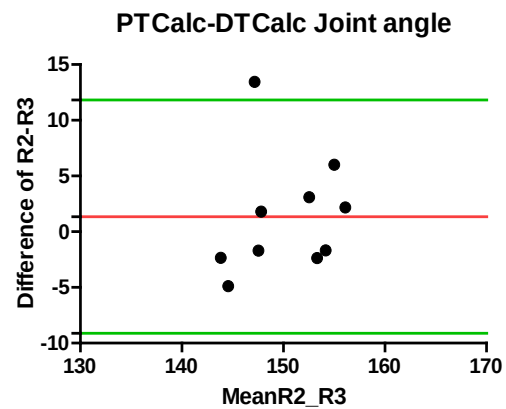
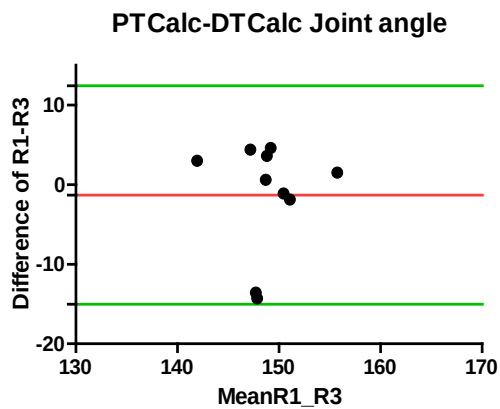
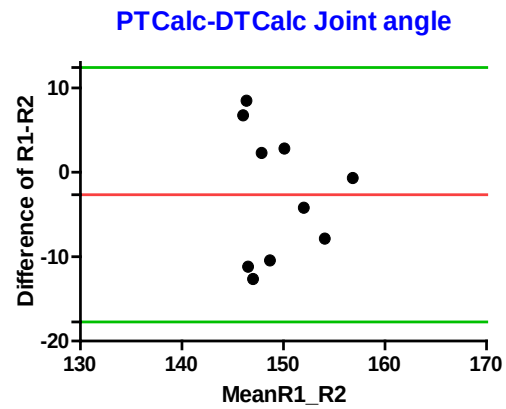
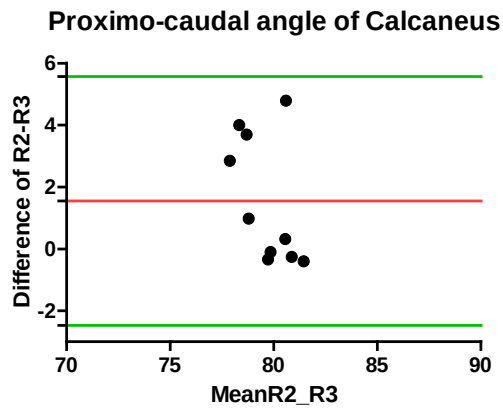
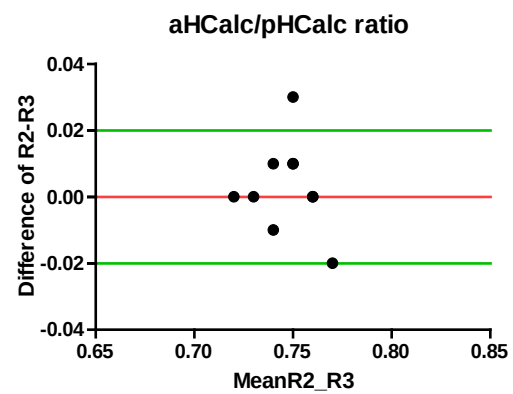
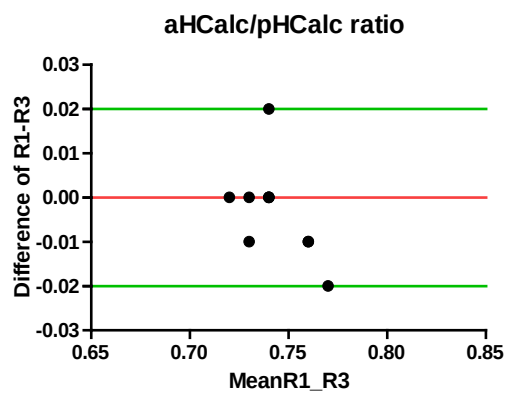
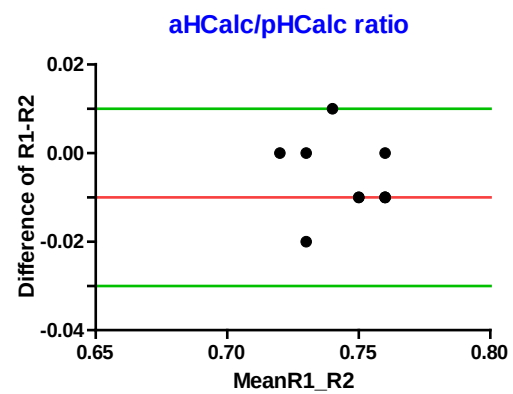
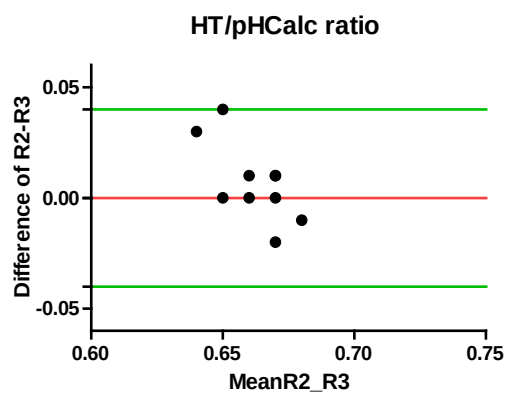
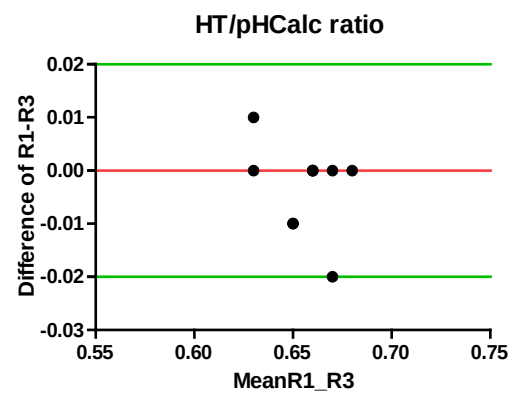
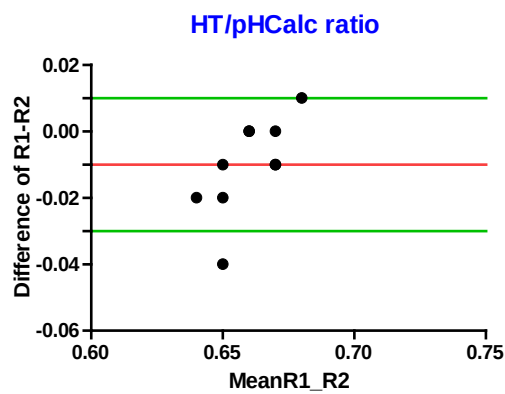
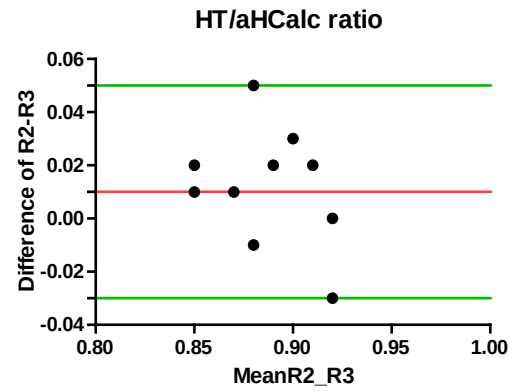
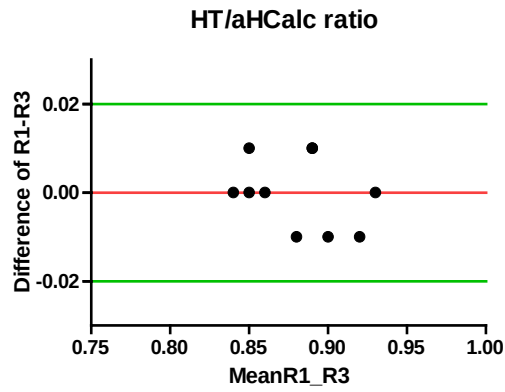


Figure 6. 2: Bland-altman plot showing the difference versus the mean of parameters of the raters R1, first rater, R2, second rater, R3, third rater in interrater reliability study.







6.4 DISCUSSION

This study assessed the reliability of the measurements among raters for only six of the parameters. The results showed that, although the reliability of these tarsal measurements varied from moderate to almost perfect ($CCC > 0.41$) except between rater 2 and 3 in the cranial-caudal (anterio-posterior) angle of proximo-caudal angle of calcaneus and HT/PHCALC ratio between rater 1 and 2 and between rater 2 and 3 where the reliability was poor ($CCC < 0.4$), the resulting measurement errors due to the variation of the landmark identification could still be clinically significant. According to Nagasaka et al. (2003) the distances between landmarks can affect the magnitude of measurement errors in linear and angular measurements. They mentioned that the closer two landmarks were, the greater the angular measurement error that resulted (Nagasaka, Fujimura, & Segoshi, 2003). Therefore, the much closer landmarks of the angular parameters may have contributed to the proportionately large SD of the angular measurements demonstrated in the results.

Variability in landmark identification was exaggerated when the angle of four landmarks were derived from two other contributed angles of four landmarks of each because the errors are cumulative. For instance, in the current study, the cranial-caudal (anterio-posterior) angle of proximo-caudal angle of calcaneus was measured by picking up three landmarks, while Ptcalc-dtcalc joint angle and calcq-dtcalc joint angle were derived angles calculated from two angles. There were moderate to almost perfect agreements between rater 1 and 2 and 1 and 3 in all parameters except the HT/PHCALC ratio where Lins's CCC for the agreement between raters 1 and 2 was 0.336, and poor to substantial agreements between rater 2 and 3. The possible cause of this different reliability was the experience of the raters. Rater 3 was regularly reading radiographs during lab work in the histological field in order to visually estimate the differences between the widths of the different layers of fascia results on radiographic film,

while rater 1 was the author of this thesis. Experience is a very important factor for reliability (Chen, Chen, Chang, & Chen, 2000). However, with a low level of agreement between the rater's measurements of these parameters, additional training seemed to make some improvement in the consistency of measurements.

The angle measurements more than the ratios were assumed to be clinically relevant in the comparison between different raters although the ratios exhibited the best agreement between rater 1 and 3 (Lin's CCC =0.946). Hence it might be more acceptable to use measurements of angles from different raters in a study and less acceptable to use measurements of ratios from different raters. In general, the close limit of agreements in all selected parameters were observed between rater 1 and 3 while wider limit of agreement of rater 2 with other raters.

The anatomical nature of the landmarks of a particular parameter and the level of training and experience of the observers could also affect the reliability of measurements as suggested by other observers as well (Rudolph et al., 1998). Human error can also occur both in recording of the measurement or in the use of the measurement devices. In some cases, missing or use of wrong landmarks for a particular parameter by mistake or not labelled them before measurement might cause additional errors. Training in measuring these parameters should include practice in the accuracy of selecting the anatomic landmarks, and practice with the measurement software used in this experiment.

In conclusion, the statistical analysis of the inter-rater reliability showed that the correlated angular and ratio parameters were more reliable and reproducible than individual angle parameters. The inter-raters repeatability test showed that HT/AHCALC and AHCALC/PHCALC ratios and PtCalc-DtCalc joint angle were the most reliable and reproducible parameters among the six selected parameters. These parameters had a high ability to identify significant

morphometrical variation between horses. In addition, sufficient training and the ability to identify landmarks are important to increase the reliability of these measurements. The use of such reliable morphometric parameters to measure any changes in the alignment of tarsal bones during loading of this joint will be investigated in the next chapter.

Chapter 7: The effect of loading/hyperextension on radiographic measurements of tarsal joint to assess the alignment and changes in the positions of tarsus under loading condition.

7.1 INTRODUCTION:

The loading stability of the equine tarsus is poorly understood as its role in transition of the large axial compressional and ground reaction forces between the crural region and the distal hindlimb during training and racing is not well defined. Normal tarsal motion is a complex well-coordinated effort as it undergoes a series of cyclic flexions and extensions during the swing and stance phases of the stride cycles. The strains experienced during exercise lead to adaptation of osteochondral tissues at each location within the joint (Rachel Clare Murray et al., 2007). Therefore, the alignment and stability of the tarsal bones is important in assessment of kinetic changes in the tarsal joint in a natural loading or full weightbearing condition using conventional radiography.

Several studies have been reported for the objective measurements of carpal alignment in humans using both goniometry and lateral radiographs to assess the functional status of the wrist and the three-dimensional kinematics of the tarsal bones in the human ankle joint under a weight-bearing condition or axial loading (Ito et al., 2017; Kaneda et al., 2019; Ryogo Nakamura, Hori, Imamura, Horii, & Miura, 1989; R Nakamura, Miura, Kino, & Horibe, 1985; Nishikawa, 1985; Nozaki, Watanabe, Kamiya, Katayose, & Ogihara, 2019). Tarsal hyperextension and instability have been reported in small animals (Buote & Reese, 2006; Guilliard, 2003; Holt, 1977) but no information is available on actual changes in the positions of tarsal bones and their direction of movement as the limb is being loaded. The objective

measurement of the loading stability of the tarsal bones has not been performed in the horse. Although there are marked anatomical differences in the morphology of the tarsus between humans and horses and importantly functional differences, the investigation of the alignment and its relationship to stability is important to understand the mechanisms of loading, force distribution and pathogenesis of tarsal damage in all species.

In bone morphometrical studies, there are several factors such as machine, object and cassette distances and alignment that can affect the accuracy of radiographic details and measurements and should be considered carefully (Curry et al., 1990; Major, Johnson, Hesse, & Glover, 1994). The importance of accurate diagnosis in musculoskeletal injury is obvious but the radiographic details might easily be changed due to the high sensitivity of the x-ray to any rotation of the limb and to changes in the relative position of the radiography machine during radiography procedures. The effect of these factors on the quality of radiographs and the consistency of bone measurements has been investigated in several studies (Han et al., 1994; Hans et al., 1995; Walter & Davies, 2001), but none concerned the effect of the tarsal joint loading on the bone alignment and other bony features on the radiographs.

The shape of the articular surfaces of a bone might provide significant information about the likely biomechanical action of a joint while detection of the effects of load distribution through the body skeleton is a major requirement for the interpretation of the mechanical influence of shape of a bone in terms of the resistance to loading (Biewener, Thomason, & Lanyon, 1983).

The congruence of the articular surfaces of each tarsal bone should affect their movements and thus conformational stability during loading. The tarsal bones are associated with a high incidence of fractures and pathologies (Baxter, Dechant, G. M. & Southwood, 2003; Dahn &

Ueltschi, 1989; Kidd, Fuller, & Barr, 2001; Pool, 1996). It is clear from the literature that there is scant morphometric information for the tarsus, especially in equine species. Previous Chapters describe how some parameters were developed to measure conformation (morphology and alignment) of the tarsal bones and then an acceptable range of rotation for each parameter was determined. Using these parameters of tarsal congruities (angles) and changes in tarsal bone alignment during load bearing, would improve our understanding of the tarsal joint biomechanics and the potential tarsal pathogenesis.

The hypothesis of this experiment was that loading affects the tarsal joint conformation, and changes in alignment and/or movements of tarsal bones would affect the parameters that were identified by the author in chapter three and four. Therefore, this experiment was designed as a pilot trial to investigate the response of tarsal bone alignment with external loading as measured on tarsal DP and LM radiographs.

The main objectives of this study were

- i) to observe the relevant tarsal bones alignment as a response to change in loading of the tarsal joint.
- ii) to measure the changes in relative positions of the tarsal bones during loading
- iii) to observe the direction of change where possible.

7.2 MATERIAL AND METHODS

7.2.1 Animal/test specimen:

Two cadaver hindlimbs were collected from one 4.5-year old Thoroughbred that had been euthanized or died for reasons not associated with this project. The tarsi were observed to be apparently healthy on visual inspection with no radiological signs of damage. Both limbs were collected from the Department of Pathology, Faculty of Veterinary and Agricultural Sciences (FVAS), The University of Melbourne. Each limb was transected at the distal one third of tibial shaft (about 10-15cm from the tarsocrural joint).

7.2.2 Loading Press/Rig:

A custom-built loading apparatus was developed from a commercial press and used for limb loading (TTi 20,000kg Hydraulic floor press, Total Tools, Derrimut, VIC 3030, Australia) with a mechanical pump, loading cup and metal platform fabricated and fixed to the rig (Figure 7.1). A squared wire mesh (12.67mm x 12.67mm) was attached to the back of the loading press to create a radiopaque measuring grid or “scale” on all the radiographs taken (Figure 7.1). The wire mesh was used to create a scale to monitor any obvious change in position of a particular bone thorough the coordinate/fixed points on the targeted bones in ZLM and ZDP and then chase up their locations in both x and y axes of scale with other tarsal bones to assess their alignment changes during loading steps.

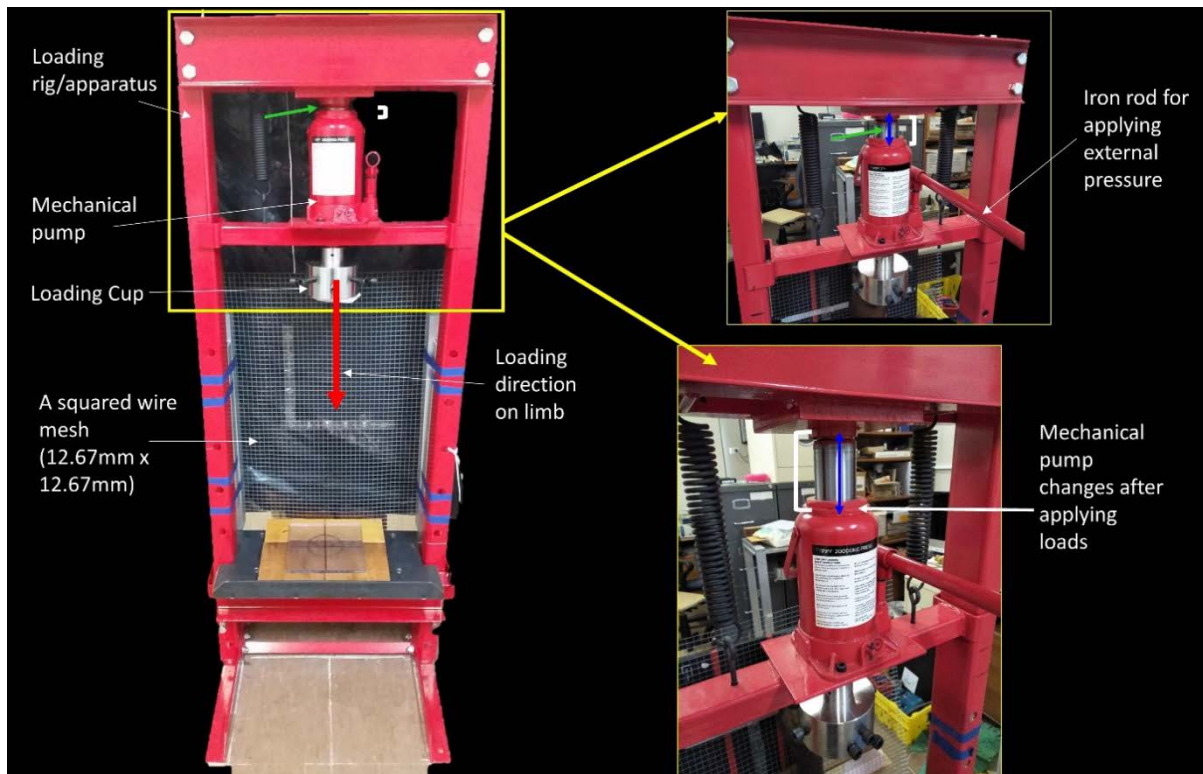


Figure 7. 1: The Loading apparatus (front views) for positioning and loading the cadaveric hindlimbs for radiography. The mechanical pump put pressure in a downward direction with the help of a loading cup, designed to fit and hold the cut end of the distal tibia. The highlighted portion indicates the changes of the mechanical pump position after applying external loads. A squared wire mesh (12.67mm x 12.67mm) was attached to the back of the loading rig.

7.2.3 Limb preparation and mounting into the loading rig

The limbs were loaded in a vertical position into the loading rig and aligned along the vertical axis of the frame, and the tibial shaft was firmly secured with folded cardboard padding and screws within the metal cup while the hoof was placed on the metal platform. Lines were drawn with chalk on the hoof and the platform so that changes in the hoof positions during loading could be traced out. Using a goniometer, the dorsal fetlock angle was then measured, and appropriate pressure was applied via the jacking system of the loading rig to achieve a dorsal fetlock angle of 170° (Figure 7.2). This position was then considered to be the initial

loading position (Step one-170). The limb was kept hydrated throughout the experiment by periodic (20 – 25 min) damping with moist drape/towel. The tarsus ZDP and ZLM views (at 0° of rotation) were achieved with the X-ray beam (machine) and radiographic plate positioned using a similar procedure to that described in chapter 3 and 4.

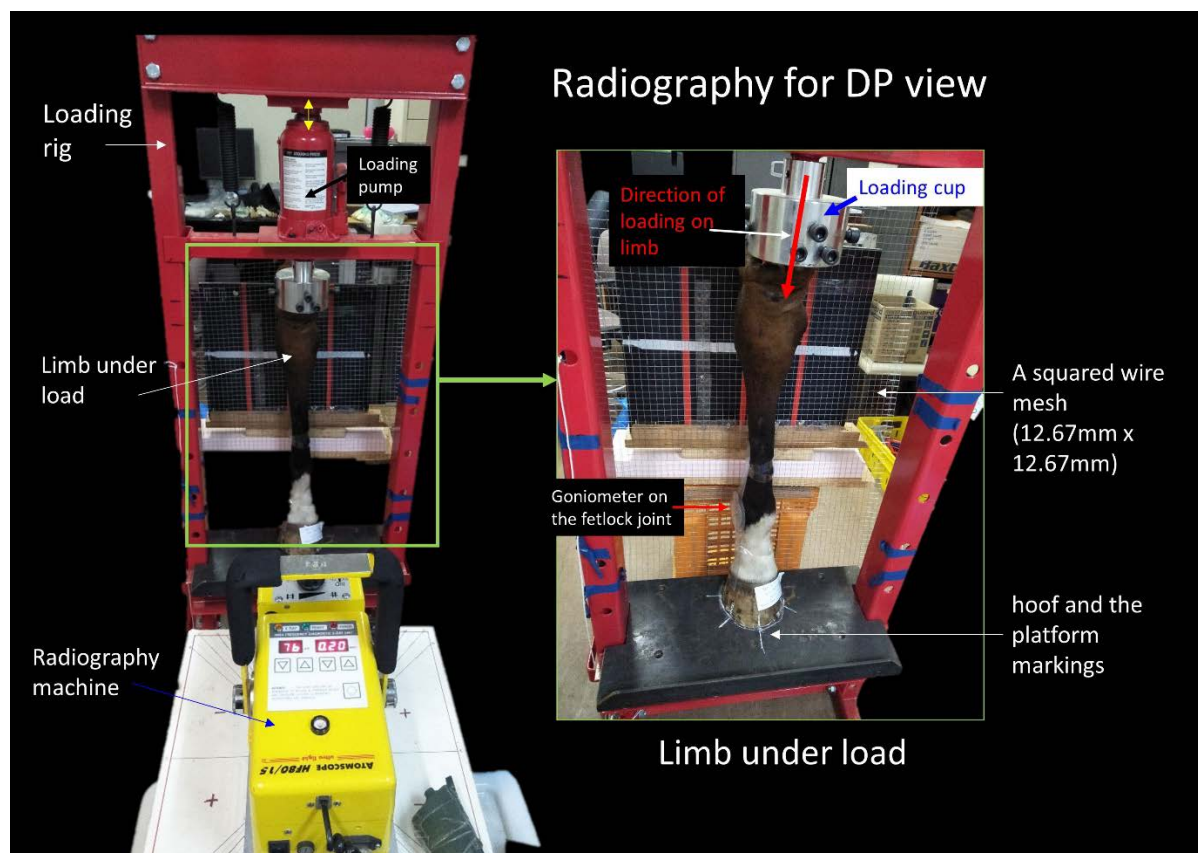


Figure 7. 2: Radiographing a right tarsus of a Thoroughbred under external loading through the distal end of the tibia via a loading cup.

The highlighted area indicates the position of the limb in the loading rig for DP views of the tarsus. The red vertical arrow shows the direction of loading. The hoof and radiographic base markings help to evaluate any change of hoof position during the loading process.

7.2.4 Radiographic set-up and position

The radiographic machine was positioned on a stable platform (stool) at about 95 cm from the mounted limb. The X-ray beam projection angle was orientated perpendicular to the tarsal region and centred at about the central tarsal and a few radiographs (1-3) were taken to ascertain the ZDP or ZLM positions. Thereafter, the center of the x-ray beam was kept at the level of the centrodistal joint for all ZDP radiographs while the x-ray beam was centred on the talus which was then marked to provide a landmark after manual palpation for positioning of the center of the x-ray beam parallel to an imaginary line joining the lateral and medial malleoli of the tibia for all ZLM radiographs. In this way the X-ray position was kept constant at marked point throughout the exposure of all the loading positions to be taken.

Starting from the initial ZLM loading position (Step one-170), serial radiographs of the limb were then taken at each loading point where the dorsal fetlock angle had decreased by 10° (Step one-170; Step two-160; Step three-150; and Step four-140) (Figure 7.4). After the Step four-140 position, the load on the limb was released. The same serial radiographs were obtained from initial ZDP loading position (Step one-170).

The loading was performed very carefully after the proper adjustment of the limb in loading apparatus, the positions of tarsus, Mt3 and fetlock was kept monitored through their position relative to the fixed loading bars/columns of the loading device, In case of any obvious change the whole process was started again to make sure no other rotations occurred during each step of loading. As the load on the limb was increased, the tarsal region gradually moved downwards, leaving the focal spot and x-ray field out of alignment with the limb. The position of radiography machine was adjusted to keep the x-ray beam position at same point during

different loading conditions and the aperture of the radiographic machine was increased to widen the collimation (If required).

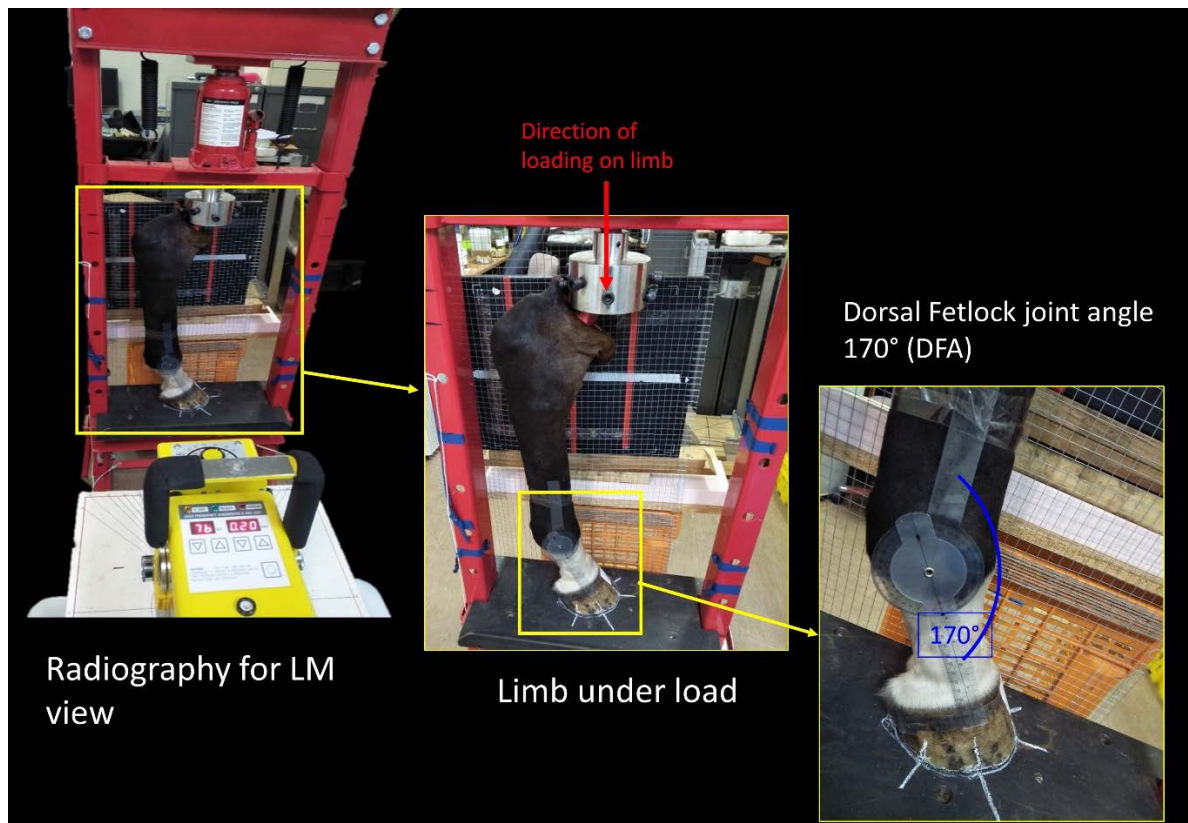


Figure 7. 3: Radiographing a right tarsus of a Thoroughbred under external loading through the distal end of the tibia via a loading cup for LM views of the tarsus.

The highlighted area indicates the position of the limb in the loading rig and dorsal fetlock joint angle measured by positioning of a goniometer on the distal limb. The red arrow shows the direction of loading. The hoof and radiographic base markings help to evaluate any change of hoof position during the loading process.

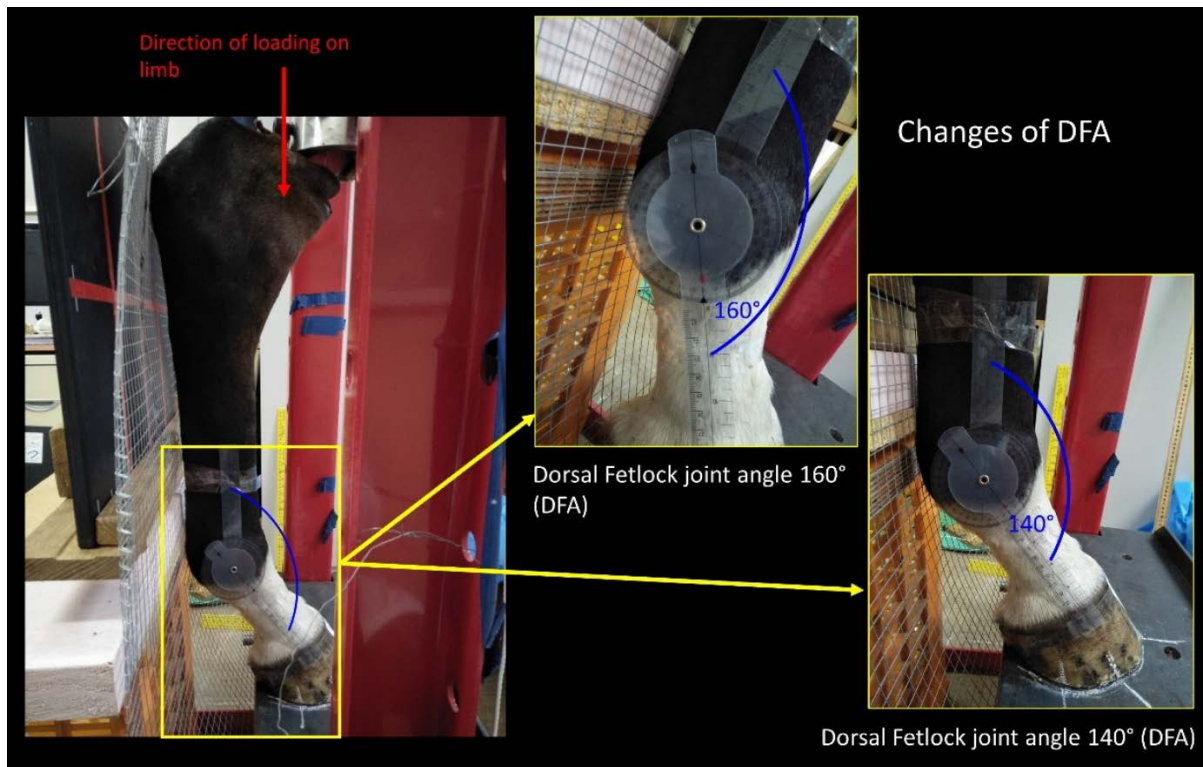


Figure 7. 4: Radiographing a right tarsus of a Thoroughbred (lateral views) with an increase of external loading resulting in a change of dorsal fetlock angles. The highlighted area indicates the changes of dorsal fetlock joint angle measured by positioning of goniometer on the distal limb.

7.2.5 Radiographic measurement

The preparation and radiography of the specimens in this experiment were similar to that described in previous Sections of Chapter 3 (for ZLM) and 4 (for ZDP). In this study, tarsal parameters were selected based upon the location of their landmarks and their ability to monitor the alignment changes of tarsal bones and measure the morphometrical details of the loaded tarsal radiographs from both LM and DP views. The measurements were performed in the same setting in DicomPACS® Rx X-Ray acquisition software (Figure 7.7). Three repeat measurements were taken for each radiographic parameter by the author.

7.2.5.1 THE SELECTED TARSAL PARAMETERS FOR THE LM VIEW WERE:

1. **Calcaneo-Metatarsus joint angle:** this angle was measured at the intersection point between the long axis of Mt3 and the long axis of calcaneus in the lateromedial view (Figure 7.5/1 and RL). The long axis of calcaneus was measured by a line joining two points (landmarks), the proximal point was the highest point or edge of the palmer border of the calcaneal tubercle and the caudo-distal edge of calcaneus at the level of the calcaneoquartal joint. The line joining both proximal and distal mid points represents the long axis of calcaneus.
2. **Proximal intertarsal-distal intertarsal joint angle:** This was the angle between a line perpendicular to the proximal intertarsal joint and a line perpendicular to the distal surface of the central tarsal or the distal intertarsal joint (Figure 7.5/2).
3. **Distal intertarsal- tarsometatarsal joint angle:** this was the angle between a line perpendicular to the distal intertarsal joint and a line perpendicular to the best fit line of the tarsometatarsal joint (Figure 7.5/3).
4. **Calcaneoquartal- distal talocalcaneal joint angle:** This angle was measured between two best-fit lines of the distal calcaneo-quartal joint and distal talocalcaneal joint (Figure 7.5/4).
5. **Talo-Calcaneal axial angle:** this angle was measured at the intersection point between the long axis of calcaneus (as described earlier) and the long axis of talus. The long axis of talus was measured by taking two mid-points. The proximal mid-point was halfway between the proximal talocalcaneal joint where it intersects the dorso-cranial border of sustentaculum tali to the dorsal boundary of the lateral and medial trochlear ridges (overlapped in this view). The second (distal) mid-point was halfway between the dorsal and plantar edges/corners of the distal surface of talus. The line joining both

mid-points represents the long axis of talus tarsal bone in the lateromedial view (Figure 7.5/5).

6. **Dorsal height of central tarsal/ dorsal height of 3rd tarsal ratio parameter:** The dorsal height of the central (Figure 7.5/dHC) and third (Figure 7.5/dHT3) tarsal bones were extended from the most dorsal proximal extremity to their most dorsal distal extremities.

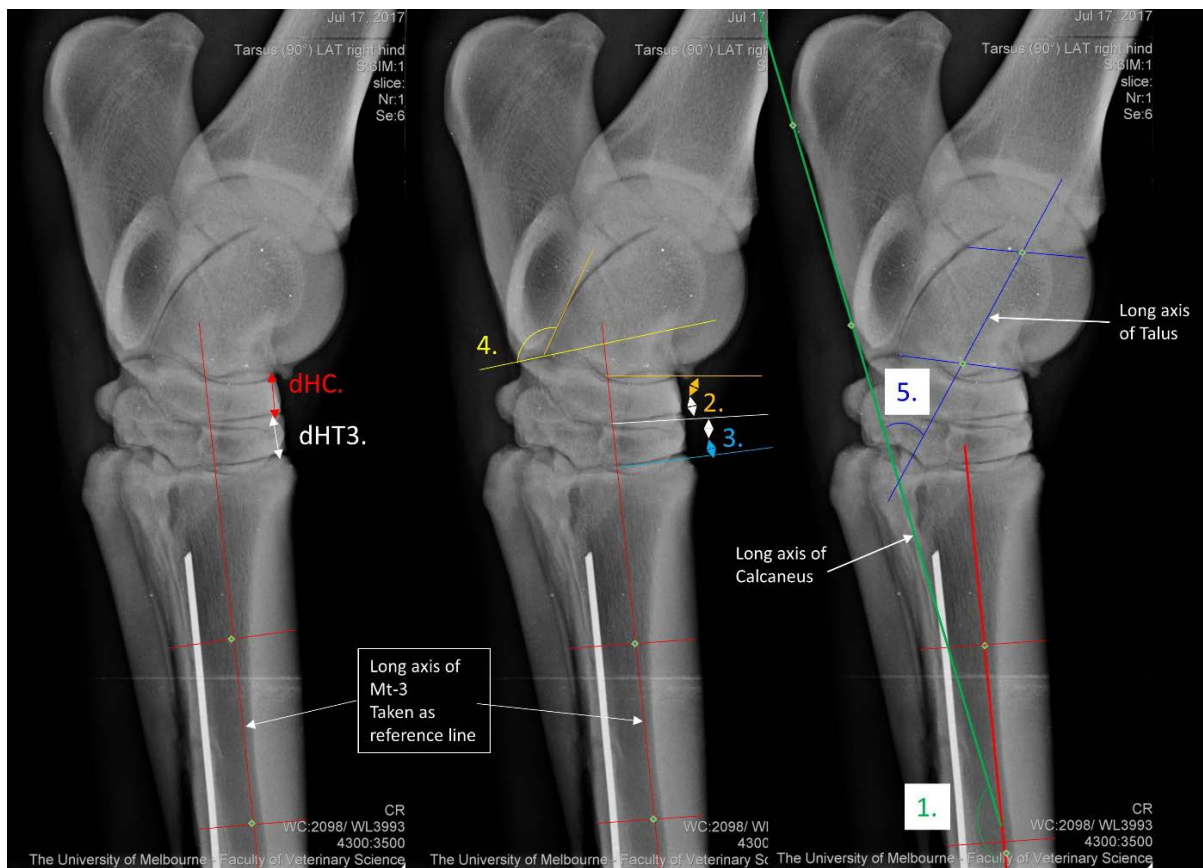


Figure 7. 5: LM radiographs of a right tarsus showing the selected tarsal parameters. Long axis of third metatarsus (Mt-3) taken as the reference line.

1. Calcaneo-Metatarsus joint angle, 2. Proximal intertarsal-Distal intertarsal joint angle, 3. Distal intertarsal- tarsometatarsal joint angle, 4. Calcaneoquartal- distal talocalcaneal joint angle, 5. Talo-Calcaneal axial angle, dHC. represent the dorsal height of the central tarsal, dH3rd. Dorsal height of the third tarsal bone.

7.2.5.2 THE SELECTED TARSAL PARAMETERS FOR THE DP VIEW WERE:

7. Tarsometatarsal- tibial metaphyseal/malleolar joint angle (TMT-Tm Joint angle):

This was the angle that was measured between a line perpendicular to the tarsometatarsal joint and a line perpendicular to a reference line (Chap 4, section 4.1 RL) between the lateral and medial malleolus of the distal extremity of the tibia as previously described (Figure 7.6/7).

8. Tarsometatarsal- tibiotalus joint angle (TMT-Tt Joint angle): This was the angle that was measured between a line perpendicular to the tarsometatarsal joint and a line perpendicular to the tibiotalus or tarsocrural joint (Figure 7.6/8).

9. Tarsometatarsal - proximal intertarsal joint angle (TMT-PIT Joint angle): This was the angle that was measured between a line perpendicular to the tarsometatarsal joint and a line perpendicular to the proximal intertarsal joint (Figure 7.6/9).

10. Tarsometatarsal - proximal central joint angle (TMT-PC joint angle): This was the angle that was measured between a line perpendicular to the tarsometatarsal joint and a line perpendicular to the proximal central joint (Figure 7.6/10).

11. Tarsometatarsal – distal intertarsal joint angle (TMT-DIT joint angle): This was the angle that was measured between a line perpendicular to the tarsometatarsal joint and a line perpendicular to the distal intertarsal joint (Figure 7.6/11).

12. Lateral/ medial height of the central tarsal (Lat. /Med.C.h ratio):

This was the ratio of lateral to medial height of the central tarsal. The lateral height was measured between the proximo-lateral end of the central tarsal at its articulation with the distal talus (forming the lateral extremity of the proximal intertarsal joint). The medial height was measured from the disto-lateral end of the central tarsal bone at its articulation with the

proximal surface of the third tarsal bone forming the distal intertarsal joint (Figure 7.6/LhC and MhC).

13. Lateral/ medial height of the third tarsal (Lat. /Med.h.T3 ratio):

This was the ratio of the lateral to medial height of the third tarsal. The lateral height was measured between the proximo-lateral extremity of the third tarsal at its articulation with the central tarsal proximally forming the distal intertarsal joint. The medial height was measured between the disto-lateral end of the third tarsal at its articulation with the third metatarsal distally (Figure 7.6/LhT3 and MhT3).

Mh or medial height of the tarsus: This was the height which extended from the medial edge of the distal surface of the tibia to the dorsomedial edge of the proximal surface of the third metatarsal (Mt3) (Figure 7.6/Mh).

Lh or lateral height of the tarsus: This was the lateral height which extended from the lateral edge of the distal surface of the tibia to the dorsolateral edge of the proximal surface of the third metatarsal (Figure 7.6/Lh).

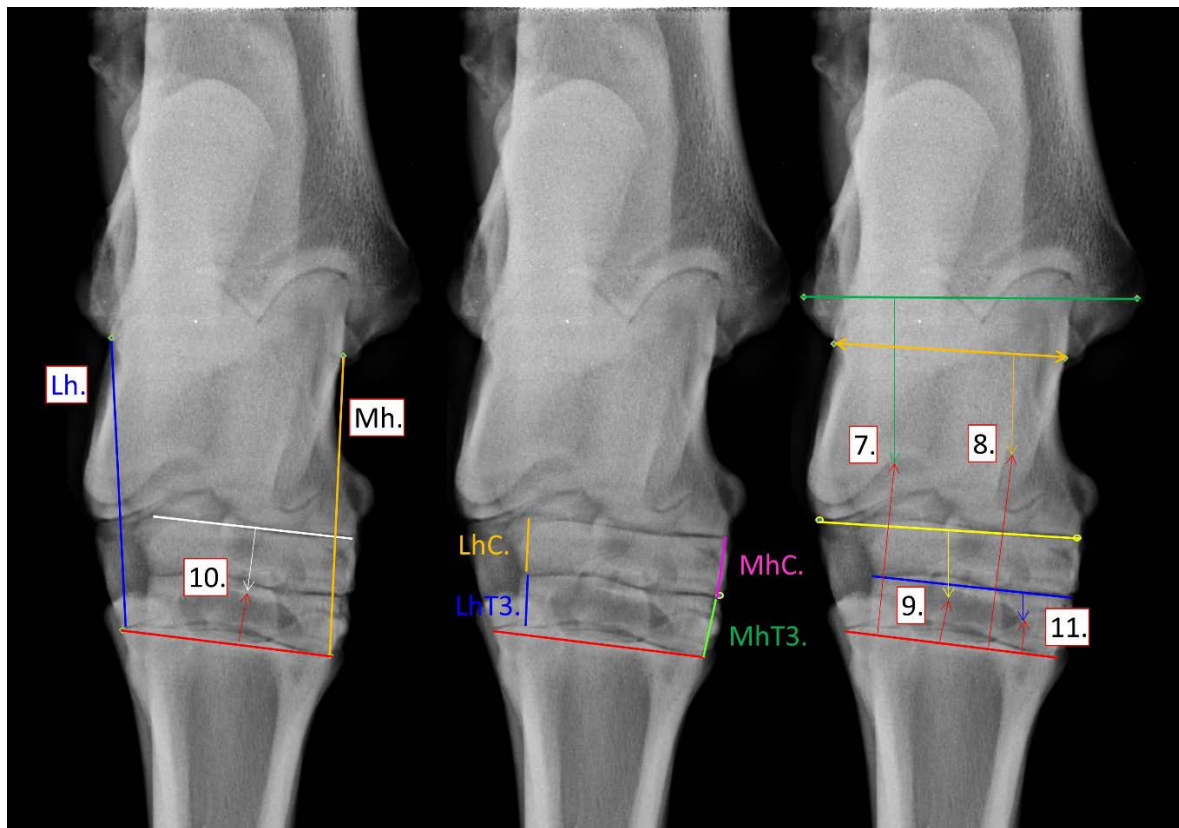


Figure 7. 6: DP radiographs of a right tarsus of a thoroughbred. The correlated angular parameters are shown in this picture.

7. Tarsometatarsal- tibial metaphyseal/malleolar joint; 8. Tarsometatarsal-tibial tibio-talus;
 9. Tarsometatarsal- proximal intertarsal joints; 10. Tarsometatarsal-proximal central joints;
 11. Distal intertarsal-tarsometatarsal joints; Lh., total lateral height of the tarsal joint; Mh.,
 total medial height of the tarsal joint; LhC, Proximodistal lateral height of central tarsal;
 MhC. Proximodistal medial height of central tarsal; LhT3. Proximodistal lateral height of 3rd
 tarsal; MhT3. Proximodistal medial height of third tarsal.

7.2.6 Statistical analysis

All parameters were presented as Mean $\pm$ SD. To compare the means of each variable (parameter) obtained from the measurement levels (i.e. DFA170 - DFA140) one-way analysis of variance (ANOVA) was used. Bonferoni adjusted t-tests were used as post-hoc tests to compare means between levels with significant effects from ANOVA. A 95% confidence interval was used and values where $P < 0.05$ were considered significant.

7.3 RESULTS

7.3.1 RADIOGRAPHIC MEASUREMENTS OF THE LOADED TARSUS IN THE LM VIEW:

Different types of parameters including individual angular parameters, correlated angular parameters and ratio parameters were included in this pilot study to avoid effects of tarsus size variation and magnification effects of radiography machine distance. There was a decrease in the values of most of the parameters when the tarsus was loaded in a loading rig (Table 7.1). This was not including the Distal Intertarsal- Tarsometatarsal Joint angle which showed a consistent increase from $177.5^{\circ} \pm 0.17^{\circ}$ with a maximum difference of 1.14° . Regardless of whether there was an increase or decrease of the values, the measurements demonstrated that the Calcaneo-Metatarsus Joint angle and Talo-Calcaneal Axial angle were the least affected angle during loading of the tarsus.

The Calcaneoquartal- Distal Talocalcaneal Joint Angle changed significantly (<0.05) from $118.6^{\circ} \pm 0.81^{\circ}$ when unloaded to $112.2^{\circ} \pm 0.03^{\circ}$ in the loaded joint with a maximum difference of 6.5° . These differences were statistically significant in some comparison to the initial loading positions (Table 7.1). A progressive increase in Distal Intertarsal- Tarsometatarsal Joint angle and decreases in Proximal Intertarsal-Distal Intertarsal Joint angle and Dorsal height of central tarsal/ Dorsal height of 3rd tarsal ratio parameter was observed as load increased on the cadaver hindlimb (i.e. DFA170 to DFA140).

The individual angular parameters Calcaneo-Metatarsus Joint angle and Talo-Calcaneal Axial angle showed non-significant changes during all loading positions of the tarsus with changes of 1° and less respectively. The correlated angular radiographic parameters showed a different response as some correlated angles changed significantly ($P < 0.05$) during increased

loading of the joint. Such as Calcaneoquartal- Distal Talocalcaneal Joint angle which showed significant differences ($P < 0.05$) in the values in more extreme loading positions of DFA160°-140°. The Dorsal height of central tarsal/ Dorsal height of 3rd tarsal ratio parameter also showed significant changes ($P < 0.05$) in loading positions DFA 170° vs. 140° and DFA 160° vs. 150°. In contrast, the Proximal Intertarsal-Distal Intertarsal Joint angle, Distal Intertarsal-Tarsometatarsal Joint angle showed non-significant changes during all loading positions of the tarsus with the smallest changes of 1° and less respectively.

The distribution of these parameters was similar for both cadaver limbs in this Thoroughbred. The graphs (Figure 7.8) show the confidence intervals for the mean differences in different loading positions with DFA angles. The bar graphs represent the pairwise comparison of each parameter at the 4 loading positions measured in both horses and are presented in Figure 7.8. The bar graphs of each tarsal radiographic parameter in the LM view of the tarsus represent the means $\pm$ SD in both horses (H1 and H2) depicting the individual variations and variations among the different loading positions. Each bar graph has their respective graphs on the right showing the confidence interval for the mean differences in different loading positions (Figure 7.8). This graphical representation of confidence interval illustrates the effectiveness of analysing the differences, when confidence intervals within the same range suggest that the difference between different loading positions is not very pronounced as the mean difference is quite narrow and vice versa (Figures 7.8).

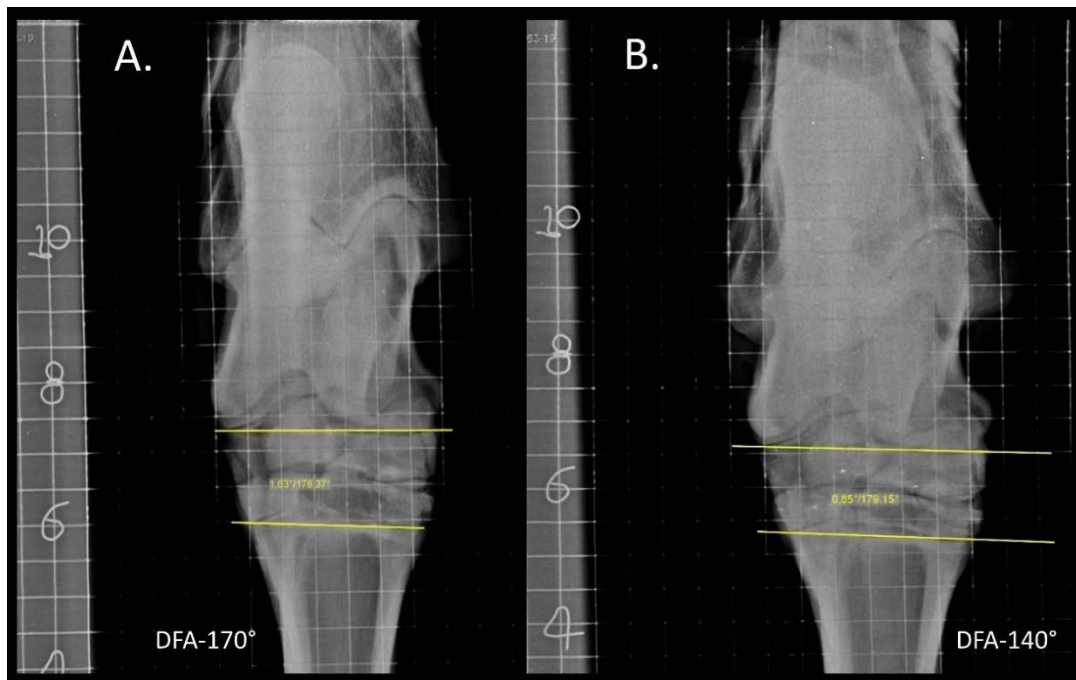


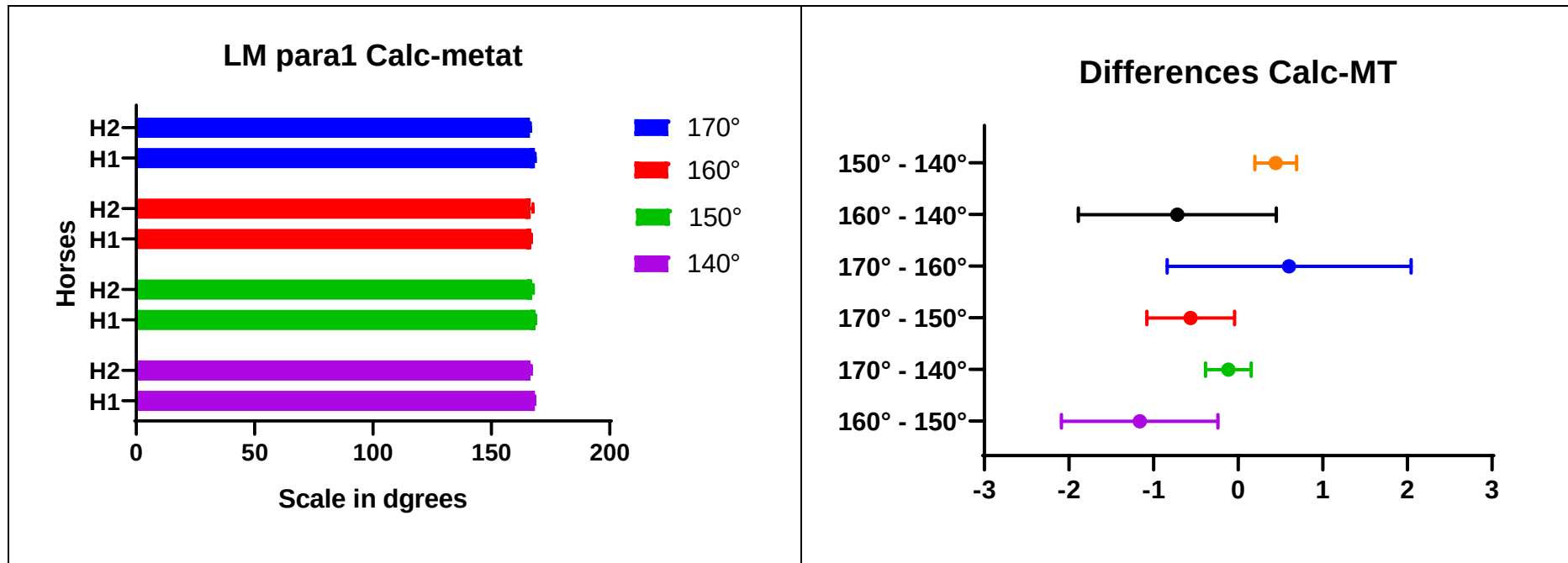
Figure 7. 7: Dorsoplantar (DP) radiograph of a right tarsus of a thoroughbred showing the measurement of the Tarsometatarsal- proximal intertarsal joint angle calculation (yellow lines) from an image acquired from loading position DFA170°(A) and DFA140°(B).

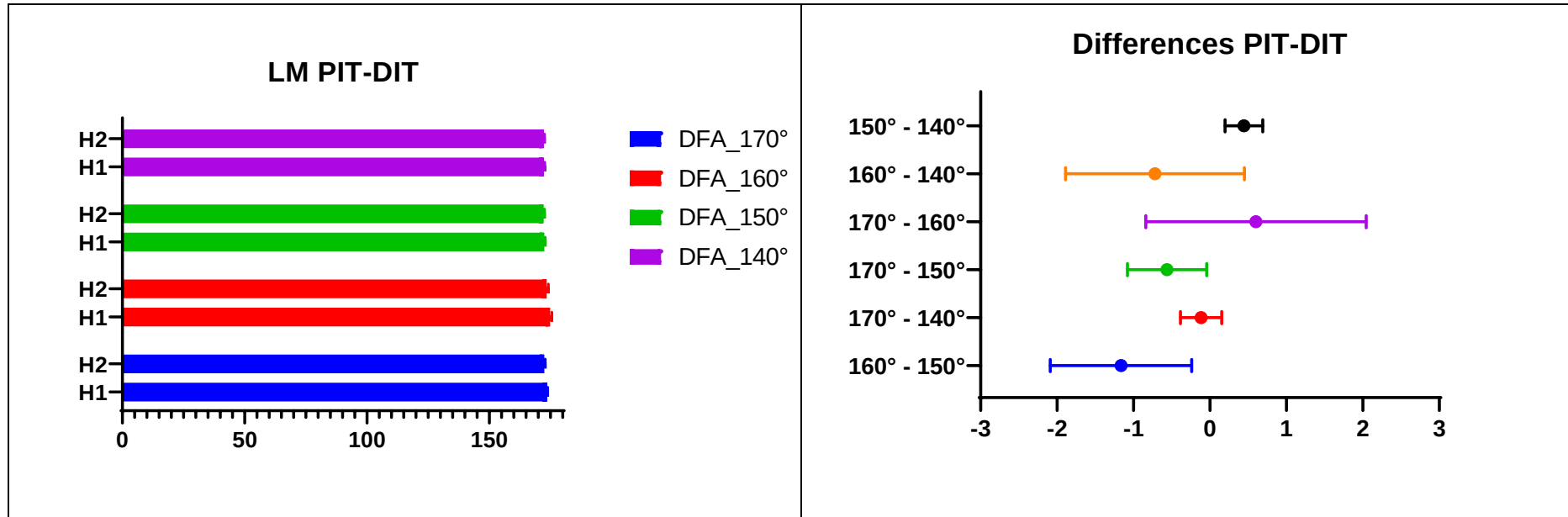
Table 7. 1: Mean, standard deviation, standard error of the mean, 95% confidence interval of the mean and the mean difference for pairwise comparisons of LM radiographic tarsal parameters measured at 170 ° and the other different loading positions (angles) of the 2 cadaver hindlimbs. The loading positions were determined by goniometric measurement of the dorsal fetlock angle (DFA) (DFA170° - DFA140°).

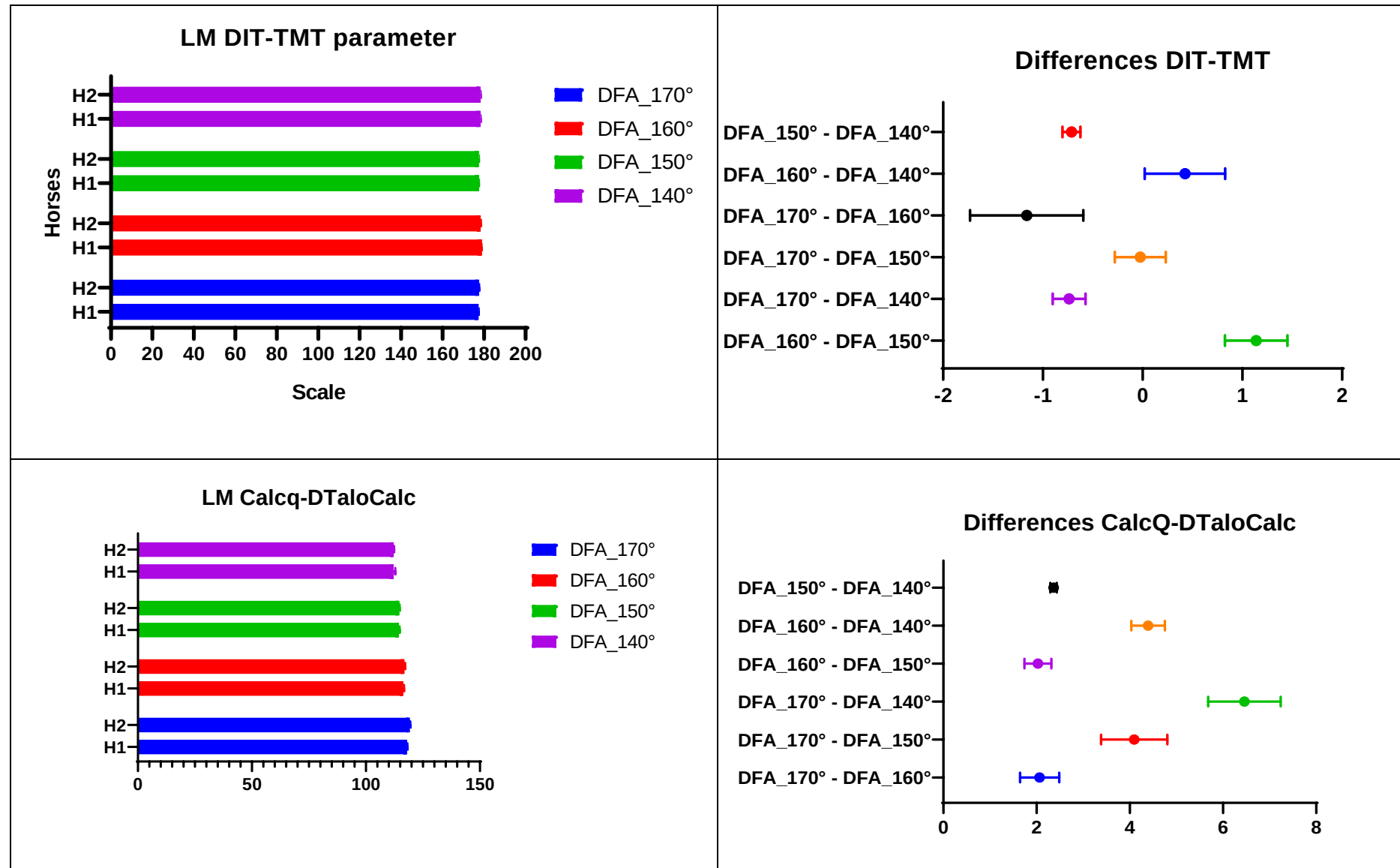
PARAMETERS		DESCRIPTIVE STATISTICS								
		DORSAL FETLOCK ANGLES (DFA)	Means ± SD	Std. Error Mean (SEM)	95 % CI of Mean		LOADING POSITIONS OF DFAs	PAIRED MEAN DIFFERENCE (ZLM-H0°- OTHERS) ± SD.	Std. Error Mean (SEM)	P value P<0.05
					Lower	Upper				
CALCaneo- METATARSUS ANGLE	JOINT	170°	167.3 ± 1.54	1.09	166.2	168.4	170° vs. 160°	0.60	1.020	0.923
		160°	166.7 ± 0.10	0.07	166.7	166.8	170° vs. 150°	-0.56	0.367	0.622
		150°	167.9 ± 1.03	0.73	167.2	168.6	170° vs. 140°	-0.12	0.192	0.921
		140°	167.4 ± 1.27	0.90	167.4	168.3	160° vs. 150°	-1.16	0.653	0.559
		-	-				160° vs. 140°	-0.72	0.828	0.833
		-	-				150° vs. 140°	0.45	0.175	0.421
PROXIMAL INTERTARSAL-DISTAL INTERTARSAL ANGLE	JOINT	170°	173 ± 0.91	0.642	165	181	170° vs. 160°	-1.13	0.055	0.05*
		160°	174 ± 0.99	0.697	165	183	170° vs. 150°	0.755	0.478	0.609
		150°	172.2 ± 0.23	0.163	170	174	170° vs. 140°	0.795	0.592	0.675
		140°	172.3 ± 0.07	0.05	172	173	160° vs. 150°	1.88	0.533	0.316
		-	-				160° vs. 140°	1.92	0.647	0.368
		-	-				150° vs. 140°	0.04	0.113	0.979
DISTAL INTERTARSAL- TARSOMETATARSAL JOINT ANGLE	170°	177.5 ± 0.17	0.117	176	179	170° vs. 160°	-1.162	0.402	0.377	
	160°	178.7 ± 0.40	0.285	175	182.3	170° vs. 150°	-0.023	0.180	0.999	
	150°	177.5 ± 0.09	0.063	176.7	178.3	170° vs. 140°	-0.736	0.117	0.181	

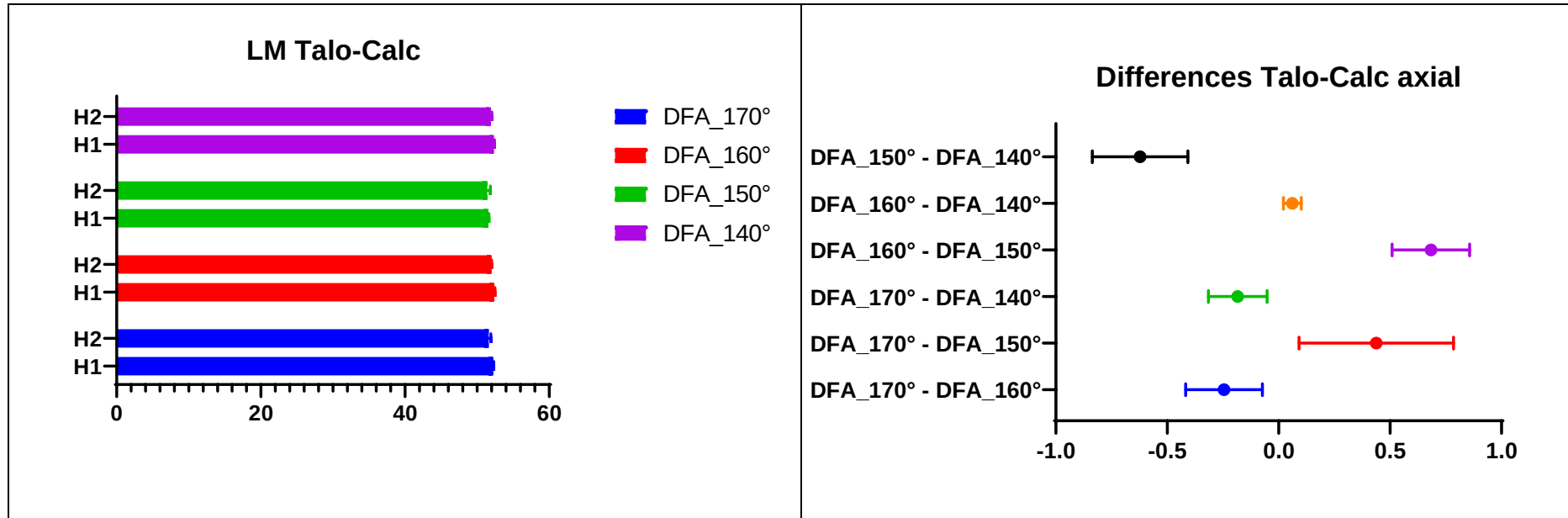
	140°	178.2 ± 0.00	0	178.2	178.2	160° vs. 150°	1.138	0.222	0.222
	-	±				160° vs. 140°	0.425	0.285	0.632
	-	±				150° vs. 140°	-0.713	0.063	0.103
CALCANEOQUARTAL-DISTAL TALOCALCANEAL JOINT ANGLE	170°	118.6 ± 0.81	0.573	111.3	125.9	170° vs. 160°	2.067	0.297	0.165
	160°	116.6 ± 0.39	0.276	113	120.1	170° vs. 150°	4.095	0.502	0.141
	150°	114.5 ± 0.10	0.071	113.6	115.4	170° vs. 140°	6.460	0.550	0.099
	140°	112.2 ± 0.03	0.023	111.9	112.5	160° vs. 150°	2.028	0.205	0.117
	-	-				160° vs. 140°	4.393	0.253	0.066*
	-	-				150° vs. 140°	2.365	0.048	0.021**
TALO-CALCANEAL AXIAL ANGLE	170°	51.91 ± 0.44	0.31	47.97	55.85	170° vs. 160°	-0.245	0.122	0.510
	160°	52.16 ± 0.27	0.188	49.76	54.55	170° vs. 150°	0.438	0.245	0.557
	150°	51.47 ± 0.09	0.065	50.65	52.3	170° vs. 140°	-0.183	0.093	0.519
	140°	52.09 ± 0.31	0.216	49.34	54.85	160° vs. 150°	0.683	0.123	0.206
	-	-				160° vs. 140°	0.062	0.028	0.479
	-	-				150° vs. 140°	-0.622	0.152	0.274
DORSAL HEIGHT OF CENTRAL TARSAL/ DORSAL HEIGHT OF 3RD TARSAL RATIO PARAMETER	170°	0.95 ± 0.01	0.007	0.865	1.035	170° vs. 160°	0.050	0.003	0.077*
	160°	0.90 ± 0.01	0.003	0.858	0.942	170° vs. 150°	0.053	0.003	0.072*
	150°	0.90 ± 0.01	0.003	0.854	0.939	170° vs. 140°	0.042	0.002	0.041**
	140°	0.91 ± 0.01	0.005	0.845	0.972	160° vs. 150°	0.003	-0.017	<0.00**
	-	-		0.865	1.035	160° vs. 140°	-0.008	0.002	0.227
	-	-				150° vs. 140°	-0.012	0.002	0.164

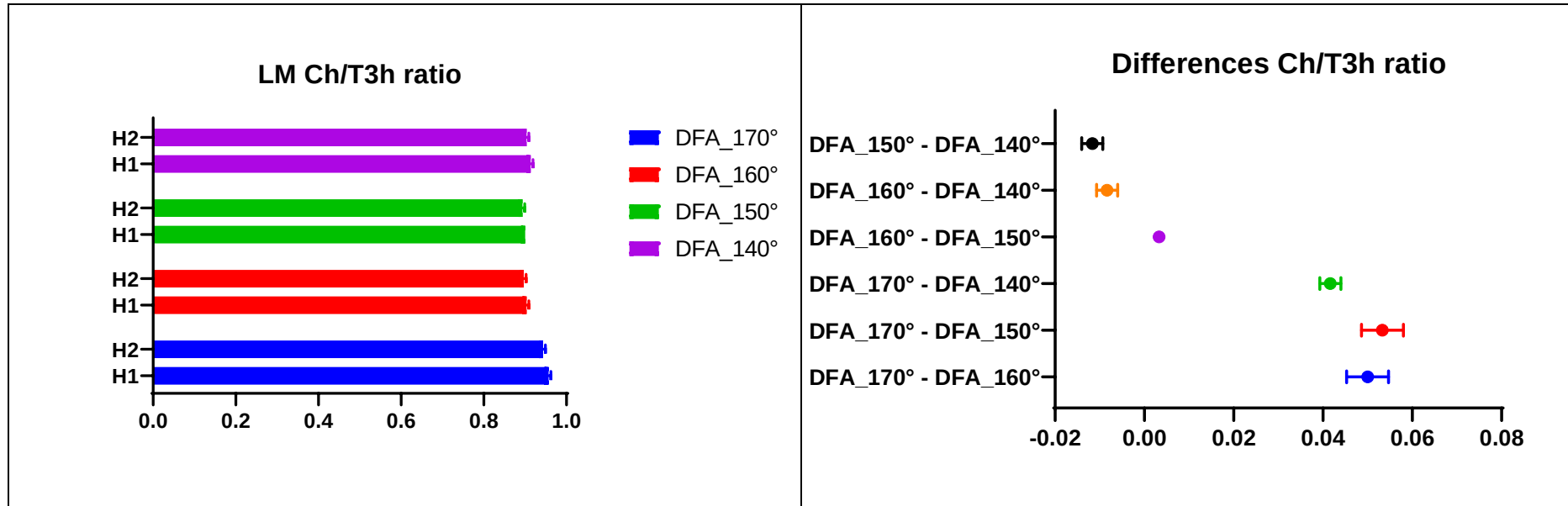
Figure 7. 8: Graphs of the tarsal LM view radiographic parameters in comparative loading positions of DFA 170° to 140° in both horses (H1 and H2) on the left with their respective graphs on the right showing the confidence interval for the mean differences in different loading positions such as 150° vs 140° to 160° vs 150° with mean differences (on x-axis).











RADIOGRAPHIC MEASUREMENTS OF THE LOADED TARSUS IN DP VIEW:

Different types of measuring parameters including correlated angular parameters and ratio parameters for the DP view were included in this pilot study. Generally, there was a decrease in the values of three out of eight DP parameters when the tarsus was loaded (Table 7.2). This was not including the TMT-Tt Joint angle which showed an increase in values from $181.3^{\circ} \pm 1.41^{\circ}$ with the dorsal fetlock angle (DFA) at 170° to $185.7^{\circ} \pm 0.68$ with the DFA at 140° so had a maximum difference of more than 4° . The TMT-DIT joint angle also showed a slight increase with a difference of 1° in different loading positions. Regardless of whether there was an increase or decrease of the values, the measurements demonstrated that the TMT-PIT joint angle and TMT-PC joint angle were the least affected angles during loading of the tarsus. The TMT-Tt Joint angle changed significantly (<0.05) during 150° vs. 140° from $184.7^{\circ} \pm 0.71^{\circ}$ to $185.7 \pm 0.68^{\circ}$ in the loaded joint. The maximum difference of 4.3° was observed in TMT-Tt Joint Angle values as the initial value was $181.3 \pm 1.41^{\circ}$ at DFA 170° position which increased to $185.7 \pm 0.68^{\circ}$ at DFA 140° . A progressive increase in TMT-Tt Joint angle, TMT-DIT Joint angle and decreases in TMT-Tm Joint angle, Lat. /Med.C.h ratio and Lh/Mh ratio parameter were observed (Figure 7.9) as load increased on the cadaver hindlimb (i.e. DFA170 to DFA140).

There were significant changes ($P < 0.05$) in the values of some correlated angular radiographic parameters during the increased loading of joint. TMT-Tm Joint angle, TMT-PIT Joint angle, TMT-DIT Joint angle showed non-significant changes during all loading positions of the tarsus with changes of less than 2° while TMT-Tt and TMT-PC Joint angles showed significant differences ($P < 0.05$) in the values in loading positions of DFA150°-140° and DFA170°-160° respectively. Among the ratio parameters, Lat./Med.h.T3 ratio showed no significant differences ($P < 0.05$) in the values in all loading positions of DFA170°-140°. In other

ratio parameters, the Lat. /Med.Ch. ratio showed a slight decrease in DFA170°-140° loading positions from 0.99 ± 0.00 to 0.95 ± 0.01 with significant difference ($P < 0.05$) in the DFA 170° vs. 140° positions. The Lh/Mh ratio parameter also showed significant changes ($P < 0.05$) in loading positions of DFA 160° vs. 140°. The distribution of these parameters was similar for both cadaver limbs. The graphs show the 95% confidence interval for the mean differences in different loading positions. The bar graphs represent the distribution of each parameter at the 4 different loading positions measured in both horses (Figure 7.9).

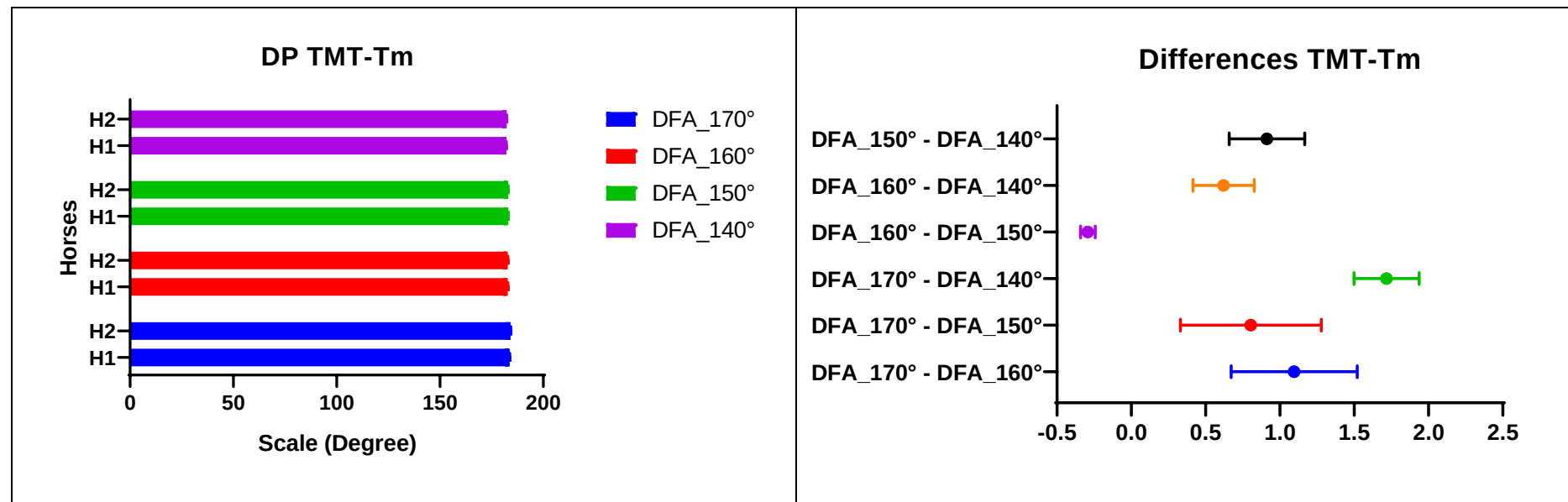
Table 7. 2: Mean, standard deviation, standard error of mean, 95% confidence interval of mean and the mean difference for pairwise comparisons of DP radiographic tarsal parameters measured at 170 ° and the other loading positions (angles) of the two cadaver hindlimbs. The loading positions were determined by goniometric measurement of the dorsal fetlock angle (DFA) (DFA170° - DFA140°).

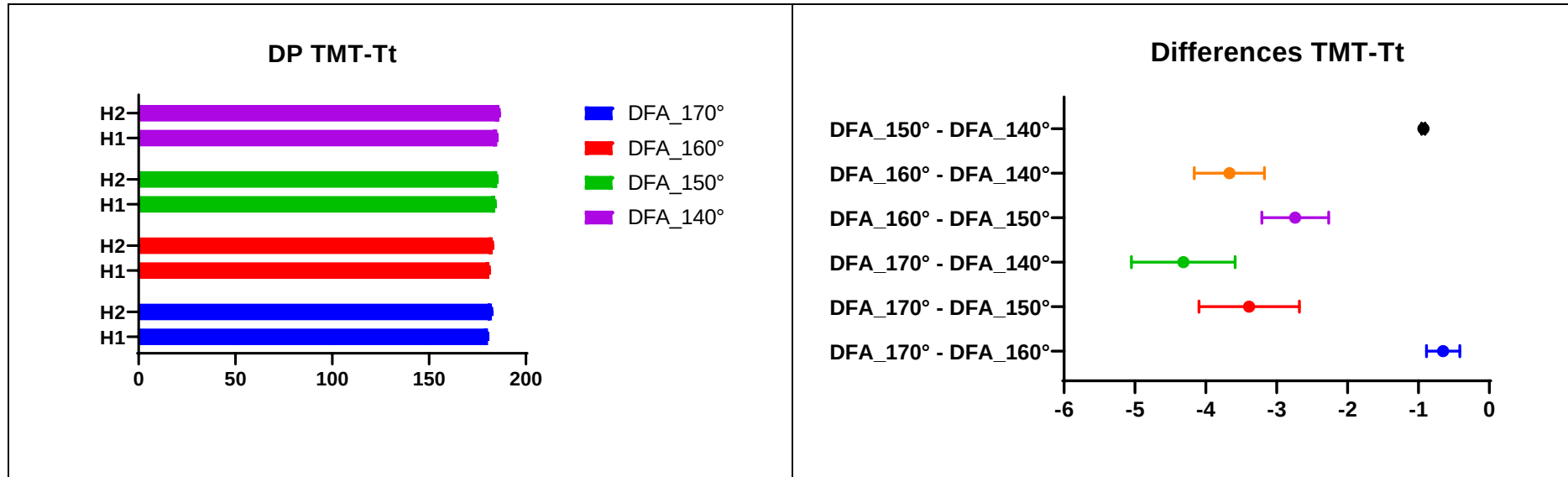
PARAMETERS		DORSAL FETLOCK ANGLES (DFA)	DESCRIPTIVE STATISTICS							
			Means ± SD	Std. Error Mean (SEM)	95 % CI of Mean		LOADING POSITIONS	PAIRED MEAN DIFFERENCE (ZLM-H0°- OTHERS) ± SD.	Std. Error Mean (SEM)	SIG. (2- TAILED) P<0.05
					Lower	Upper				
TMT-Tm Angle	Joint	170°	183.9 ± 0.42	0.3	180.1	187.7	170° vs. 160°	1.097	0.300	0.305
		160°	182.8 ± 0.00	0	182.8	182.8	170° vs. 150°	0.805	0.335	0.442
		150°	183.1 ± 0.05	0.035	182.6	183.5	170° vs. 140°	1.718	0.155	0.104
		140°	182.2 ± 0.21	0.145	180.3	184	160° vs. 150°	-0.292	0.035	0.138
		-	-				160° vs. 140°	0.622	0.145	0.263
		-	-				150° vs. 140°	0.913	0.180	0.224
TMT-Tt Angle	Joint	170°	181.3 ± 1.41	1	168.6	194	170° vs. 160°	-0.650	0.167	0.287
		160°	182 ± 1.18	0.8333	171.4	192.6	170° vs. 150°	-3.387	0.500	0.169
		150°	184.7 ± 0.71	0.5	178.4	191.1	170° vs. 140°	-4.317	0.517	0.138
		140°	185.7 ± 0.68	0.4833	179.5	191.8	160° vs. 150°	-2.737	0.333	0.140
		-	-				160° vs. 140°	-3.667	0.350	0.110
		-	-				150° vs. 140°	-0.930	0.017	0.021**
TMT-PIT ANGLE	JOINT	170°	181.3 ± 0.53	0.3717	176.6	186	170° vs. 160°	0.408	0.305	0.676
		160°	180.9 ± 0.09	0.06667	180	181.7	170° vs. 150°	0.122	0.488	0.992
		150°	181.2 ± 0.17	0.1167	179.7	182.6	170° vs. 140°	-0.048	0.395	0.999
		140°	181.3 ± 0.03	0.02333	181	181.6	160° vs. 150°	-0.287	0.183	0.613

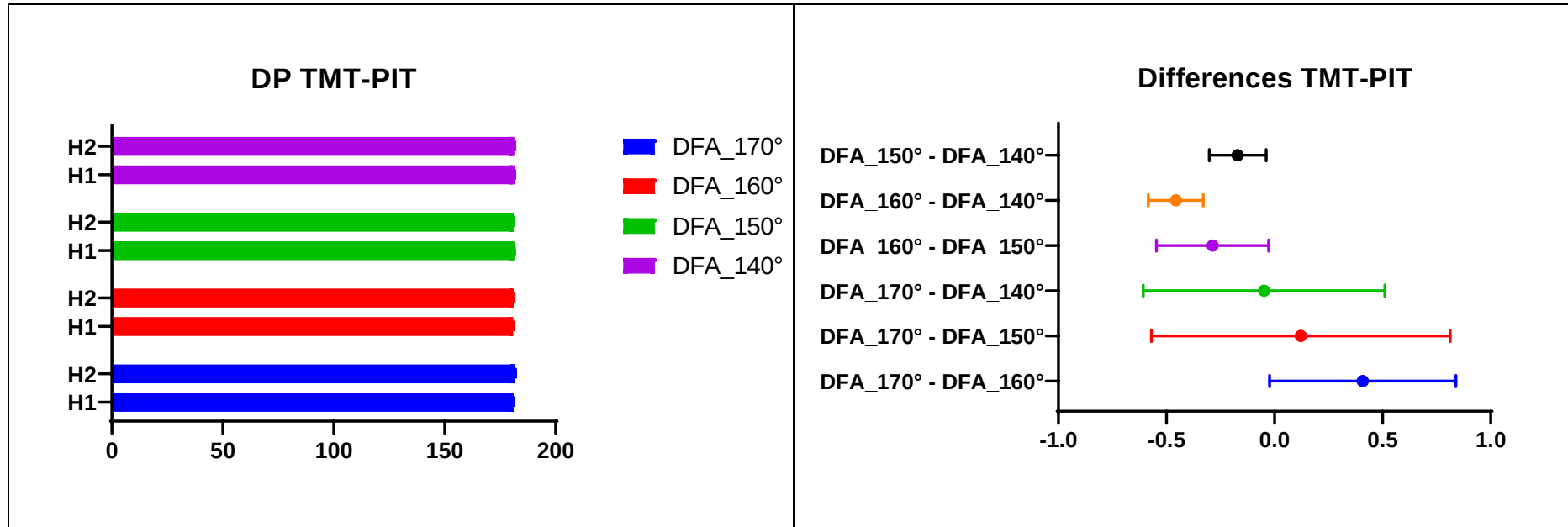
	-	-				160° vs. 140°	-0.457	0.090	0.224
	-	-				150° vs. 140°	-0.170	0.093	0.550
TMT-PC JOINT ANGLE	170°	178.9 ± 0.71	0.5	172.5	185.2	170° vs. 160°	-0.240	0.000	<0.000**
	160°	179.1 ± 0.71	0.5	172.7	185.5	170° vs. 150°	-1.473	0.300	0.231
	150°	180.3 ± 0.28	0.2	177.8	182.9	170° vs. 140°	-1.000	0.483	0.499
	140°	179.9 ± 0.02	0.01667	179.6	180.1	160° vs. 150°	-1.233	0.300	0.274
	-	-				160° vs. 140°	-0.760	0.483	0.610
	-	-				150° vs. 140°	0.473	0.183	0.416
TMT-DIT JOINT ANGLE	170°	178.2 ± 0.81	0.575	170.9	185.5	170° vs. 160°	0.242	0.425	0.930
	160°	178 ± 0.21	0.15	176	179.9	170° vs. 150°	-0.932	0.465	0.512
	150°	179.1 ± 0.16	0.11	177.7	180.5	170° vs. 140°	-0.882	0.382	0.457
	140°	179.1 ± 0.27	0.1933	176.6	181.5	160° vs. 150°	-1.173	0.040	0.032**
	-	-				160° vs. 140°	-1.123	0.043	0.038**
	-	-				150° vs. 140°	0.050	0.083	0.921
Lat. ratio /Med.C.h	170°	0.99 ± 0.00	0.001667	0.9738	1.016	170° vs. 160°	0.017	0.013	0.703
	160°	0.98 ± 0.02	0.015	0.7877	1.169	170° vs. 150°	0.043	0.003	0.089
	150°	0.95 ± 0.01	0.005	0.8881	1.015	170° vs. 140°	0.003	0.000	<0.000**
	140°	0.99 ± 0.00	0.001667	0.9705	1.013	160° vs. 150°	0.027	0.010	0.405
	-	-				160° vs. 140°	-0.013	0.013	0.787
	-	-				150° vs. 140°	-0.040	0.003	0.096
Lat. ratio /Med.h.T3	170°	0.89 ± 0.00	0.003333	0.851	0.9357	170° vs. 160°	-0.013	0.003	0.281
	160°	0.91 ± 0.00	0	0.9067	0.9067	170° vs. 150°	-0.003	0.017	0.996
	150°	0.90 ± 0.02	0.01333	0.7273	1.066	170° vs. 140°	0.008	0.008	0.787
	140°	0.89 ± 0.02	0.01167	0.7368	1.033	160° vs. 150°	0.010	0.013	0.873
	-	-				160° vs. 140°	0.022	0.012	0.542
	-	-				150° vs. 140°	0.012	0.025	0.956

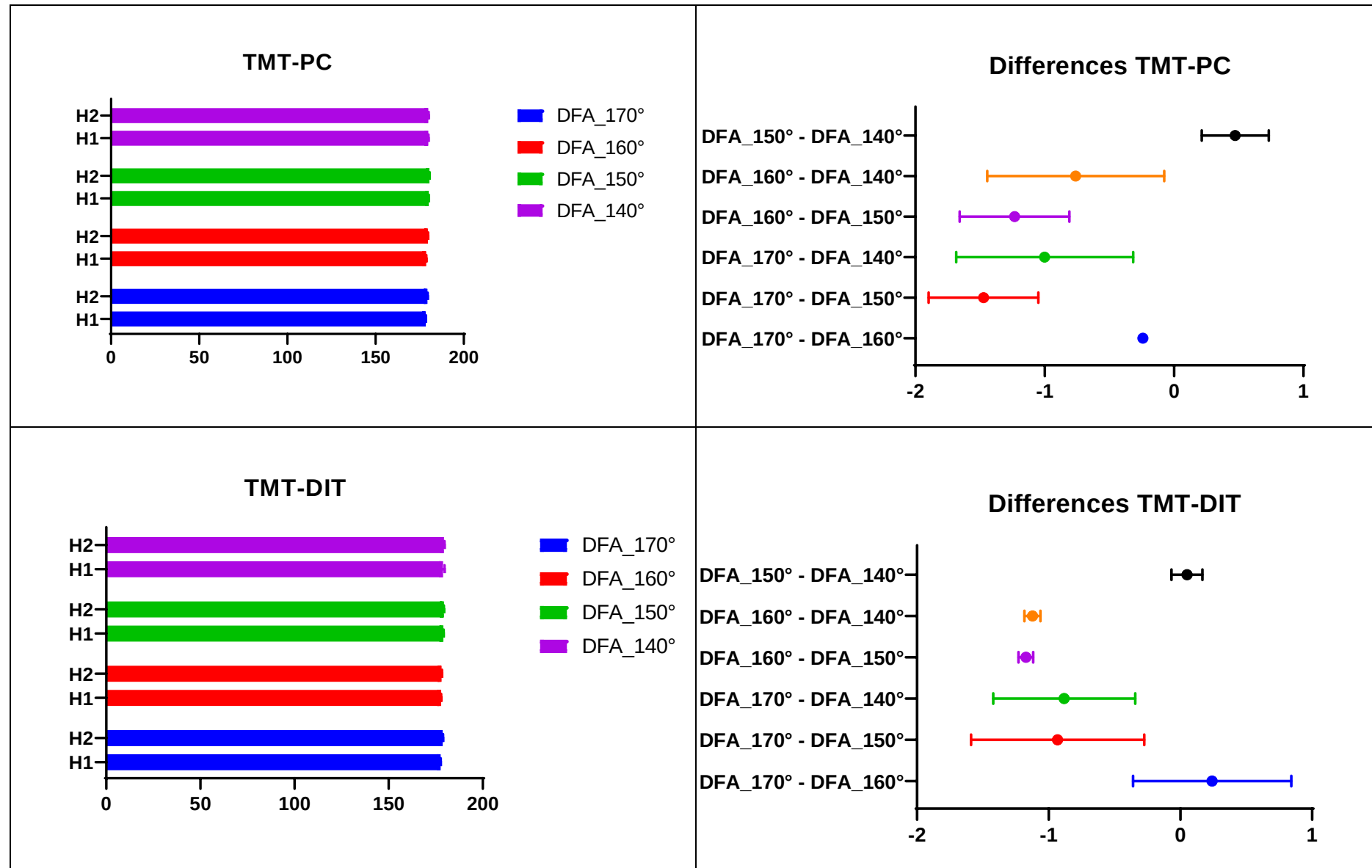
Lh/Mh	170°	1.01 ± 0.01	0.005	0.9415	1.069	170° vs. 160°	0.013	0.007	0.512
	160°	0.99 ± 0.00	0.001667	0.9705	1.013	170° vs. 150°	0.063	0.013	0.239
	150°	0.94 ± 0.01	0.008333	0.8358	1.048	170° vs. 140°	0.100	0.010	0.116
	140°	0.91 ± 0.01	0.005	0.8415	0.9685	160° vs. 150°	0.050	0.007	0.153
	-	-				160° vs. 140°	0.087	0.003	0.038**
	-	-				150° vs. 140°	0.037	0.003	0.105

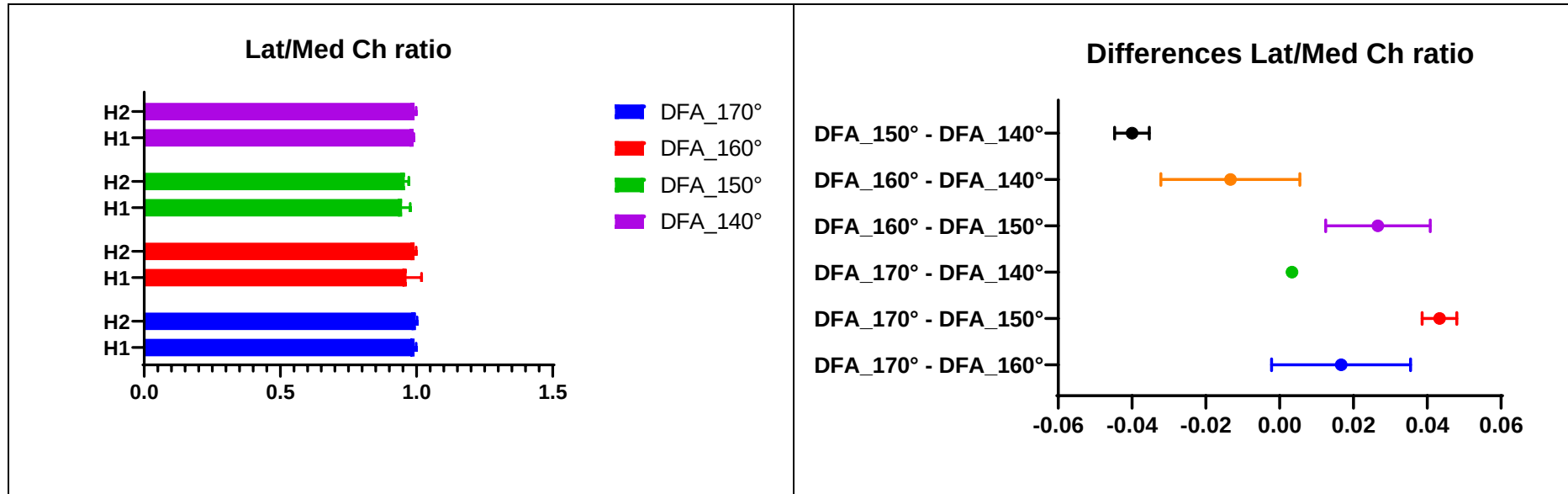
Figure 7. 9: Graphs of tarsal DP view radiographic parameters in comparative loading positions of DFA 170° - 140° in both horses (H1 and H2) on the left with their respective graphs on the right showing the confidence interval for the mean differences in different loading positions such as 150° vs 140° to 160° vs 150° with mean differences (on x-axis).

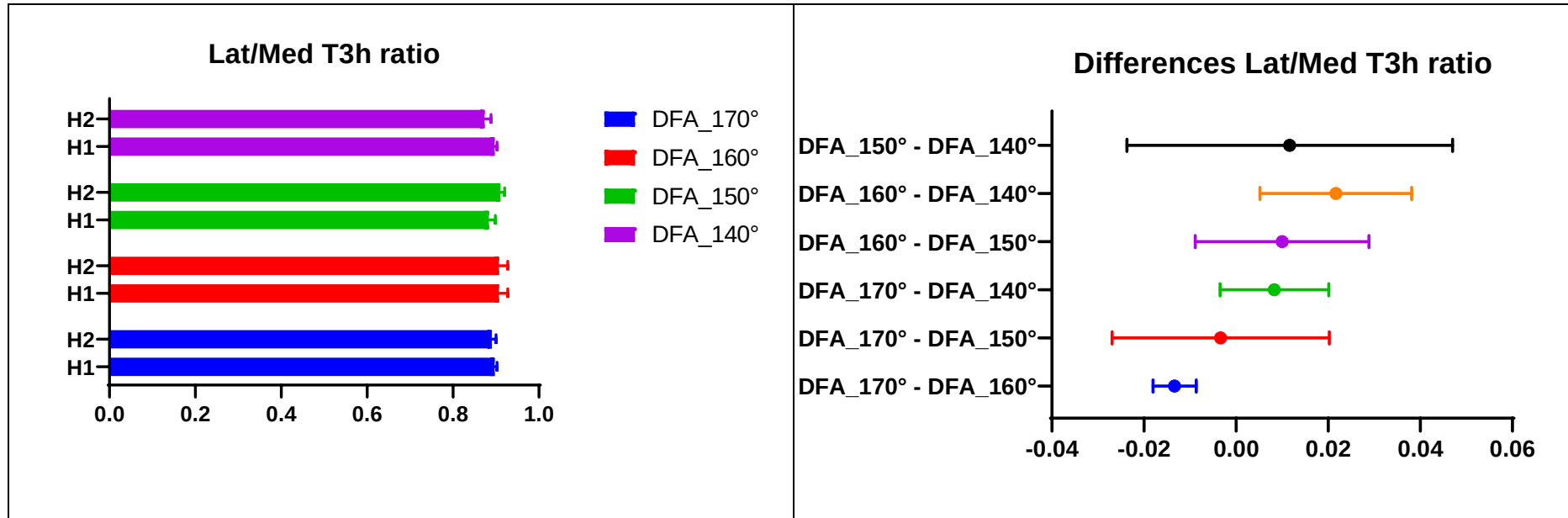












7.4 Discussion

The studies in previous chapters were conducted to identify several landmarks on tarsal LM (Chapter 3) and DP radiographs (Chapter 4) and developed some parameters that can be measured consistently. Effects of rotations were considered for each developed parameter. The better understanding of the relationship between measurable parameters and the limb orientation of the acquired images provide a range of acceptable tarsal parameters. Loading produced measurable radiographic changes in the equine tarsus. Changes in the general alignment of the tarsus and movement of tarsal bones were observed in this study. Bone movements in vivo are generated as a consequence of the neural control of forces which are generated by the muscles. The use of cadaver limb helps to control such factors, possibly leading to a deeper understanding of the natural pattern of the tarsal bone movements during joint loading. Tarsus morphometrical details might provide an opportunity to predict the likelihood of musculoskeletal injury and further imbalanced loading in the distal hindlimbs.

This experiment showed that there were significant changes in parameters including the individual, correlated and ratio measurements in different tarsus loading positions. On the other hand, almost all the individual angular parameters did not change significantly as the loading of tarsus proceeded. Hence, archival radiographs within the range of acceptable orientations may be used to investigate the relationship between morphology and bone response under incremental loads. Therefore, the effect of the loading on bone positions and any change in the acceptable range of developed parameters should be evaluated to facilitate studying the morphometry of this joint on radiographs.

The loading apparatus provided an opportunity to exert simple axial loading on the cadaveric hind limbs that was somewhat constrained because of the angle of the tibia, and

representation of various stages of the axial loading of the tarsus was evaluated through measuring the dorsal fetlock angle. The dorsal fetlock angle (DFA) measures the extension of the fetlock joint. This angle has been measured through photography, radiography and by using goniometers in live animals (Adair III, Marcellin-Little, & Levine, 2016; Alrtib AM, 2015; Liljebrink & Bergh, 2010; C. W. McIlwraith, Anderson, & Sanschi, 2003; Weller, Pfau, May, & Wilson, 2006). The DFA has been reliably used in other studies to give an indication of loading on the carpus (Colahan, Turner, Poulos, & Piotrowski, 1988; Palmer, Bertone, & Litsky, 1994).

The loaded tarsal joints were captured radiographically and subsequently studied. The use of cadaveric limbs was helpful to avoid rotations of tarsal bones because of complex sensory and other control mechanisms. In several studies of bone geometry, alignment, morphology and biomechanics of joints in humans and animals such specimens have provide necessary materials (Brama, Karssenbergh, Barneveld, & Van Weeren, 2001; Colahan et al., 1988; Garcia-Elias, An, Amadio, Cooney, & Linscheid, 1989; Palmer et al., 1994; Sakane et al., 1999).

The current experiment was conducted to identify any changes of alignment and tarsal bone movement under loading in absence of any external control mechanism using certain developed parameters in both LM and DP views. The calcaneoquartal- Distal Talocalcaneal joint angle, TMT-Tt Joint angle and Dorsal Height of Central Tarsal/ Dorsal Height of 3rd Tarsal ratio parameter were quite helpful in monitoring changes under loading because of their location, their landmarks and relative positions. These parameters showed significant changes in response to increases of loading. The calcaneo-metatarsus joint angle measures the general alignment of the tarsal region viewed from the lateral plane (measured on the LM radiographs) using the intersection of the long axes of the calcaneus and third metatarsal bone. Lanovaz et al. 2002 performed a study to describe the 3D kinematics of the tarsal joint

by measuring the motion of the tibia and third metatarsus. Their targets were attached directly to the bones via steel pins in four sound Quarter horses. The 3D motion of the tibial and third tarsal bones were recorded as each subject trotted in hand. Translations and rotations were observed within the tarsal joint, although the majority of tarsal motion occurred in the tarsocrural joint (Lanovaz et al., 2002), but that study lacked information regarding the small tarsal bones movements within the joint and any changes in their alignment.

The tarsal joint angular motion has been measured in many studies but there have been a limited number of studies into the translational motions within the tarsal joint. Ito et al. 2017 demonstrated that the talus is medio-inferiorly translated in the human foot, causing internal rotation of the tibial bone while the cuboid was everted concurrently with the calcaneus bone because of the osseous locking mechanism in the calcaneocuboid joint under axial loading (Ito et al., 2017). The wedge shapes of the short cuboidal carpal bones are believed to facilitate and ease sliding movements of the joint components into a “locked up position” during weight bearing. This shape also acts as a stop or barrier to limit overextension of the joints when fully slid into position. This sort of mechanism is believed to provide loading stability to the carpus (Deane & Davies, 1995a). This might be a reason that the load is transmitted through the carpal joint via the articular surfaces as they were in contact with each other rather than through the subchondral bone and the soft tissues (Palmer et al., 1994). The proximal Intertarsal, Distal Intertarsal and Tarsometatarsal joint angles were measured by the best fit lines formed by proximal and distal articular surfaces of the tarsal bones. There was a statistically significant change ($P < 0.05$) of the proximal intertarsal-distal intertarsal joint angle between loading positions DFA 170°-DFA160° which highlights a

significant shift of internal alignment of this joint. The proximal surfaces of all the central tarsal, third tarsal and third metatarsal bones are concave while the distal surfaces of the first two are slightly convex suggested that slight lateromedial rotations might change the angle values. A decrease in Calcaneoquartal- Distal Talocalcaneal Joint angle and Proximal Intertarsal-Distal Intertarsal Joint angles also indicated that increased loading is associated with movement of the tarsal bones and ultimately changes in their respective internal angulation within the tarsal joint.

Generally small changes in measurements of the majority of the parameters were observed and non-significant changes as the loading of the tarsus proceeded in this study. Schamhardt et al. (1984) performed an x-ray stereophotogrammetric (in vitro) study of the tarsal joint under static loading. Only submillimetre translations were found in any of the bones even when the limb was loaded more than bodyweight. However, no detailed information regarding the loading procedure was provided which might have potential errors in this study (Schamhardt, Hartman, & De Lange, 1984). Other studies have used multiple high-resolution cameras to capture joint angles from horses on treadmills or towards the finish line of a race to show the degree of joint extension (Burn, Portus, & Brockington, 2006; Deane & Davies, 1995b; Johnston, Drevemo, & Roepstorff, 1997). Above mentioned methods have some drawbacks because of associated errors, such as the positions of cameras, placement of skin markers or steel pins with their associated errors. In addition, these methods are more expensive and require extensive image processing and software analysis, and the inherent likelihood of noise in the kinematic data.

The cranial margin of the third tarsal was believed to fracture when medial rotation of the third tarsal bone commenced before the central tarsal thus causing compression on its cranial

margin (Rooney, 1969). This kind of asynchronous movement among tarsal bones might be initiated by interosseous ligament damage or faulty conformation of articular surfaces (Lindsay, McMartin, & McClure, 1982; Rooney, 1969). A decrease in the value of the Dorsal Height of Central Tarsal/ Dorsal Height Of 3rd Tarsal Ratio Parameter was expected as loading increased from DFA160° to DFA110°, a similar decrease of this ratio parameter was observed in the current study. The continuous convex dorsomedial borders of the central and third tarsals and any slight lateromedial movement during loading might be another reason for change in relative heights projections of dorsal heights in LM or DP views. Although complete mediolateral rotations of tarsal bones were difficult to evaluate in one aspect only so, the combined use of lateromedial and dorsoplantar views is suggested for evaluation of such changes.

In a previous study, Dahn and Ueltschi 1989 estimated the heights of the central and third tarsal bones and compared between male and female. The heights of the central tarsal and the third tarsal bone was slightly lower in the mares (14.2 and 14.0 mm respectively) compared to the males (14.8 and 14.6 mm respectively) (Dahn & Ueltschi, 1989). However, the measurements taken were linear, and no information was provided for reliable landmarks, effect of distance of radiography on acquired images and effects of different sized tarsal joints within the sample population was not addressed.

The investigation of differences in joint morphometry also offer easily measurable parameters like Proximal intertarsal-Distal intertarsal joint angle, Distal intertarsal- tarsometatarsal joint angle and dorsal height of central tarsal/ dorsal height of 3rd tarsal ratio parameter of LM view which responded to the influence of load changes through the joint. Such findings suggest the importance of conformation of articular surfaces and their internal alignment for

the proper handling of load transmission through this region. Tarsometatarsal- proximal intertarsal joint, Tarsometatarsal-proximal central joint, Distal intertarsal-tarsometatarsal joint and Tarsometatarsal- tibial metaphyseal/malleolar joint angles might all be useful to indicate the likelihood of certain pathologies as based on their locations of landmarks which are commonly effected areas of tarsus and assist in the choice of good performance horses but needs further detailed investigations.

Several limitations of the present pilot study should be considered. The proper static axial loading is still difficult because of the angle of the tibia as compared to the forelimb. However, the static loading regime in this study probably does not accurately simulate physiological conditions and it does not address the action of the joint during load bearing standing position of the hind limb. It must also be acknowledged that equine cadaver limbs do not represent the complete function of the hindlimb because of dealing with a complex biological system where other components of the hindlimb will also affect the tarsal joint biomechanics. In this study, equine cadaver hindlimbs were collected based on availability.

The results were found consistent in both limbs but the collection of racing or athletic history and information on each equine such as age and medical history, would have been more beneficial addition to this study. However, more samples from thoroughbred and other breeds could provide better representation of loading changes and alignment of tarsus along with their normal variations among a larger population of horses. The use of any scale with labelled values in both x any y axes was found to be helpful in additional observation of any changes in the coordinate tarsal positions and change of tarsal alignment like in the direction of tarsal movement (displacements).

The loading procedure is also worth commenting on in regard to axial loading on the joint. The fresh specimens were used in this study without previous freezing or preservation. The loads were applied to a certain extent because of the tibial bone angulation. More sophisticated equipment can address such issues to apply loads on bone and plantar structures, closer to in vivo situation. In addition, a single investigator was responsible for all procedures, measurements and recordings in this study which could have led to a chance of unintentional bias. However, it may also be questioned whether the same or significant different results could be obtained by another investigator performing the same study. Repeat measurements for each parameter were taken and in a previous study interrater repeatability and variability of radiographic measurements were assessed. Those results demonstrated that there were no significant differences in measurements between the three independent investigators. In addition, our method using a radiographic technique can be a powerful tool to investigate kinematic changes of the tarsus. The clinical relevance of the hindlimb motion during natural full weightbearing should be further studied.

In conclusion, the overall combined results from the current pilot study suggest that the movements of tarsal bones within the tarsal joint and their support for joint stability is more complex than what is currently understood in terms of tarsal bones responses to loading and the anatomy of the joint. We successfully clarified the changes in tarsal bones alignment occurring during increased loading of the tarsus, although the present study cannot assess whether movements of the tarsal bones occurred because of the articular surfaces of the bones and their osseous morphology, along with distribution and configuration of soft tissues like ligaments and fascia. The possible contribution of the soft tissue morphology in normal biomechanics of tarsal joint has generally been overlooked and is not well understood. Such

detailed descriptions about the innate mobility of the tarsus along with the support of soft tissue during the loading and stress bearing conditions will contribute to clarifying functional adaptation and pathogenic mechanisms of this complex joint. In addition, the role of supporting soft tissue structures of the joint and their connectivity with other structures in the hindlimb may provide a more detailed understanding of how the tarsal joint responds to load. Hence, these factors should be investigated in future studies.

Chapter 8: Morphometric and observational analysis of the intertarsal ligaments and functional simulation of osteoligamentous preparations of the equine tarsal joint

8.1 INTRODUCTION

The loading of tarsal joint is complex. The function of the equine tarsal joint also largely dependent on the several intertarsal ligaments that provide both stability and elasticity besides the morphology of the tarsal bones (Getty, 1975b; Updike 1984).

The tarsal bones are held together in intimate contact through the attachments of soft tissue, including different types of ligaments such as the intertarsal ligaments, collateral ligaments, plantar tarsal ligament along with the fibrous joint capsule. The ligaments are bands of tough elastic tissue around the joint connecting bone to bone, giving joint support and strength and limiting the joint movement. The medial and lateral collateral ligaments consist of two distinct two parts or bands which cross each other (Auer, 2012). These two bands are termed the long and a short part. The long lateral collateral ligament connects the distal tibia (lateral malleolus) and is attached to the calcaneus and fourth tarsal bones and third and fourth metatarsal bones, but the short lateral ligament is deeper and arises on the cranial part of the lateral malleolus and ends on the rough excavation on lateral surface of talus and calcaneus.

The medial collateral ligament including both longer and shorter parts originates from the medial malleolus, superficially and deep respectively. The long medial collateral ligament arises on the caudal part while the short medial collateral ligament extends from the cranial part of the medial malleolus. The plantar tarsal ligament covers the latero-plantar surface of the tarsus and attaches to the plantar surface of the calcaneus. The dorsal tarsal ligament is

a triangular sheet attached to the distal tuberosity on the medial face of talus proximally, and onto the central and third tarsal bones as well as the proximal ends of the third and second metatarsal bones.

A joint or articulation is formed by the union of two bones or cartilages by other tissue chiefly cartilage or fibrous tissue or a mixture of these (Getty, 1975b). The joints can be classified based on their anatomy or function or by a combination of the foregoing considerations. The anatomical classification focuses on the nature of the uniting medium binding the bones together, and the shape of the joint surfaces and whether a joint cavity is present or not, while the functional classification is based on the amount and kind of movement or the absence of mobility in the joint. The degree of mobility between two bones depends entirely on the shape of gap between them (Getty, 1975b; König & Liebich, 2013).

The anatomic structural classification includes fibrous, cartilaginous, and synovial joints while synarthroses, amphiarthroses and diarthroses are examples of joints based on classifications according to physiological functions (Getty, 1975b). The equine tarsal or hock joint is a composite joint, made up of a number of articulations between tibia and fibula, the tarsal bones and the metatarsal bones. These are surrounded by a common fibrous joint capsule which encloses separate synovial sacs (Nickel et al., 1986b). The fibrous layer of the joint capsule covers the whole tarsus extending from the distal end of the crus to the proximal part of the metatarsus. The synovial membrane forms 3-4 synovial sacs for the four levels of articulations including the tarsocrural, intertarsal and the tarsometatarsal articulations (Dyce, K. W. Sack, & C. Wensing, 1987; König & Liebich, 2013).

The tarsus is a composite joint and composed of five joints: the tarsocrural (TC), proximal intertarsal (PIT), distal intertarsal (DIT), tarsometatarsal (TMT), and talocalcaneal (TCal) joints.

Four of these five joints are considered to be almost immobile and the tarsocrural articulation is the only typical mobile joint. Micromovements are reported to occur in the others during ambulation and weight bearing by several authors despite no specific measurement technique for such movements being described in these references (Auer, 2012; Getty, 1975b; Updike, 1984).

These movements are likely to be fundamental to the effective functioning of the tarsal joint. The range of tibiotarsal joint motions is considered very important in humans. Every joint has a normal (ideal) range of motion. As the talus moves laterally, dorsally, and posteriorly on the calcaneus, supination occurs. This supinatory motion was observed to strengthen the joints of the human foot in activities involving weightbearing (Graham, 2018). The equine carpus is also a complex composite joint with 3 major joints and several minor intercarpal joints. The sliding motions of the wedge-shaped carpal bones facilitates smooth transfer of load from bone to ligaments as the intercarpal ligaments attenuate, absorb and redistribute high compressional loading forces. This mechanism is believed to provide loading stability and thus prevent damage to the equine carpus (Boening, 1981; Bramlage, Schneider, & Gabel, 1988; Deane & Davies, 1995a).

Similarly, the directions of the constituent bones of the tarsal joint and the amount of movement that will be allowed within a joint along with tarsal bones displacements during movements depends on the length, elasticity and orientations of the connecting fibres of the ligaments but no detailed study of tarsal joint intertarsal ligaments is available in literature. The better understanding of the passive and dynamic roles of such anatomical structures which contribute to the normal function of the tarsal joint might be very helpful for determination of their role in support and stabilisation of joint during loading and other stress

conditions. This could also provide insight into the effects of surgical procedures on the function of the joint. Furthermore, investigating the anatomical constraints of joint movements, could identify the presence and significance of mechanical stabilisers which would be likely to play a vital role during joint function, and may be involved in the pathology or injury to the tarsal joint.

The aims of this study were:

- i) to monitor the changes in the direction of movements of tarsal bones during simulation of tarsal movements.
- ii) to observe the fiber orientations of these ligaments during simulation of flexions and extensions of ligamentous preparations of the equine tarsal joint
- iii) to provide gross morphometric data for the significant intertarsal ligaments of the tarsus.
- iv) investigate how the range of flexion and extension of the tarsal joint is constrained by the mechanical connections and attachments of ligaments/soft tissue structures.

8.2 MATERIALS AND METHODS

8.2.1 Collection of specimens:

The equine cadaveric hindlimbs (6 hind limbs from 4 Thoroughbreds aged approximately between 3-7 years) were collected from the department of pathology, Faculty of Veterinary and Agricultural Sciences, University of Melbourne. These animals were deceased for reasons unrelated to the study, hence no animal ethics approval was required for this study. All specimens appeared to be healthy upon physical examination and had no previous history of tarsal damage. The hindlimbs were transected through the tibia at about 20-25 cm proximal to the tarsocrural joint and stored in a chiller (5-6 °C) until further processing. No hindlimb was subjected to deep freeze until all ligament measurements had been taken.

8.2.2 Ligamentous preparations of the equine tarsal joint (dissection procedure):

The skin was removed from the pastern region by making a skin incision that encircled the limb at about 25 mm proximal to the coronet and a second longitudinal incision on the dorsal aspect from the leg proximally to the first incision (where the leg was removed). The skin was then sharply dissected away from the subcutaneous tissue (mostly fascia). The remaining soft tissue structures (mainly the extensor retinaculum that envelopes the tarsus) were dissected away from their deeper attachments to reveal the distal tendinous part of the cranial tibial muscle and the other muscles and their tendons (including the peroneus tertius, long digital extensor and lateral digital extensor muscles) on the dorsolateral aspect of the tarsus. The extensor muscles and their tendons were then dissected away to clearly expose the joint capsule.

The dorsal aspect of the joint capsule was exposed after reflection of the distal portion of the cranial tibial and peroneus tertius tendons. The incisions were made to gain access into the tarsocrural and dorsal part of the middle tarsal joint. The joint capsule tissues were then carefully dissected away from the underlying dorsal intertarsal ligaments, and from the adjacent medial and lateral collateral ligaments. The three rows of tarsal bones, and the tibiotalus and other tarsal joint spaces were then exposed from the dorsal aspect (Figure 8.1A).

To gain access to the plantar side of the tarsus, most soft tissues on the medio-plantar aspect of the tarsus and structures that extended through the tendon sheath of the united tendons of the lateral digital flexor and caudal tibial muscles had to be dissected away. The flexor muscle group including the medial digital flexor was excised at the level of their tendinous insertion in the tarsal region.

The superficial digital flexor tendon (SDFT) and deep digital flexor tendon (DDFT) were transected at about 5-6 cm from the fetlock (i.e. proximal to the fetlock) and, along with the biceps femoris or semitendinosus muscle tendons. The common calcaneal tendon comprises the tendons of gastrocnemius and soleus and was transected. The flexor digitorum longus, flexor digitorum profundus, nerves, blood vessels and tarsal sheath were then carefully dissected away from within the tarsal region (Figure 8.1B).

The structures remaining from this dissection were the bones and the ligaments associated with the articulations of the tarsal joint (an osteo-ligamentous preparation of the tarsus) from which measurements of the significant intertarsal ligaments and simulations of flexion and extension movements were made (Figure 8.1 and 8.2).

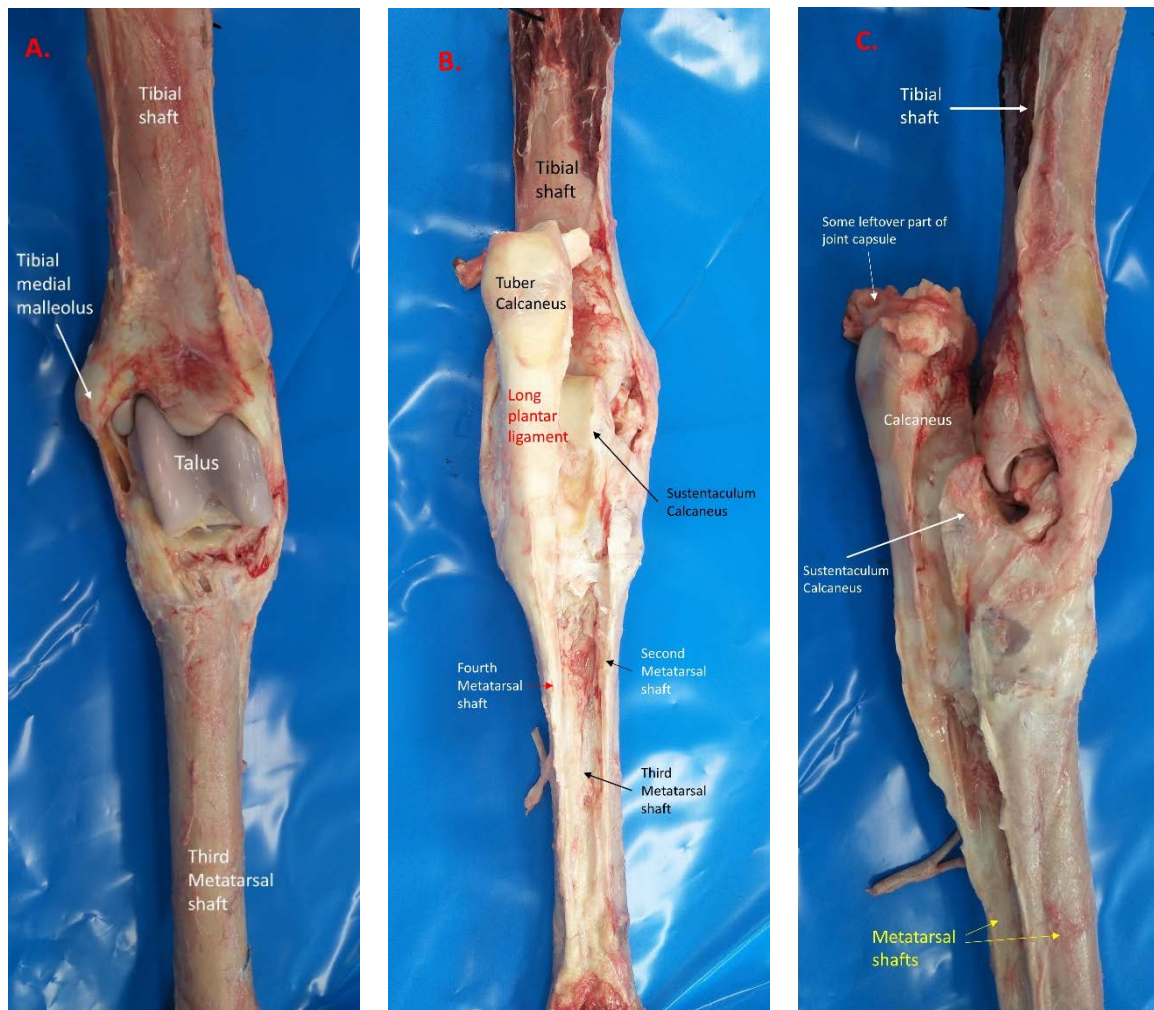


Figure 8. 1: Left equine tarsal joint ligamentous preparation; A. Dorsal view, B. Plantar view, C. Medial view.

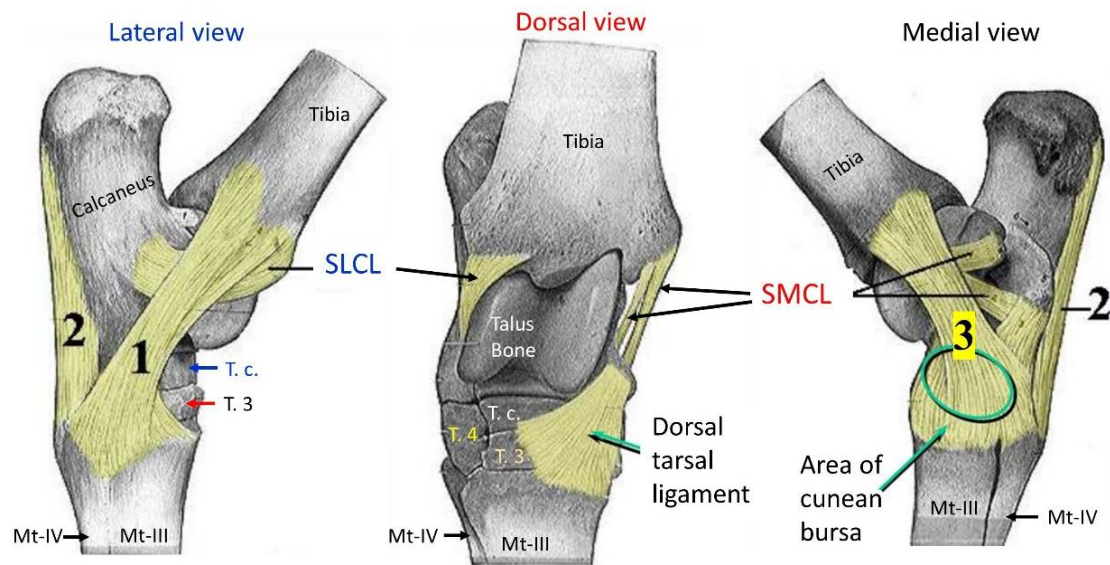


Figure 8.2: Right tarsal joint of horse, images were adapted from (<https://www.slideserve.com/MikeCarlo/equine-hind-limb>) and labelled for demonstration of major tarsal ligaments; lateral, dorsal and medial views.

1. Long lateral collateral ligament, 2. Long plantar ligament, 3. Long medial collateral ligament, SLCL. Short lateral collateral ligament, SMCL. Short medial collateral ligament, T. c. Central tarsal bone, T. 3. Third tarsal bone, T. 4. Fourth tarsal bone, Mt-III. Third metatarsal bone, Mt-IV. Fourth metatarsal bone.

8.2.3 Measurement protocol

8.2.3.1 Gross morphometry of intertarsal ligaments of the tarsal joint:

The length, width and depth (thickness) of the intertarsal ligaments were measured using a string and then calculated by digital Vernier calliper. After measuring the length and width of selected sites for each ligament, further dissections were carried out as necessary to measure the depth of the ligaments and to observe fibre orientations. The lengths were obtained mostly from the points of origin and insertions mostly in a proximodistal direction. Attempts were made where feasible to measure the width of each ligament at three locations viz: at the origin, at mid-length and at its insertion (Figure 8.3A-F). The depth of each ligament was

measure through the depth measuring blade of the Vernier's caliper at three different points along the length and then the average of these measurements was recorded as the depth for each ligament (Figure 8.4).

The following ligaments were measured:

a) Long lateral collateral ligament:

The length of the lateral collateral ligament (LCL) was measured from its origin on the distal lateral tibial malleolus to the insertion point on the talus and fourth and third metatarsal bones. The widths were measured at the point of origin, middle and insertion. The middle width was taken at approximately half of the total length (Figure 8.3A).

b) Short lateral collateral ligament:

The length was taken from the lateral malleolus to the point of insertion where it attaches with one branch to the calcaneus and one branch to the talus. This short ligament runs deep to the long lateral collateral ligament and can be further divided into following two parts (Figure 8.3B).

i, Short lateral Collateral ligament-I (with calcaneal insertion)

ii, Short lateral Collateral ligament-II (with talus insertion)

c) Long medial collateral ligament:

The length was taken between the medial malleolus to the base of the medial metatarsal bones, attaching also to the medial tarsal bones along its course (Figure 8.3C).

d) Short medial collateral ligament:

The length was measured from the medial malleolus to deep to the insertion point on the talus and the branch attached to the calcaneus (Figure 8.3D).

i, Short medial collateral ligament-I (with calcaneal insertion)

ii, Short medial collateral ligament-II (with talus insertion)

e) **Dorsal tarsal ligament:**

The length was measured between the medial aspect of the talus and the dorsomedial surface of the third and fourth metatarsal bones. It is a triangular sheath, which spreads out at the dorsomedial aspect of the tarsus (Figure 8.3E).

f) **Long plantar ligament:**

A very strong, flat band of ligament lies on the plantar aspect of the hock. The length was taken between the distal tuber calcanei to the proximal end of the third and fourth metatarsal bones (Figure 8.3E).

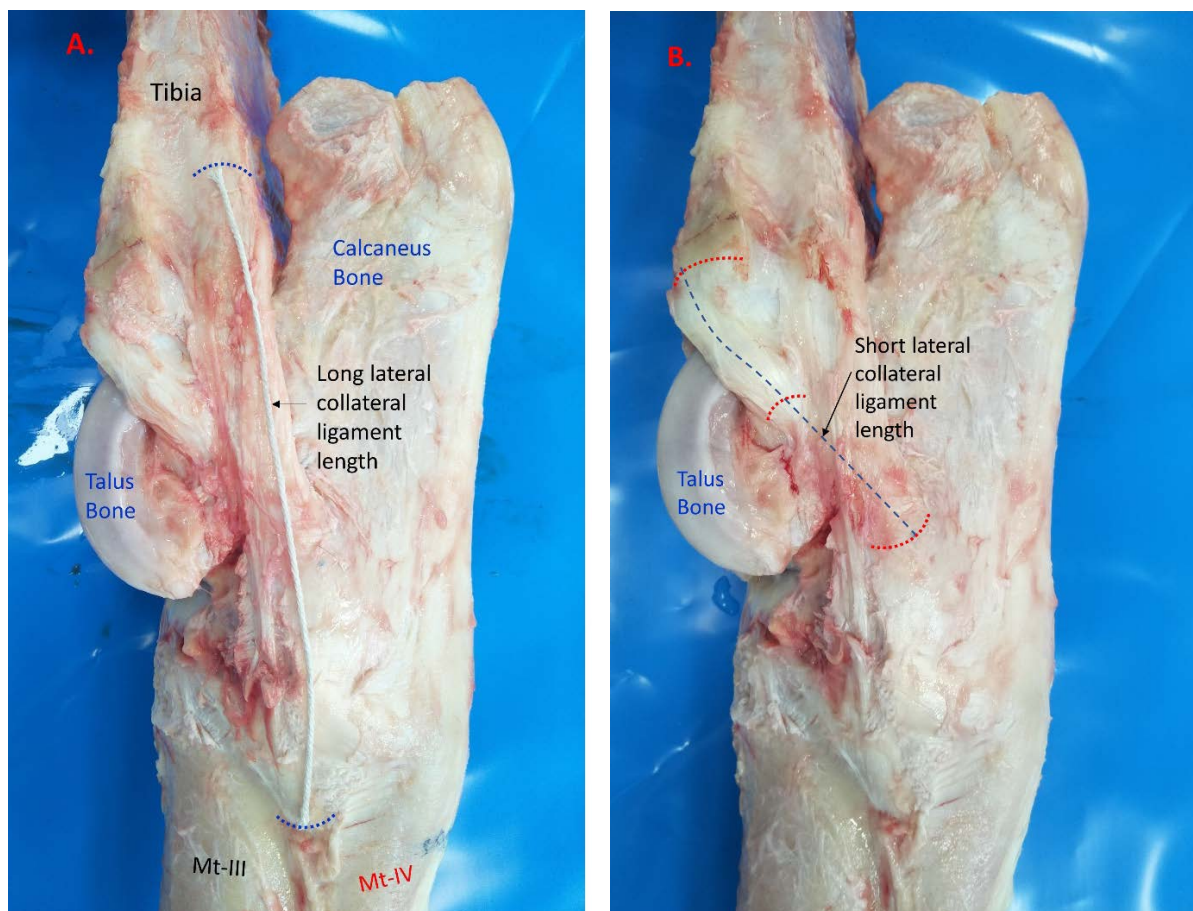




Figure 8. 3: Left tarsal joint of horse; A and B. lateral views of tarsus, the length and widths (at three sites as indicated in the picture) of the long and short lateral collateral ligaments

were measured from their origin at the distal tibial malleolus to its insertion points. C and D. Medial views of tarsus, the length and widths (at three sites as indicated in the picture) of the long and short medial collateral ligaments were measured from their origin at the distal tibial malleolus to their insertions. E, Dorsal view of tarsus, the length and widths (at three sites as indicated in the picture) of the dorsal tarsal ligament were measured from the origin to the insertion as indicated. F, Plantar view of tarsus, the length and widths (at three sites as indicated in the picture) of the long plantar tarsal ligament were measured from the origin to the insertion as indicated.

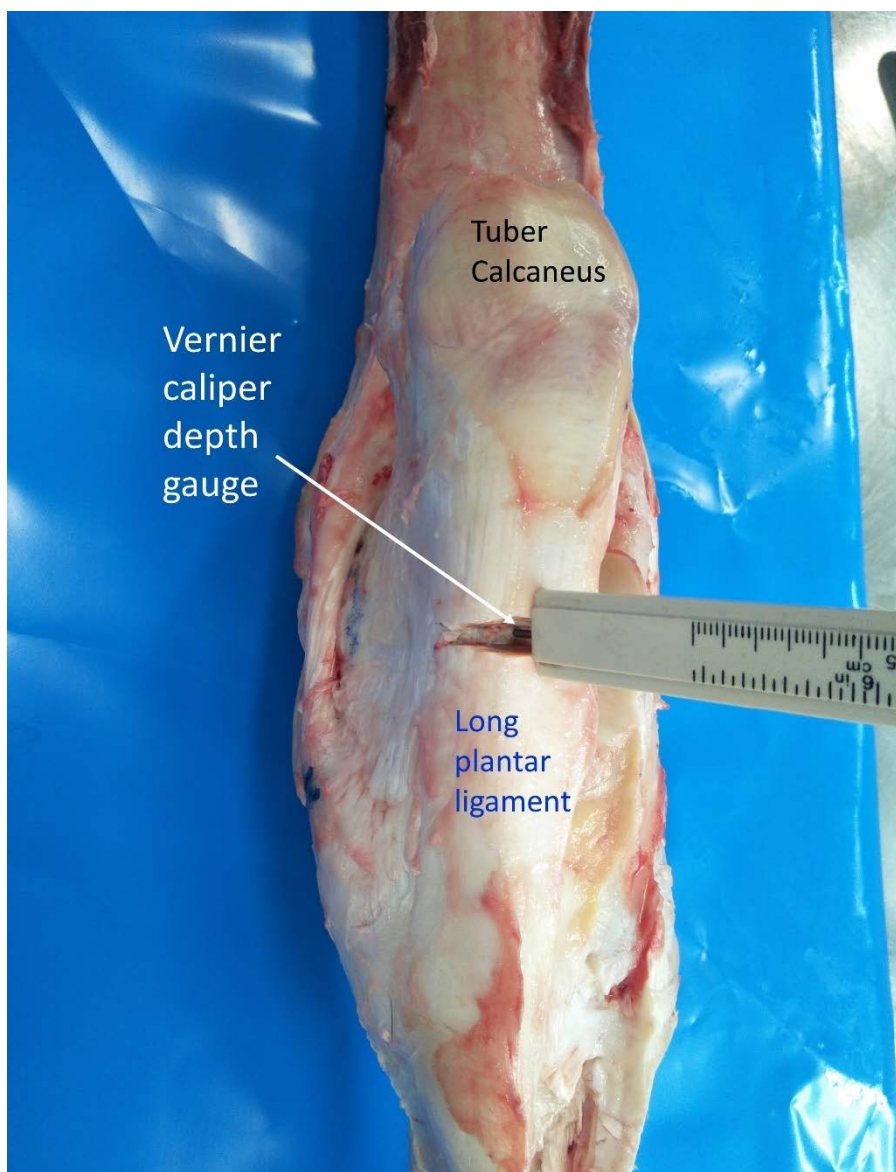


Figure 8. 4: Left tarsal joint of horse; Plantar view of tarsus, demonstration of measuring the depth of the long plantar ligament from the middle of its length.

8.2.4 Simulation of flexions and extensions:

A transparent plastic Prestige Medical® 360° 25-cm goniometer with 1° gradations was used to measure the range of prepared tarsal extensions and flexions. Measurements of extension and flexion of tarsal joint were taken with the hindlimb placed on a flat table, and the lateral side of the hindlimb was on the upper surface. The arms of the goniometer were aligned with the long axis of the tibia (repeatable) over the tibial shaft and the other on the third metatarsal bone by marking the mid-point of the width of shaft at the end of arms of goniometer. The centre of the goniometer was placed onto the centre of the lateral most prominent point (insertion of short collateral ligament) at the calcaneus. The angle between the two arms of the goniometer was recorded for each step.

Prior to the dissection, the investigator took time to initially flex and extend the limbs enough to reduce any rigidity (rigor mortis) which might have caused inconsistency in values following residual effects of external force applied for maximum extension and flexion. To understand the range of motions of the tarsal joint, maximum extension of the joint was initially measured, and was defined as the point of extension at which there was a force applied on the crus and metatarsus, without the use of excessive force that could compromise the integrity of the limb. Both flexion and extension of the tarsal joint were measured following a single motion at the point where a large change in resistance to the joint motion occurred and reached to a point where further joint movement would have required considerable force beyond the normal range in 2 pilot samples to gain some experience and consistency in this operation. The positioning of the goniometers on the tarsal joint landmarks and the handling of the tibia and long third metatarsal during flexion and extension is shown (Figures 8.5-8.8). All measurements were performed after all the dissections listed below. A single investigator

dissected, measured and recorded both the left and right prepared osteoligamentous tarsal joint from a single horse in one session or within the same day. During the measurements with the goniometer, the author (investigator) measured and recorded the range of flexion and extension of one side (left), followed by the other side (right), and then alternating between the two sides. The investigator would only read the angle readings on the goniometer when placed on prepared joint in its respective position. Three measurements were recorded to get the mean value and recorded on separate sheets of paper and replaced every time, so the investigator could not read the previous recordings.

During the course of this study, any specimen with any obvious signs of pathology were noted. The dissection was chosen based on the major ligaments which play an active role in the stability of tarsal joint. The cutting of ligaments was based on their attachments sites which can be easily accessible and longer ligaments were cut at approximately half their length. The list below outlines the steps and order of dissections and measurements, which were identified to be most suitable in this study:

1. Skin of the hindlimb was removed from crural region at approximately half the length of the tibia distally to the hoof.
2. An incision around the crus was made at the level of first skin incision. The incision penetrated through the superficial and upper layers of deep crural fascia covering all the crural muscles.
3. This incision was deepened to include all the connections between the crural region and tarsus including all muscular, tendons and fascial support (Figures 8.5-8.8).

4. The osteoligamentous preparation was made by removing all the tendons with their retinacular bands, joint capsule, and remaining soft tissues crossing the tarsal joint leaving only the tarsal ligaments.
5. The next incision was made on the plantar aspect of the long plantar ligament at approximately half of its length as described previously (Figures 8.3F and 8.4).
6. This incision was made on the dorsal aspect of the dorsal tarsal ligament at approximately half of its length.
7. This incision was made on the lateral aspect of the longer part of the short lateral collateral ligament (attachment of insertion onto calcaneus) at approximately half of its length.
8. This incision was made on the lateral aspect of the shorter part of the short lateral collateral ligament (talus attachment of insertion) at approximately half of its length.
9. This incision was made on the lateral aspect of the longer part of the short medial collateral ligament (calcaneus attachment of insertion) at approximately half of its length.
10. The last incision was made on the lateral aspect of the shorter part of the short medial collateral ligament (talus attachment of insertion) at approximately half of its length.

The range of flexion and extension of the tarsal joint was measured 5 times during each step and recorded with the goniometer as described. Images (while taking measurements at flexion and extension, and all aspects of each limb after each dissection) were taken during each step using a Canon IXUS 170 camera and android Phone camera.



Figure 8. 5: Left tarsal joint of horse; showing an example of how the Liberty® 360-degree 25cm goniometer was aligned and used to measure the extension angle for step 1 of the dissection. The lateral aspect of the hindlimb is facing up.



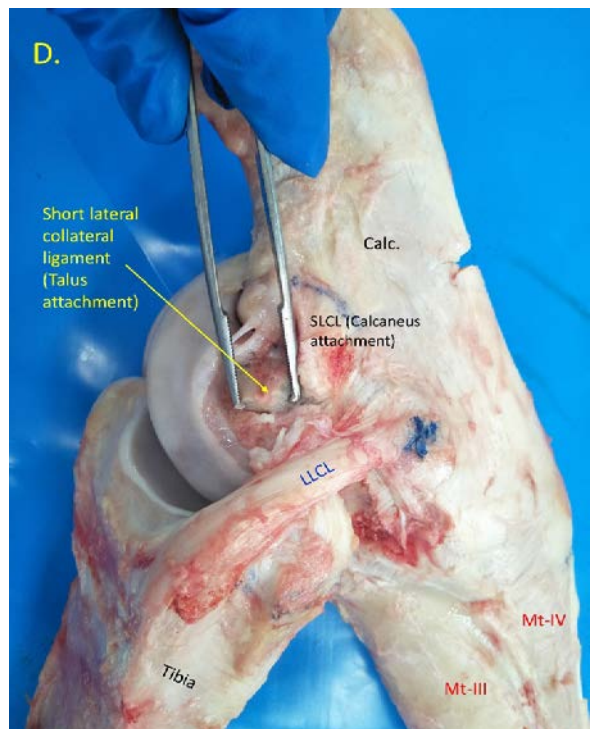
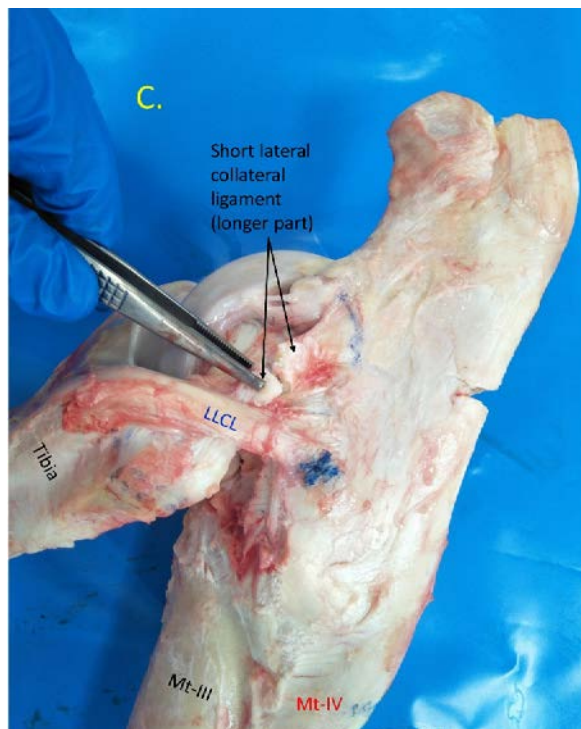
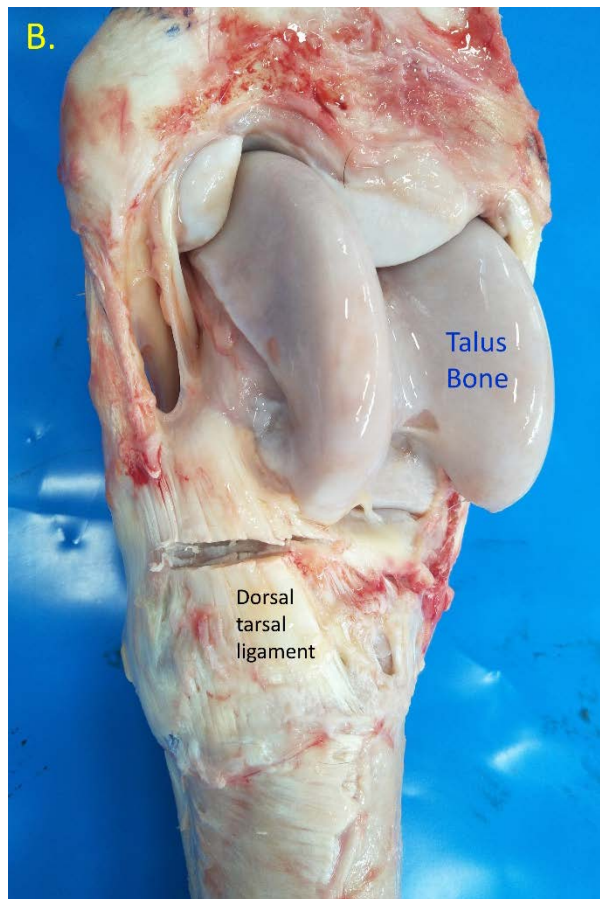
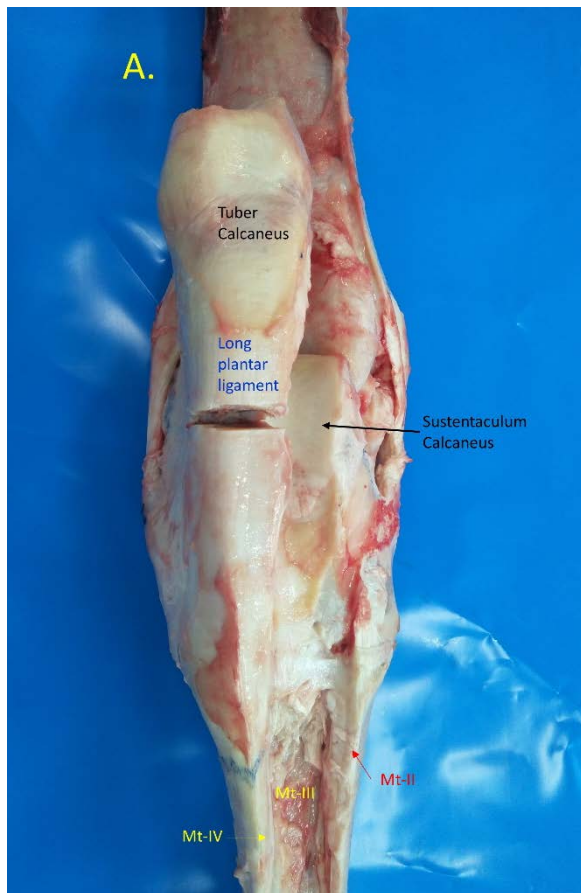
Figure 8. 6: Left tarsal joint of a horse; showing an example of how the Liberty® 360-degree 25cm goniometer was aligned and used to measure the extension angle for step 2 of the dissection. The lateral aspect of the hindlimb is facing up.



Figure 8. 7: Left tarsal joint of horse; showing an example of how the Liberty® 360-degree 25cm goniometer was aligned and used to measure the extension angle during step 3 of the dissection and extension in each subsequent step of the dissection. The lateral aspect of the hindlimb is facing up.



Figure 8. 8: Left tarsal joint of horse; showing an example of how the Liberty® 360-degree 25cm goniometer was aligned and used to measure the flexion angle for step 3 and flexion angle in further dissection steps. The lateral aspect of the hindlimb is facing up.



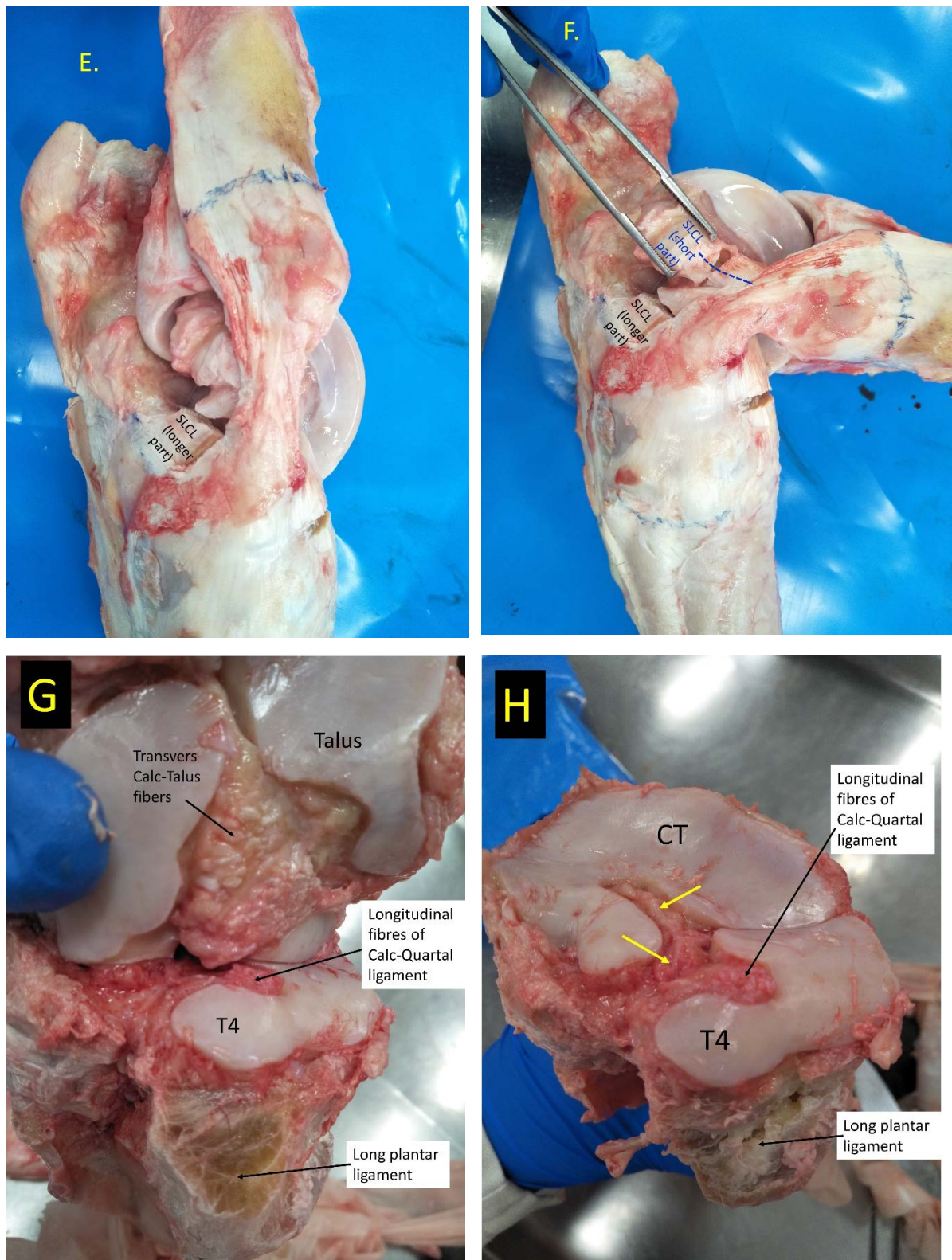


Figure 8. 9: A, The plantar aspect of a left hindlimb showing step 5 of the dissection where an incision was made approximately half of length of the long plantar ligament. B, shows the 6th step of the dissection process, where an incision was made through the dorsal tarsal ligament. C, shows the 7th step of the dissection process, where an incision was made

through the longer part of the short lateral collateral ligament (Calcaneus attachment). D, shows the 8th step of the dissection process, where an incision was made through the short part of the short lateral collateral ligament (Talus attachment). E, shows the 9th step of the dissection process, where an incision was made through the longer part of the short medial collateral ligament (Calcaneus attachment). F, shows the 10th step of the dissection process, where an incision was made through the shorter part of the short medial collateral ligament (Talus attachment). G, Plantar aspect of talus showing transverse and longitudinal fibers of calcaneus talus intertarsal ligament (Calc-talus ITL), T4, fourth tarsal. H, proximal aspect of central tarsal (CT) and T4 aspects. Arrows, showing the intertarsal ligaments and in-between connections of intertarsal ligaments.

8.2.5 Anatomical investigation of the tarsal retinacular fibres and joint capsule

The equine cadaveric hindlimbs (6 hind limbs from 3 Thoroughbreds aged approximately between 2.5-6 years) were collected from the department of pathology, Faculty of Veterinary and Agricultural Sciences, University of Melbourne. These animals were deceased for reasons unrelated to the study, hence no animal ethics approval was required for this study. All specimens appeared to be healthy upon physical examination and had no previous history of tarsal damage.

8.2.5.1 Dissection and anatomical description

Each equine hindlimb was dissected to observe and describe the anatomy of the retinacular fibres and joint capsule network of fibres around the tarsal joint. Thickness of the fibres, direction of collagen fibres, attachments to bone and its distribution were noted with naked eye or using magnifying glass (If required). Using a Panasonic Lumix DMC-LXS camera and mobile camera, images from all the aspects were taken and PHOTOSHOP used to add labels to the images. Images and video footage were taken.

8.2.5.2 Statistical analysis:

All ligament morphometric measurements were expressed in simple descriptive statistics and presented as Mean $\pm$ SD. IBM SPSS Statistics version 24 software was used to analyse the data collected from this study. The mean was calculated using the five goniometry measurements taken for each step and for each horse hindlimb. These means were then compiled and, using a paired t-test, changes in flexion and extension were analysed between each step of the dissection. Confidence intervals (95%) of the mean differences between each step were also calculated. Significance was set as $P < 0.05$. Microsoft Excel 2016 and Prism-GraphPad 8 were

used to plot multiple line graphs depicting separately the flexion and extension of each pair of horse hindlimbs (mean of 4 measurements of left and right) for each step of the dissection.

8.3 RESULTS

8.3.1 Major tarsal ligaments of the tarsal joint

Gross morphometric data of the measured major tarsal ligaments are presented (Tables 8.1 and 8.2). The superficial and deep layers of the medial and lateral collateral ligaments including long and short parts respectively provided a medial and lateral boundary that limited tarsal joint movement to a single plane of flexion and extension. Both the short lateral and medial collateral ligament were further identified into two branches with different insertion points as elaborated in figure 8.9 C-F and table 8.1.

The average length of dorsal tarsal ligament (DTL) was measured as $6.10 \pm 0.33\text{cm}$. It coursed from its attachment to the distal tuberosity of the medial surface of talus proximally and spreads out distally as a triangular sheet covering different bones of the tarsus including the attachments to the central and third tarsal, and the proximal ends of the third and second metatarsal bones (Figure 8.3E). The fibres ran proximodistally starting from a narrow region of the tuberosity and spread out to multiple attachments to give a strong connection and dorsal stability to the central and third tarsals on the dorsomedial aspect at the level of the proximal ends of the second and third metatarsals of the tarsus.

The plantar ligament was a very strong, flat band ($13.91 \pm 0.37\text{cm}$) which covered the lateral aspect of the plantar surface of the tarsal joint. It was attached to the plantar surface of the calcaneus along with the fourth tarsal bones and the proximal end of the lateral metatarsal bone. This ligament has the maximum depth ($0.88 \pm 0.06\text{cm}$) among all other tarsal ligaments. The alignment of fibers was Proximodistal direction, the fibers got stretched being more

tensed during the flexion and extension, to keep the alignment of the calcaneus with the metatarsals of the distal limb.

There are a considerable number of short ligaments which connect adjacent bones of the tarsus and metatarsus. Among these intertarsal short ligaments some are quite distinct while some are difficult to isolate. The tibial and calcaneal tarsal bones are united by four calcaneal ligamentous bands. The first band extends from the sustentaculum tali of calcaneus to the adjacent part of the tibial tarsal, and also blends with the short collateral ligament. The second band extends from the cochlear process of the calcaneus to the adjacent part of the lateral ridge of the trochlea of the talus bone. The third band extends from the caudal (posterior) margin of the trochlea to the calcaneal tarsal bone. The interosseous ligament is deeply placed in the sinus tarsi between the two bones. The smaller tarsal bones are attached to each other by interosseous short ligaments. The tarsometatarsal ligaments connect tarsal bones of distal row with the metatarsus and are not distinct from the common ligaments, except in the case of the interosseous ligament between the third tarsal and third metatarsal bones.

Table 8. 1: Morphometric measurements (mean dimensions) of the intertarsal ligaments of the equine tarsal joint.

S/no	Parameters (n=14)		Mean $\pm$ SD (cm)	95% Confidence interval for mean	
				Lower	Upper
1	Long lateral Collateral ligament	Length	14.5 $\pm$ 1.27	13.21	15.88
		Depth	0.62 $\pm$ 0.08	0.54	0.71
		Width origin	1.78 $\pm$ 0.11	1.67	1.89
		Width at midpoint	1.38 $\pm$ 0.20	1.18	1.59
		Width at insertion	3.44 $\pm$ 0.39	3.03	3.85
2	Short lateral Collateral ligament-I (calcaneal insertion)	Length	7.63 $\pm$ 0.74	6.86	8.41
		Depth	0.59 $\pm$ 0.05	0.54	0.64
		Width at origin	2.42 $\pm$ 0.18	2.22	2.61
		Width at midpoint	1.20 $\pm$ 0.08	0.11	1.29
		Width at insertion	2.46 $\pm$ 0.23	2.21	2.70
3	Short lateral Collateral ligament-II (Talus insertion)	Length	5.95 $\pm$ 0.65	5.26	6.64
		Depth	0.45 $\pm$ 0.06	0.39	0.51
		Width at origin	2.17 $\pm$ 0.29	1.86	2.47
		Width at midpoint	1.34 $\pm$ 0.06	1.29	1.40
		Width at insertion	2.24 $\pm$ 0.11	2.12	2.36
4	Long Medial Collateral ligament	Length	12.14 $\pm$ 1.31	10.76	13.52
		Depth	0.61 $\pm$ 0.05	0.57	0.66
		Width at origin	3.09 $\pm$ 0.27	2.80	3.37
		Width at midpoint	1.69 $\pm$ 0.20	1.47	1.90
		Width at insertion	3.47 $\pm$ 0.28	3.17	3.76
5	Short Medial Collateral ligament-I (calcaneal insertion)	Length	8.18 $\pm$ 1.03	7.09	9.27
		Depth	0.55 $\pm$ 0.12	0.42	0.68
		Width at origin	2.54 $\pm$ 0.05	2.49	2.59
		Width at midpoint	1.57 $\pm$ 0.33	1.22	1.92
		Width at insertion	1.92 $\pm$ 0.10	1.81	2.01
6		Length	6.55 $\pm$ 0.48	6.05	7.05

	Short Medial Collateral ligament-II (Talus insertion)	Depth	0.57 ± 0.06	0.50	0.63
		Width at origin	2.26 ± 0.10	2.16	2.37
		Width at midpoint	1.03 ± 0.05	0.97	1.09
		Width at insertion	1.55 ± 0.05	1.50	1.61
7	Dorsal tarsal ligament	Length	6.10 ± 0.33	0.36	0.42
		Depth	0.39 ± 0.03	0.36	0.42
		Width at origin	1.77 ± 0.24	1.51	2.02
		Width at midpoint	2.60 ± 0.15	2.44	2.76
		Width at insertion	3.97 ± 0.09	3.87	4.06
8	Long plantar ligament	Length	13.91 ± 0.37	13.52	14.30
		Depth	0.88 ± 0.06	0.81	0.94
		Width at origin	2.99 ± 0.21	2.77	3.22
		Width at midpoint	2.27 ± 0.25	2.01	2.54
		Width at insertion	1.97 ± 0.20	1.76	2.18

The length of the long lateral collateral ligament ($14.5 \pm 1.27\text{cm}$) appeared longer than that of the long medial collateral ligament ($12.14 \pm 1.31\text{cm}$) and the difference was found to be statistically significant ($p = 0.000$). The lateral short collateral ligament-I (Calcaneus insertion), the length of this ligament appeared shorter ($7.63 \pm 0.74\text{cm}$) than that of its counterpart medial short collateral ligament-I (Calcaneus insertion) ($8.18 \pm 1.03\text{cm}$) on the medial side although the difference was not found to be statistically significant ($p = 0.064$) but the P value was very close to 0.05. The orientations of the fibres were diagonally interwoven in a criss-cross manner cranio-proximally to caudo-distally.

Table 8. 2: Comparison of length between the long and short medial collateral ligament (MCL) and the lateral collateral ligament (LCL).

Length of collateral ligament	Mean $\pm$ SD (cm)	Paired differences (t-test)			
		Mean Diff. $\pm$ SD	95% CI of the difference		Sig (p<0.05)
			lower	upper	
Lateral collateral ligament length (LLCL)	14.55 $\pm$ 1.27	2.41 $\pm$ 0.53	1.85	2.96	0.000*
Medial collateral ligament length (LMCL)	12.14 $\pm$ 1.31				
Lateral short collateral ligament-I length (LLCL)	7.63 $\pm$ 0.74	-0.55 $\pm$ 0.57	-1.15	0.05	0.064
Medial short collateral ligament-I (SMCL)	8.18 $\pm$ 1.03				

8.3.2 Simulation of flexion and extension of the tarsal joint:

The results are presented (differences during each step in Table 8.3, and the actual means $\pm$ SD measured at each step in Figures 8.10-8.11), which depict changes to flexion and extension following each step of the dissection in the osteoligamentous preparations. Flexion and extension take place at the tibiotarsal joint. The movements within the tarsal bones, and between the metatarsal bones were difficult to observe so far as the action of the joint as a whole was concerned. Tension of the collateral ligaments prevent maximum or complete extension of the joint. Flexion was checked only by contact of the metatarsus with the tibia. The lower part of the limb deviated somewhat outward during flexion because of a slightly oblique axis of motion (due to angle of trochlear ridges). The long collateral ligaments were tense in extension while the short ones were tense during flexion.

There were marginal changes found during limb extension in most of the dissection steps in the osteoligamentous preparations. The significant effects were found during flexion. Commencing with the intact hindlimbs there was an increase in hock extension with the following steps: when the skin was removed (step 2, $-0.5^{\circ} \pm 0.1$, $P < 0.000$), with transection of the deep fascia (step 3, $-0.4^{\circ} \pm 0.2$, $P < 0.006$), and cutting of crural muscles support (step 4, $-5^{\circ} \pm 0.7$, $P < 0.000$). There was a non-significant difference observed in step 5, in the ligamentous preparation by removing all the tendons, retinacular bands, fibrous joint capsule and remaining soft tissues around the tarsal joint leaving only tarsal ligaments and bones (step 5, $-0.8^{\circ} \pm 1.3$, $P < 0.185$). No clear change in trends or differences between the measurements/observations between left and right hindlimbs were identified in these hock joint measurements.

The incision around the crus to remove all the muscular connections between the crural region and the tarsus including all muscular, tendons and fascial support around the distal tibia ($-5^{\circ} \pm 0.7$, $P < 0.000$) showed individually variable, but overall significant, maximum changes to hock extension in all samples (Figure 8.11). After this step, the maximum extension was achieved in the next step after removing all the tendons, retinacular bands, fibrous joint capsule and remaining soft tissues around the tarsal joint, leaving only the tarsal ligaments and bones. In contrast, cutting all the major ligaments produced non-significant ($P > 0.05$) changes to the range of further extension of the hock joint as the movement of the tibial bone was restricted because of the calcaneal tarsal bone.

Severing all the major tarsal ligaments from the tarsus and their connections across the hock joint resulted in significant changes in hock joint flexion in all steps of dissections (Step 5-11) ($P < 0.001$). The incision on the plantar aspect of the long plantar ligament at approximately

half of its length from its connections across the hock joint resulted in the largest significant change in hock extension of $2.3^\circ \pm 0.5$ ($P < 0.000$).

Table 8. 3: Mean $\pm$ standard error of the mean (SEM) of the difference in flexion and extension of the equine tarsus (n=6) before and after the completion of each step in the dissection. Differences in flexion and extension were also calculated for before (step 1) and after (step 13) of the dissection, to compare any overall changes in the study. + or - indicates the direction of change in angle of the joint during flexion or extension.

Dissection Steps	95% CI of the difference			
	Mean difference ± SEM (°)	(°)		P-value
		Lower	Upper	
Step 1 vs. Step 2				
Extension	-0.45 ± 0.10	-0.56	-0.34	0.000**
Step 2 vs. Step 3				
Extension	-0.35 ± 0.19	-0.55	-0.15	0.006*
Step 3 vs. Step 4				
Extension	-5.03 ± 0.71	-5.78	-4.29	0.000**
Step 4 vs. Step 5				
Extension	-0.83 ± 1.33	-2.23	0.56	0.185
Flexion	2.25 ± 0.52	1.70	2.80	0.000**
Step 5 vs. Step 6				
Extension	0	0	0	0
Flexion	1.58 ± 0.66	0.89	2.28	0.002**
Step 6 vs. Step 7				
Extension	0	0	0	0
Flexion	1.83 ± 0.75	1.04	2.62	0.002**
Step 7 vs. Step 8				
Extension	0	0	0	0
Flexion	0.83 ± 0.41	0.40	1.26	0.004**

Step 8 vs. Step 9					
Extension		0	0	0	0
Flexion		1.33 ± 0.52	0.79	1.88	0.001**
Step 9 vs. Step 10					
Extension		0	0	0	0
Flexion		1.33 ± 0.41	0.90	1.76	0.000**

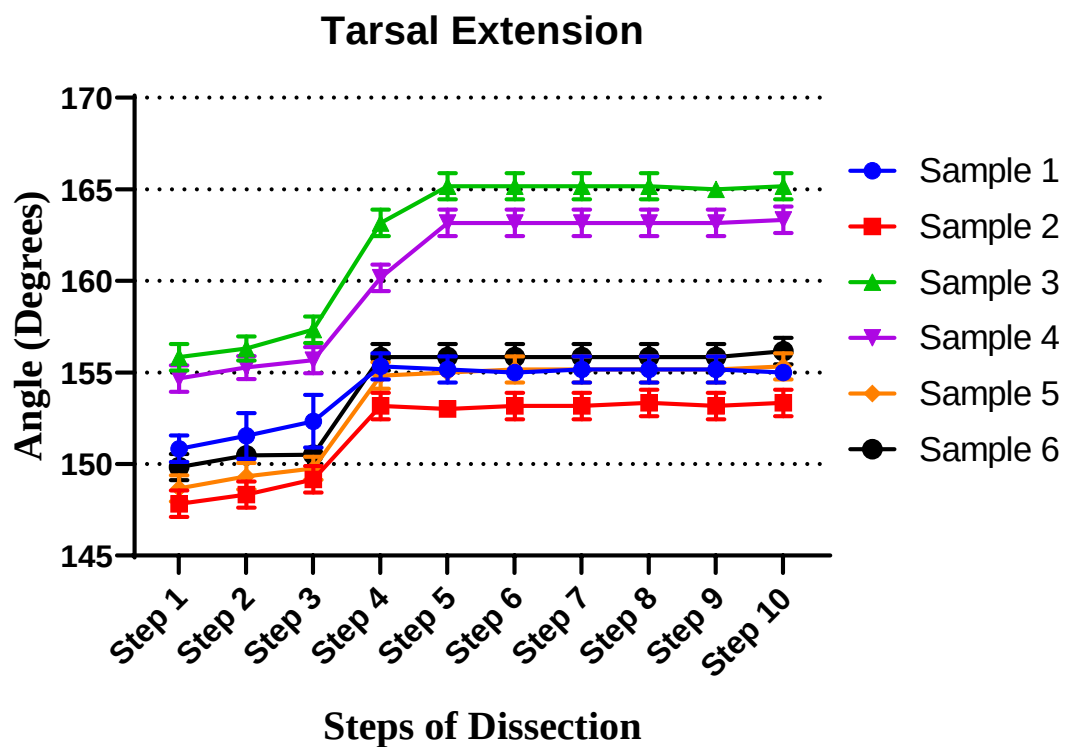


Figure 8. 10: Multiple line graph showing the mean $\pm$ SD of repeated goniometer measurements (five repeats for each measurement of each limb) for the extension of the tarsal joint (degrees) from steps 1 to 10 for dissected hindlimbs ($n = 6$). Significant changes in extension can be seen at steps 2, 3 and 4.

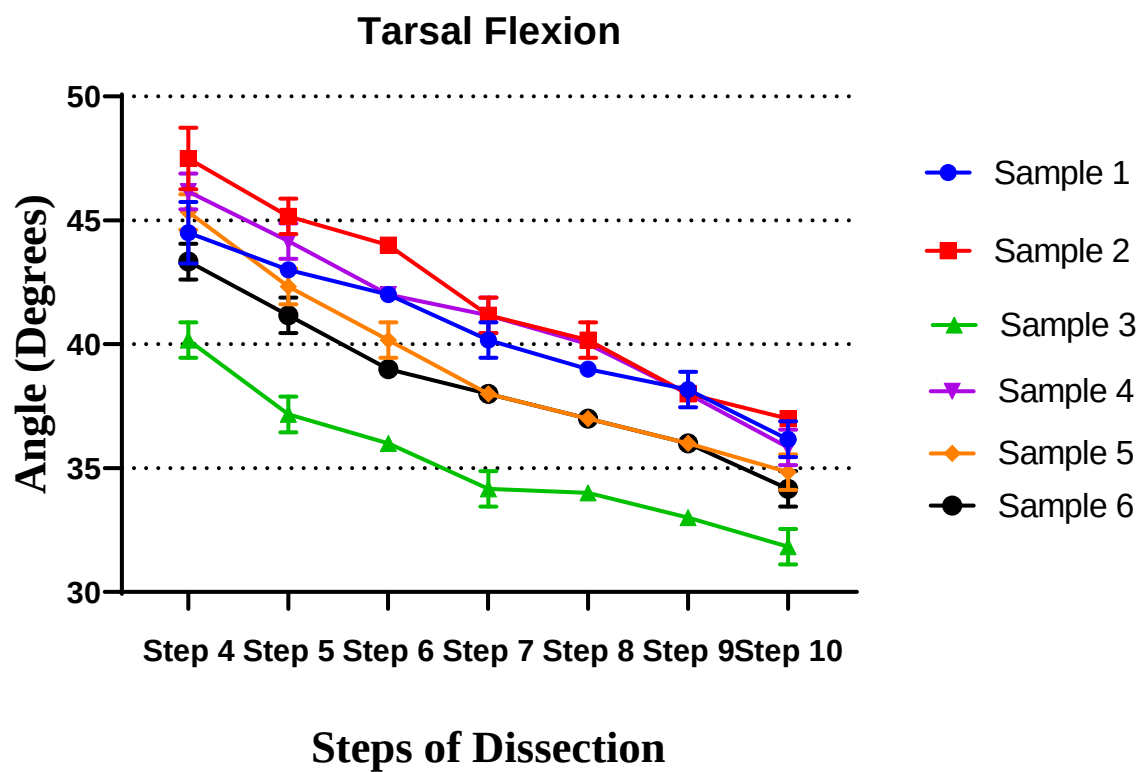


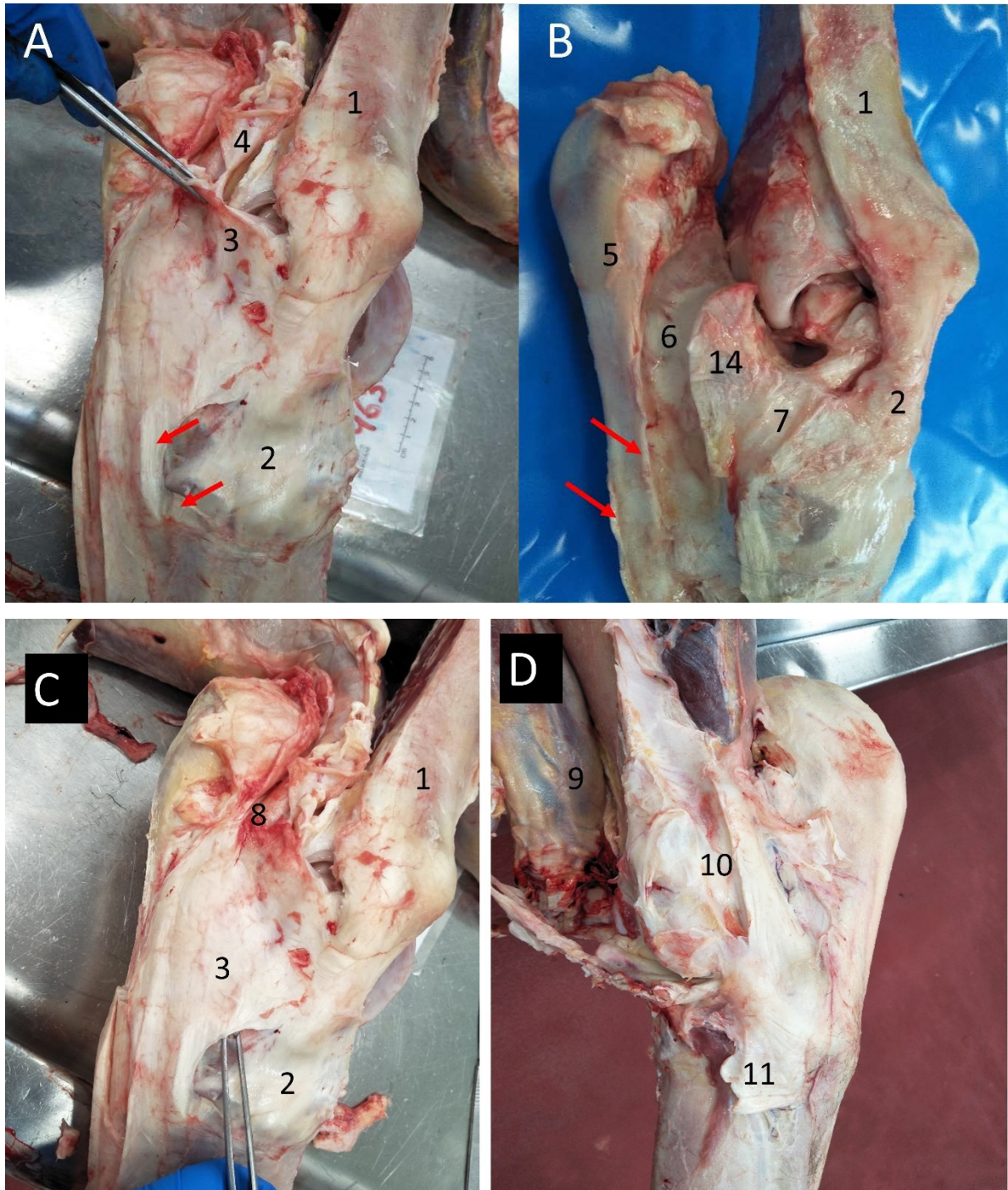
Figure 8. 11: Multiple line graph showing the mean $\pm$ SD of repeated goniometer measurements (three repeats for each measurement of each limb) for the flexion of the tarsal joint (degrees) starting from steps 4 to 10 for dissected hindlimbs ($n = 6$). Significant changes in flexion can be seen at all the steps of dissections.

8.3.3 Observation and description of the deep fascia, joint capsule, retinacular bands and major ligaments restricting hock joint movements and their role in joint stability

To understand where and to what extent the fascia, fibrous joint capsule and ligaments were restricting the movement or extension of the tarsal joint, the joint was flexed and extended while the fascia was being palpated and observed as per methodology (Section 8.2.5). The flexion of the tarsal joint resulted in a lack of tension and stress throughout the fascia in this area of the hindlimb.

Removal of the skin revealed a layer of superficial fascia that was attached to the skin, and the deep fascial coverings could be identified deep to this layer of superficial fascia. Incising through this layer of superficial fascia on the lateral aspect of the hindlimb in the crural region over the extensor muscles further exposed a layer of deep fascia. This section of deep fascia, which appeared to be thicker than other areas of crural fascia, extended distally along the extensor muscles and tendons where it merged with, and became part of, the metatarsal fascia extending to the distal hindlimb.

Deep to the fascial layers covering on the tarsal joint, the joint capsule surrounds the tarsus proximally and distally. The joint capsule can be divided into two major parts, synovial and fibrous. The fibrous part was composed of an outer layer with thick strong fibers covering the whole joint capsule. The thick fibers have strong attachments with ligaments and retinacular bands for stronger stability (Figure 8.12).



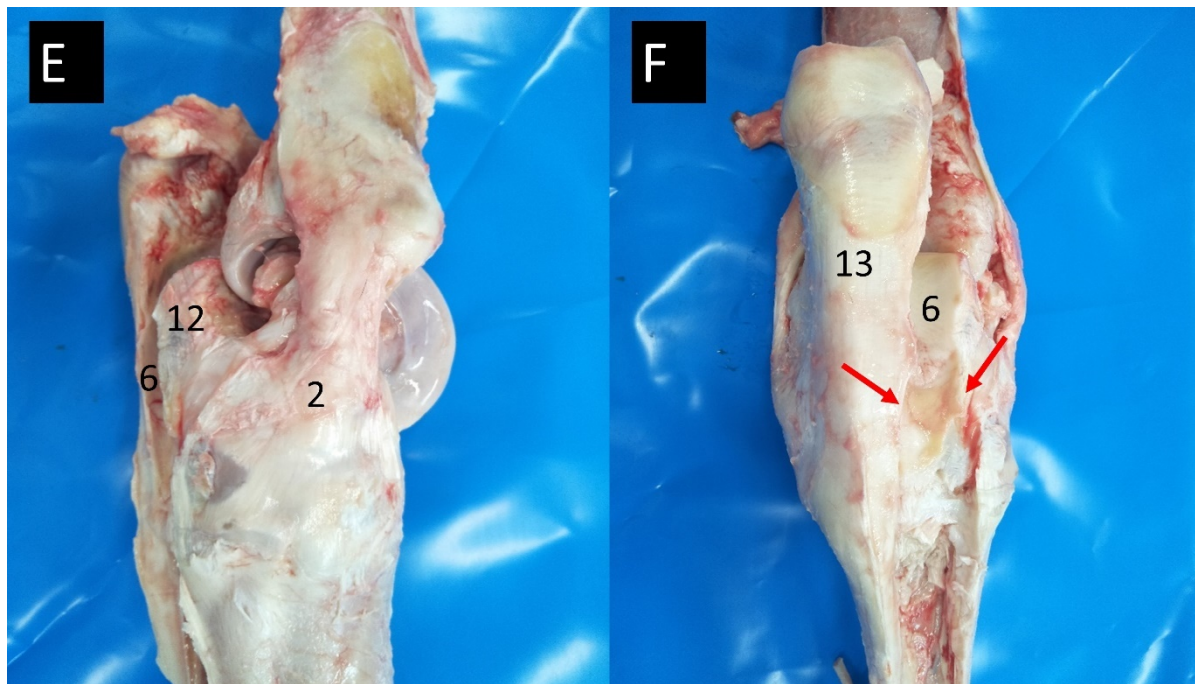


Figure 8. 12: The medial aspect of a left hindlimb showing the fibrous joint capsule where an incision was made in the proximal portion of the medial collateral ligament, showing the proximal and distal opening of the tendon sheath of the united tendons of the lateral digital flexor and caudal tibial muscles, 1. Tibia, 2. Medial collateral ligament, 3 and 4. Fibrous (3) and Synovial (4) parts of the joint capsule, 5. Calcaneus, 6. Tarsal sheath passage of flexor digitorum longus (number on the cartilage within the joint capsule), 7. Tarsal sheath passage of flexor digitorum profundus (pointed in figure C), 8. Tendon of flexor digitorum longus, D. Lateral aspect of a left hindlimb showing the fibrous joint capsule where an incision was made in the proximal portion of the lateral collateral ligament showing the removal of the joint capsule, 9. Extensor digitorum longus muscle after removal of its tendon from the dorsal aspect of the tarsus, 10. Tarsal sheath passage of extensor digitorum lateralis, 11. distal extensor retinacular bands, E-F, shows the remaining structures and passages of tendon sheaths on the medial and plantar aspects after complete removal of the joint capsule, 12. Sustentaculum tali of calcaneus, 13. Long plantar ligament, arrows indicates the attachment points of the fibrous sheath.

Both superficial and deep fascial layers were identified within the equine crural region and extending to the distal limb. The superficial fascia encased the crural region in a stocking-like manner. Proximally, it was continuous with the superficial fascia of the fascia lata, whilst distally it was continuous with the superficial fascia of the metatarsus. It could generally be separated from the overlying skin relatively easily due to the delicate nature of the loose connective tissue in between the two layers. The deep fascia could be isolated as two to three separate laminae along different aspects of the crural fascia and generally invested between muscles to create isolated muscular compartments. The fascial layers thickness and consistency was quite variable between specimens, but overall fascial connectivity was similar. The detailed description of fascia has been discussed in the next chapter.

8.4 DISCUSSION

The previous study in Chapter Seven was conducted to identify the effect of increased loading on the tarsus on the general alignment of the tarsus. Parameters were developed using certain reliable landmarks and to assess any change in alignment of the tarsus that could be traced on sequential radiographs. Previous findings suggest that the support of the tarsal joint is more complex than what is currently understood in terms of the tarsal bones' responses to loading. In addition, the tarsal bones are held together in intimate contact through the attachments of soft tissue, including different types of ligaments around the joint connecting bone to bone, and which play a vital role in joint support and stability by limiting the bone movements during loading. Flexion and extension of the equine tarsal joint is an important component in function of the hindlimb and soft tissue (ligaments, fascia and other structures) provide mechanical connections for better coordination and influence the function. The current understanding of normal joint function in reference to mechanical influence of the soft tissue structures remains unclear. Also, there is a clear role of ligaments in the change of internal alignment of the tarsal bones in response to loading. Intercarpal ligaments have been shown to play a part in attenuation, absorption and redistribution of high compressional loading forces in the carpus of equines (Boening, 1981; Bramlage et al., 1988; Deane & Davies, 1995a). Limited studies are available regarding the tarsal ligaments either describing their morphometry or their role in loading biomechanics and joint stability, apart from the general anatomic details of the major tarsal ligaments (Oakley, 2012; Updike, 1984; J. Vilar, Santana, Espinosa, & Spinella, 2011; I. M. Wright, 1995). As a result, the objective of the current study was to investigate the detailed morphology of the major tarsal ligaments and determine how the range of flexion and extension of the equine tarsal joint was constrained by the mechanical connections and attachments of these ligaments as well as other soft tissue

structures (Fascia, tendons, ligaments). In addition, further observational investigation was made into soft tissue structures like the joint capsule, deep fascia and retinacular fibres in the proximity of the tarsal joint that may contribute to both the stability and passive mechanical coordination of hindlimb movement.

The tarsus is a ginglymus type of joint because of the unidirectional movement. The tibia can be considered as one of the functional components of the hind limb skeleton and the tibial shaft is covered by thick muscles on its craniolateral and caudal aspects but subcutaneous fascial layers on medial aspect. The presence of a large muscular mass on the latero-caudal aspects and a thick cutaneous fascial layer surrounding the tibial bone medially indicates different patterns of muscular forces in different aspects of the tibial shaft and variations in the way such forces are transmitted to their distal portion. In addition, the movement of the tarsus is also linked to the movement of the stifle joint. These joints work in co-ordination due to the reciprocal mechanism because of two opposing sets of tendinous attachments (Kallista, 2019; Schuurman, Kersten, & Weijs, 2003). All of these connections play an important role in the movements of the hock joint in an intact limb and correspond with those of the stifle on account of the tendinous bands cranially and caudally (peroneus tertius and flexor superficialis), which extend from the distal femur to the tarsus and metatarsus. Sequential cutting of structures providing mechanical support to the tarsal joint such as muscular, tendons and fascia demonstrated changes in the normal range of extension and flexion of joint.

The abduction of the distal part of the hind limb causes hock flexion in an oblique axis of motion (Gnagey et al., 2006). The distal articular surface of the tibia (the cochlea) was noted to be comprised of two grooves separated by a ridge, all with a craniolateral inclination while

the cochlea was flanked by medial and lateral malleoli. The trochlear part of the talus bone was also important for the articulation with the distal tibia. The oblique articular surfaces of the tibia and talus play a vital role in ensuring that the distal part of the limb is carried outward as well as forward when the hock joint is flexed while the distal limb is pulled slightly to one side due to the trochlear ridges slanting laterally. The length of the long lateral collateral ligament was significantly longer ($p = 0.000$) than its counterpart, the long medial collateral ligament, and seems to provide more support of this motion. This difference of length indicates that the lateral ligament secures more area of the lateral side of the tarsus to keep the tibia aligned with the metatarsus during extension and flexion of tarsus joint even in an oblique axis. Similar findings were observed in previous studies (Dyce et al., 2010a; Schuurman et al., 2003) where this lateral movement of the distal hindlimb is described as helping the hooves to avoid hitting the forelimbs or abdomen.

There were different processes and ridges observed on the different aspects of the tarsal bones that fitted into the corresponding fossae on the opposite articulating surfaces, most significantly in between the talus, central tarsal, third tarsal and third metatarsal (Mt3). These bony structures could be very helpful in bearing and transferring of the loads and maintenance of the stability because such topography would increase the loading surface area while the congruity provides limits to excessive extension and thus greater stability. These findings were in line with previous studies where the most distal tarsal joints were classified as plane joints based on the orientation of the small, flat articular surfaces (K. M. Dyce, W. O. Sack, & C. J. G. Wensing, 1987).

These bony processes and/or ridges of the articular surfaces are considered important in the transfer of load while maintaining the original orientation (Bramlage et al., 1988; Deane

& Davies, 1995a). In addition, the entire compressional loads would have been transmitted distally without overstretching of the intertarsal ligaments between the distal tarsal bones in particular the third tarsal bone. Rooney (1969) described in a previous study that a premature rotation of the third tarsal bone during progression may permit abnormal compression between the central tarsal and third metatarsal bones. In addition, excessive rotations might lead to wobbling of the tarsus, so there should be a mechanism of locking such rotational movements of tarsal bones particularly the central and third tarsal (Lindsay et al., 1982; Rooney, 1969). The counter-directional slight rotational movements of the antebrachium and the metacarpus appeared to fit into a concept like the carpal wedge concept in which the sliding into a locked up wedge position of the carpal bones during loading is believed to transfer sudden application of forces from the large axial loading, ground reaction forces and the transverse limb torsional forces and pass them to the elastic intercarpal ligaments as well (Bramlage et al., 1988; Deane & Davies, 1995a).

In a similar context, the dorsal oblique ligament seems likely to play an important role in limiting the motion of these tarsal bones. The dorsal oblique ligament had different widths at the origin (1.77 ± 0.24), midpoint (2.60 ± 0.15) and insertion (3.97 ± 0.09). It was observed to commence from a small origin on the talus and extend distally while it spread widely across the central and third tarsal bones. This wide spread of the ligament distally can restrict any major rotation or movements of these bones during flexion and extension of the tarsal joint (S. J. Dyson & M. W. Ross, 2011). There are collateral and dorsal ligaments which also limit the movements of tarsal bones to a minimum level (limited to slight gliding movements). The collateral ligaments were arranged in a fan shape and the fibres were arranged in a spiral pattern while the linear pattern of fibres was not as distinct as in most of the tendons. The

eccentric attachment of the various collateral ligaments and the spiral pattern of the collateral ligaments are likely to be useful for this joint in returning to its neutral position again. The orientation of fibres and tension was observed by flexing and extending the tarsal joint. The findings of the current study on the anatomy and morphometry of the medial and lateral ligaments were in accordance with previous studies. The long collateral ligaments became tense in an extended or standing posture whereas the short collateral ligaments remained in tension during flexion of the tarsal joint (Oakley, 2012; Updike, 1984). Sequential cutting of the major tarsal ligaments demonstrated an increase in the normal range of extension and flexion of joint.

The plantar tarsal ligament fibres cover the latero-plantar aspect with strong attachments not only to the calcaneus and fourth tarsal bones, but also link with the proximal end of the fourth metatarsal more superficially. The maximum thickness and larger attachment area of this ligament highlights the importance of a strong connection and alignment of calcaneus with the distal tarsal and metatarsal bones to provide a strong base for connections of the common calcaneal tendons. Hence the plantar ligament can play a vital role in the stay apparatus. The interosseous talocalcaneal ligament from deeper in the joint provides extra stabilization of the calcaneus in opposition to the tensions that the extensor musculature can apply to the tuber calcaneus. The dissection of all the connections between the crural region and tarsus including all muscular, tendons and fascial support produced the maximum change (5°) in the range of extension. But the dissection of the long plantar ligament in the osteoligamentous preparation led to some further extension which was non-significant statistically (-0.83 ± 1.33) but it indicates that this ligament probably still plays a role in preventing the overextension of the tarsal joint. This also suggests that the

fascial architecture and attachments of the muscle and tendons contribute to the mechanical restriction of the tarsal joint during extension. After cutting this ligament no further extension was noted. It was indicated that the lower joint moments associated with a large tarsal angle may reduce the likelihood of desmitis of the plantar tarsal ligament while a small lateral tarsal angle ($>155\text{-}165^\circ$) of the tarsus predisposes to plantar ligament desmitis (Gnagay et al., 2006; T. S. Stashak, 1987).

In the current study short collateral ligaments were observed to be tense predominantly during flexion of the tarsal joint in contrast to the long collateral ligaments. The superficial short lateral ligament was reported to be under tension between flexion and extension of the joint, while the deep medial ligament remained under tension throughout movement of the tarsocrural joint (Updike, 1984). Previous injuries of the short lateral collateral ligament were considered more common than injuries to the medial collateral ligament (C. McIlwraith, Nixon, Wright, & Boening, 2005; I. M. Wright, 1995) whereas the opposite trend was also reported (Barker, Smith, Minshall, & Wright, 2013).

The tarsocrural joint is assumed to dominate the motion of the tarsus as most of the tarsal joint motion can be attributed to the tarsocrural joint. There were also some additional measurable translations and rotations, occurring during both swing and stance, in other locations within the complex tarsal joint in a study of 3D motion of the bones recorded as each subject trotted in hand (Lanovaz et al., 2002). However, the flexion and/or extension axis of the tarsal joint was not necessarily perpendicular to the sagittal plane of the horse as defined in the current study. Only a few studies have assessed tarsal joint range of movement, and they suggested that the tarsal angle may affect the performance of a horse by influencing the range of motion (Back et al., 1995; Holmström, Fredricson, & Drevemo, 1995). The range of tibiotarsal joint motions is considered very important in humans. Every

joint has a normal (ideal) range of motion. Supination occurs as the talus moves laterally, dorsally, and posteriorly on the calcaneus. This supinatory motion was observed to strengthen the joints of the human foot in activities of weightbearing (Graham, 2018).

The intertarsal ligaments are not known for any specific importance other than participating in the generalized ligamentous support and elasticity of the tarsal joint (Getty, 1975b). However, a closer look at the ligaments during dissection and functional simulation of joint motion suggested that these ligaments provide an additional stability to restrict the movements of these bones in the distal tarsal joints. This observation appears reasonable and consistent with the structure of a ligament that absorbs tensions related to movements associated with concussion forces, because a ligament under too high a strain cannot transfer and redistribute loading pressure, a core function that the intercarpal ligaments are believed to perform during carpal loading (Bramlage et al., 1988; Deane & Davies, 1995a).

The tarsal interosseous ligaments were plentiful and spanned the small vertical spaces between the neighbouring tarsal bones (Düsterdieck et al., 2002). This could suggest that movement of the tarsal joint may be mechanically constrained primarily by structures forming the joint such as the bones, ligaments or joint capsule. However, several of the intertarsal ligaments which were not dissected in this study could have some passive mechanical effect on the flexion of the tarsal joint. Ultimately, further investigation into these structures is required.

The joint is supported and stabilised by a complex interaction of passive and dynamic connective tissues as it appears to serve as an integrating structure for general functional movements of the hindlimb during locomotion. This indicated that the mechanical restrictions to tarsal joint movement, as a result of the collagenous framework and

attachments of deep fascia and muscles, may have an important role in function of the hind limb. The results also demonstrated that the transection of crural fascial connections of cadaver hindlimbs led to changes to the range of tarsal joint extension when measured with a goniometer. The fibrous part of the joint capsule surrounds the tarsus and has strong connections with ligaments and retinacular bands. This fibrous part was also thicker in composition than other fascial structures in the region and composed of thick collagenous fibres which not only provided support to retinacular bands for securing tendons around the tarsus but also reinforced the whole region. The thick fibers of tendon sheaths provide additional support to the tarsal ligaments and can bear some of the loading on the tarsus, and thus may play a role in loading or stress management along with the ligaments of the tarsus. Further detailed role of fascia has been discussed in next chapter.

In conclusion, the directional movement of the tarsal bones especially the central and third tarsals are secured through dorsal oblique and lateromedial collateral ligaments externally while internally the bones are secured by the small intertarsal ligaments during cyclic repetitive flexion and extension of the tarsus. The detailed morphology of the major ligaments and their effects on the range of extension and flexion have now been described. These directional movements corresponded to the fibre orientations of respective supporting intertarsal ligaments between the tarsal bones. This study also highlights the presence of mechanical restrictors of the tarsal joint. These constraints included the skin, deep fascia, muscular and tendinous support, and major tarsal ligaments which all contributed to restricting the tarsal joint during extension. These findings suggest that they are involved in maintaining stability of the tarsal joint particularly during extension. The intertarsal ligaments facilitate and secure distal sliding movements within the tarsus while enabling better stability

of the tarsal joint. These observations will improve our understanding of the normal tarsal functions and the pathogenesis of tarsus damage. Further research into these structures is warranted, especially the role of the deep fascia and the small intertarsal ligaments in maintaining proper function of the hind limb. The morphometric data on some tarsal ligaments provided by this study could be useful to help assess the mechanical properties of these ligaments. This may be of some value while planning for surgical repair of ligament rupture. Furthermore, this information would be of particular interest for 3-D modelling of the equine tarsus which may provide a further useful complementary method to study the complex functional mechanism and pathogenesis of tarsal damage in horses.

Chapter 9: Anatomical exploration of fascia and its role in maintaining the normal range of joint motion and coordination.

9.1 INTRODUCTION:

Previous findings in Chapter 8 demonstrated that support and loading of the tarsal joint is more complex than what is currently understood in terms of internal movements of tarsal bones in response to loading of the joint. The tarsal bones are held together in intimate contact through the ligaments (connecting bones together) which play a vital role in support and stability of joint during loading through their connections and mechanical restriction to prevent over flexion and over extension of the joint. Thus, ligaments play a part in maintaining the normal range of motion of the tarsal joint.

Other soft tissue like fascia and tendons were also observed to have multiple strong connections with bones and muscles and can impact on the range of motion and stability of the tarsal joint. Research focusing on the significance of fascia in equine hind limb loading biomechanics is noticeably lacking in the literature. The self-perception of stretch tolerance along with sensations which are associated with, and generated through, the central nervous system appeared to have a decisive role (Weppeler & Magnusson, 2010) but it has been also argued that the mechanical connection and flexibility of the associated soft tissues play an important role (Nordez et al., 2017).

The joint flexibility is also dependent on the soft tissue across a joint, such as tendinous insertions of muscles over bones and other connective tissues connections, more specifically the deep fascial layers which have dual connections with underlying muscles along its full peripheral length, as well as tendons and bones (Yucesoy, 2010). Thus, any

changes to the mechanical properties of deep fascia can impact the range of motion (ROM) of joints because of their morphological connections (attachments with other layers, muscles or bones) and by restricting the muscular extensibility.

Recent research in the field of injury management and human locomotion have begun to consider injuries with respect to the biomechanical functions and the connectivity of fascia which integrate muscles and bones. This concept highlights the role of fascia in establishing a connective tissue matrix body-wide that integrates all parts and systems of the body functionally and mechanically (Helene M Langevin, 2006; Myers, 1997; C. Stecco, 2014).

In some previous studies, mechanical reactions to the landing in the leg of humans have found that the landing was caused by distribution of tension through the linked segments of the body which contribute to physical adjustments more rapidly than the neuro reflex mechanisms (Moritz & Farley, 2004; Patla & Prentice, 1995). Thus, the importance of these mechanical links and connections provided by the fascia is now an emerging concept which is beginning to filter slowly into the literature.

In the field of veterinary medicine, the interest in fasciae is growing but precise and detailed anatomical and histological description of particular fascial structures has not available in many animals. Although, knowledge of the deep fascial structure and physiology of the limbs and body trunk attains more importance especially in horses but these types of mechanical connections have not been considered previously in the field of equine biomechanics. Such details can provide an effective solution to respond to the ground perturbations during rapid locomotion, where the neural reflex may be slow or too late to initiate the stimuli to respond accordingly.

The current knowledge of equine hind limb biomechanics is still lacking basic information

of simple characteristics of fascia, so it was hypothesized that fascia plays an important role in complex loading mechanism of tarsus through its mechanical connectivity, internal composition (histological) and proprioception. It is deemed necessary to evaluate the possible simple anatomical fascial characteristics like fibers directions, orientations and points of attachments in this chapter. These findings can be quite helpful in defining the role of hind limb fascia in the joint's range of motion, coordination, movement perception and directions of tension distribution.

9.2 MATERIALS AND METHODS:

9.2.1 Fascial exploration:

Twelve hind limbs (both right and left) were collected from six horses (5 Thoroughbred and 1 Standardbred) of 2-8 years age. All limbs were collected from the Pathology department of The University of Melbourne, Veterinary Science and disarticulated at coxofemoral/hip joint. The horses were euthanized or died for reasons that were not associated with this study. The age, breed and gender, and any additional available history was collected, and the limbs were physically examined to avoid any obvious pathology. Breeds investigated in this study included Thoroughbreds, Standardbreds and ponies. The limbs were dissected within 24 hours after the disarticulation of limbs from the horses being euthanized for the reasons not related with current study. Two extra foal limbs of unknown age were also dissected for the fascial network to observe any differences between them and the mature samples. The limbs were dissected as soon as possible after their removal (24hrs), and no frozen samples were used for the fascial dissection.

Throughout each dissection, fascial connections and characteristics were observed, described, and recorded using photography and videography (Samsung galaxy Alpha, 12-mega pixel camera and Sony digital camera 20 megapixels). The specimens were placed on the table, and the dissection was made according to the following protocol: the incision of the skin was along the lateral or medial midline from the stifle joint to the hoof junction or in different planes (If required) for better exploration of targeted areas. The skin margins were carefully removed to expose the superficial fascia and note its connection with the skin. A dissection plane was the external aspect of the superficial fascia and followed

cranially and caudally, laterally and medially. In addition, a dissection plane for exploring the deep aspect of the superficial fascia was followed laterally and medially, cranially and caudally exposing the connections of the superficial fascia with the deep fascia. The deep fascia was studied in similar procedure.

9.3 RESULTS:

9.3.1 Summary

In general, the distal hind limb is enclosed by many extensive sheets of connective tissue. The sheets of fasciae are interposed between the skin and deep structures as well as covering and passing between the muscles. These sheets form attachments for muscles and facilitate their movement across each other. Many of the deeper structures are also surrounded by fasciae and provide routes for the passage of blood vessels, lymphatics and nerves.

The overall anatomy of fascia pertaining to the equine distal hindlimb was relatively consistent in all twelve cadaveric specimens investigated in this study. The fascial system comprised of two layers (a superficial and a deep layer). These could be further subdivided according to their location: The superficial fascia of the crural region formed a mask like covering over the whole region connecting the fascia lata proximally and continuing to the distal hind limb as a cylinder. The superficial layer lay directly beneath the skin. The crural fascia enclosed the extensor and flexor muscles of the crus and continued to distal regions of hindlimb.

In the equine crural region below the stifle joint, both superficial and deep fascial layers were identified. The superficial fascia encased the whole crus (Figure 9.1) with particular areas (Figure 9.2) of notable attachments described below. Proximally, it was continuous with the superficial fascia of the fascia lata (Figures 9.3- 9.5), whilst distally it was continuous with the superficial fascia of the tarsus and metatarsus. It could generally be separated from the overlying skin relatively easily due to the delicate nature of the loose

connective tissue in between the two layers. In contrast, more fibrous connectivity was observed between the skin and superficial fascia over the tuber calcaneus of the tarsus (Figures 9.6-9.8).

The thickness or viscosity (consistency) of the fascial layers was quite variable between specimens, but overall fascial connectivity was similar although mature samples differed in having more thick and clear fibers with different directions/orientation as compared to immature foal samples. No obvious differences were observed between the limbs of right and left sides or between the breeds.

Thorough exploration of both the superficial and deep fascial layers was completed throughout the crural region to the distal digit of hindlimb as described below. The superficial fascial layers were identified over the crural region, tarsus, metatarsus, fetlock and proximal digit. The deep fascia could be isolated as two to three separate laminae along different aspects of the crus and generally invested between the muscles to create separate muscular compartments.

9.3.2 Superficial fascial layer:

The superficial fascia layer was identified over the metatarsal, fetlock and proximal digit, and was continuous proximally with the superficial fascia of the tarsus and crus (Figure 9.2-9.3). The superficial fascia over the medial aspect of the crural region covered the medial surface of the tibia and the flexor muscular bellies. The saphenous vessels and nerves could be found encased within the superficial fascia over the medial surface of the tibia. The anastomotic branches of saphenous vessels were situated caudal to the main branch and were, in contrast, encased within the deep fascia. As it coursed distally, the superficial

fascia became slightly thinner and could be separated from the deep fascia over the medial aspect of the crural region. The connection was not particularly strong between the superficial and deep fascial layers over the crural region.

The superficial layer could be peeled away from all the cranial and medial aspects of crus with care and showed slight differences of thickness proximo-distally. The fascia was thicker proximally than distally, and it remained continuous over the cranio-medial aspect of the distal leg and tarsus. The orientation of collagen fibers comprising the superficial fascia over the lateral aspect of the crural region was mostly irregular. These fibers were directed cranio-distally and provided little opportunity for stretch in response to traction applied distally (Figures 9.9 - 9.10) along with distinct perpendicular arrangement of fibers (observed proximally from the stifle to the distal third of the tibial bone) with lateromedial orientation (Figure 9.11). The superficial fascia has tight adherence with the skin and strong attachments with deeper fascia as well (Figures 9.12-9.15).

Following the superficial fascia around from the medial to caudal aspects, the attachment between the superficial and deep fascial layers began to differ noticeably. Over the position of the medial and lateral heads of gastrocnemius, the superficial fascia thinned and became much closer and more limited in terms of the relative amount of sliding possible between the two layers. With careful dissection, it could still be isolated over most of the caudal and lateral aspects. However, areas where the layers fused or became integrated via traversing collagen fibers were not uncommon.

In all dissected limbs, it appeared to consist of two delicate sheets macroscopically, which were not readily separated. Both sheets had an irregular and unspecialized pattern of arrangement of collagenous fibers, and the alignment suggest the direction of tensions

applied.

In the metatarsal region, the superficial fascia was observed to exist as a relatively elastic medium between the skin and the underlying structures as per palpation and tractions. Attachment of superficial fascia to the skin over most of the distal hindlimb was via loose connective tissue in most areas allowing a relative easier separation of the two layers and from the deep fascial layers (Figure 9.6). In addition to this, the fluidic consistency of superficial fascia also permitted a smaller amount of gliding between these tissue planes over the equine metatarsal region as per palpating this area.

The superficial fascia was observed to be intricately connected to, as well as separable from, the underlying fascia over the plantar aspect of the metatarsal region. An incision was made vertically along the mid length of the superficial digital flexor tendon (SDFT) from the proximal end to the middle of the annular ligament of the fetlock joint and demonstrated an easy separation of superficial fascia from the underlying deep fascia which covered the entire plantar surface of the SDFT.

On the dorsal aspect of the fetlock joint region, the superficial fascia was much more strongly attached to the underlying deep fascia over the juncture between the common digital extensor tendon (CDET) and the lateral digital extensor tendon (LDET). The superficial fascia could still be separated away as a distinct layer in all the specimens and it thinned over the surface of the CDET as it reached at the hoof capsule and then became inseparable from this tendon at a short distance above from the coronary band of the hoof capsule (Figure 9.33).

On the medio- and latero-dorsal aspects at the level of the proximal phalanx, fibrous connective tissue attached the superficial fascia to the deep fascia at the region proximally

to the point where the CDET and the extensor branches of the interosseous ligament (IL) united (Fig. 9.33). The fusion between the superficial and deep fascia was also observed plantar to the interosseous ligament's extensor branches (Figure 9.31).

The superficial fascia was continuous with the deep fascia at the level of the fetlock (covering the flexor tendons) in the metatarsal region on the plantar aspect. The superficial and deep fascia formed together a dense mass of connective tissue which corresponded in position to the external cornification of skin that is called the ergot tissue (Figure 9.35).

This dense fibrous cushion was intimately connected with the skin and had a very strong and inelastic adherence point to the annular ligament of the fetlock joint, and also served as the origin point for the ergot ligaments which ensured the mechanical integration of these tissue layers (Figure 9.37).

Both the medio-plantar and latero-plantar ergot ligaments were clearly defined structures in all the observed specimens (9.35).

Each of these ligaments had their proximal halves closely embedded within the superficial fascial layer and were also closely integrated with their overlying skin (Figure 9.33 and 9.36). The ergot ligaments were continuous distally with a wide reinforced band of fascia. The superficial fascia was loosely attached via loose connective tissue over the digital annular ligament and then integrated distally with the digital cushion and perichondrium of the hoof cartilage.

The superficial fascia became a much tighter distal to the fetlock joint and was more tightly adhered to the skin in this area where it provided a relatively inelastic support sheet for the structures of the proximal digit.

9.3.3 Deep fascia:

Generally, the deep fascia of the crus (crural fascia) was a thick aponeurotic sheet encasing mainly the muscle. This layer also forms the separation between muscle by comprising muscular compartments in the crural region. The deep fascia had a very limited degree of stretching as compared to superficial fascia. This fascia also had a much more definite fiber arrangement in contrast to the superficial fascia. In the distal third of the tibia, the crural fascia could be followed from its attachments and covering periosteum around the medial aspect of the tibial bone. The deep fascia was involved in formation of intermuscular septa between the muscles of the crus.

The deep fascia could be isolated as two to three separate laminae along the proximal aspects of the crural fascia and was generally invested between muscles to create isolated muscular compartments (Figures 9.23). Here, the superficial fascia was intimately connected with the thick collagenous deep fascial layers (9.23). The orientation of collagen fibers comprising the deep fascia over the lateral aspect of the crural region was mostly irregular. However, a distinct crisscross arrangement of fibers was observed proximally extending from the stifle to the proximal and distal third of the tibial bone and showing lateromedial and other orientations in the upper middle part of the crus (Figures 9.11-9.15). As these fibers were directed in different directions and provided little opportunity for stretch in response to traction applied to the specimen and provide support and stability of around the major muscle mass of crus. Similarly, fiber directions varied on the medial aspect of the crus (Figure 9.16). On the lateral aspect of the proximal third of the tibia, the fibers from different layers were crossing each other making it difficult to isolate different layers (Figure 9.12-9.15).

The other noteworthy characteristic of the fascia relates to its connectivity on the lateral aspect of the proximal crural region where the intermuscular septum between the extensor digitorum longus and extensor digitorum lateralis contains the superficial fibular nerve (Figure 9.11). The crural region was not visually different in respect to the consistency of collagen fibers, and thickness of the deep fascial layer on both lateral and medial aspects in foals. In contrast there was a thick banded pattern over the lateral and medial aspects of the crus in mature samples suggesting that there are both longitudinal and horizontal orientations of thick collagen fibers which are quite visible macroscopically with naked eye or with the help of some magnifying glasses (Figures 9.11-9.17).

The crural fascia on the lateral and caudal aspects of the crus was made complicated by the fusion of several layers. Over the caudal aspect, where the calcaneal tendon was situated, two layers of deep fascia could be isolated, but all layers of fascia showed tight adherence as compared to the dorsal aspect. The most superficial of these, as briefly alluded to above, was a continuation of the crural fascia extending from the gastrocnemius muscle (Figure 9.23).

Following this fascial layer around the plantar aspect of the tarsus, showed that all fascial layers were tightly adhered with underlying structures along with variable directions of fibers. It was observed that some layers had a diagonally oriented fiber arrangement directed towards the calcaneus. It was along this diagonal line that outermost deep fascial layer (layer-I) eventually merged with the second, deeper, layer of aponeurotic fascia over the caudolateral aspect.

The deep fascia was involved in organization and formation of tendons sheaths and incorporated ligaments, joint capsule and fascial reinforcement of retinacular bands

supporting the tarsus structures. The tarsal fascia was a direct distal extension of the crural fascia. Over the dorsal aspect, it formed tendon sheaths for the long digital extensor tendon (lgDET), and lateral digital extensor (LDE) tendons which maintained their position with manual flexion and extension of the tarsal joint. Over the flexor aspect, the tarsal fascia formed the tarsal flexor tendon sheath (Figure 9.25).

The deep fascia penetrated between and surrounded the muscles, tendons, bones, nerves and blood vessels of the hind limb through the different regions of the hindlimb. It was the dense fibrous connective tissue in the form of a fibrous sheet which invested the hind limb beneath the superficial fascia (Figures 9.17-9.18). It was comprised of multilayers in the tarsal region, was mostly devoid of fat, was usually inelastic and tough (Figure 9.18) and further extended to where fibers inserted directly to CDET. The deep fascial aponeurotic fibers over the dorsal aspect of the distal tarsus and proximal metatarsus was observed to be multilayered (1-2 layers), but each layer had their own connections to CDET through their strong link of fibrous insertions into the CDET (Figures 9.17-9.20).

Deep to the superficial fascia around the metatarsal region, additional fascial layers (1-2) were observed and could be isolated on the dorsal aspect of the metatarsal region (Figure 9.18). The most superficial of these layers (layer-I) was called the superficial lamina of deep fascia. It was easily identifiable as it enveloped the common and lateral digital extensor tendons (Fig. 9.26). Just like superficial fascia, this layer grossly appeared to consist of two very closely associated sheets of fascia, which created a clearly defined membrane independent of the overlying superficial fascia.

It was tightly adhered at the junction of the third and the respective metatarsal bones on both the medial and lateral aspects (Figure 9.28). The superficial lamina of deep fascia had

a largely unspecialized arrangement of fibers over the proximal third of the dorsal third metatarsal similar to the superficial fascia in this region, the collagen fibers had direct attachments with the common digital extensor tendon on the dorso-lateral aspect of the proximal third metatarsal (Figure 9.17). In the distal two thirds and situated laterally to the common digital extensor tendon (CDET), the fibers of this fascia became thicker and oriented in a proximodistal direction. While approaching the fetlock joint, they formed a tendon-like band of fascia (Figures 9.18-9.20).

A similar oblique orientation of fibers was observed on the medial aspect of the fetlock joint. The fibers on the medial side were of the same thickness as those observed on the lateral aspect. These fibers also appeared to be continuous with the distal extremity of the lateral digital extensor tendon (LDET) over the dorsolateral aspect of the fetlock joint macroscopically (Figure 9.22).

The dorsolateral aspect of fetlock joint contained 1-2 bursae associated with the fascia and tendons. A subfascial bursa was observed occasionally on the medial side. A constant subtendinous bursa was observed deep to the combined common and lateral digital extensor tendons in all specimens dissected (Figure 9.32).

A longitudinal incision was made along the plantar aspect (plantar length of the superficial and deep flexor tendons (SDFT)) and revealed that the deep fascia was intricately organized along the length of the metatarsals while surrounding the flexor tendons. Fascia over this region was a clear distal extension of the tarsal fascia when followed proximally, which had reinforced fibers which were involved in the formation of the flexor retinaculum bands of the tarsal joint. When followed distally, the fascia continued distally and at the level of fetlock joint, the deep fascia merged with the superficial fascial layer. The ergot was

comprised of a dense connective tissue mass which had strong and inelastic attachments to the plantar annular ligament of the fetlock joint. At this point, both superficial and deep fascia formed a prominent juncture while the superficial and deep fascia kept expanding over the distal hindlimb (Figure 9.37).

The two ergot ligaments were embedded within this superficial fascia and had their origin points in the fibrous tissue mass over the annular ligament of the fetlock joint. These ligaments did not directly insert into the medial and lateral extensions of the distal digital annular ligament (DDAL). These ligaments were inserted into fascial bands which spanned across from the margins (medial and lateral) of the common digital extensor tendon (CDET) on the dorsal surface to the distal digital annular ligament on both the medio-plantar and latero-plantar aspects of the pastern. In addition, the bands of pastern fascia had strong attachment to the digital cushion and cartilages of the hoof.

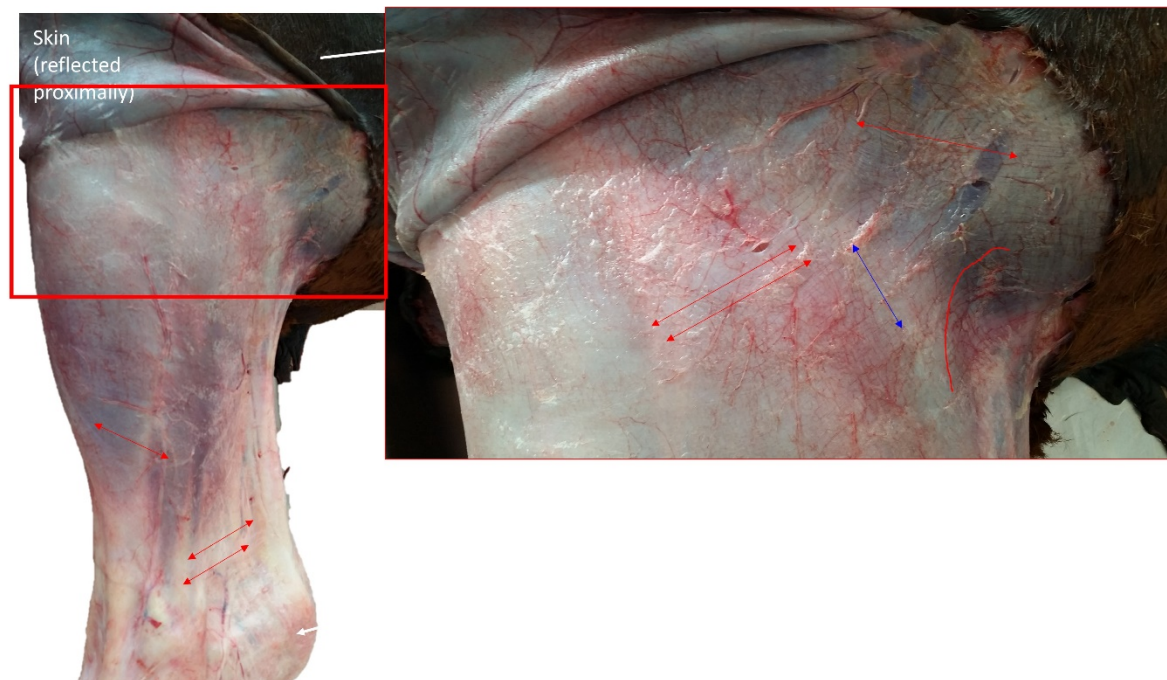
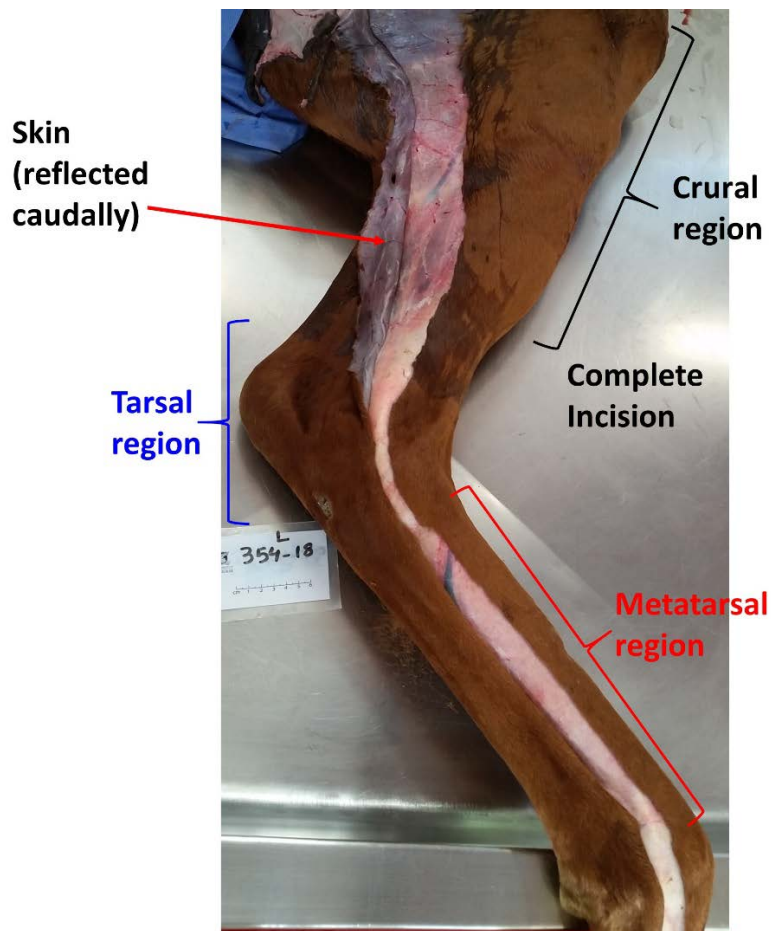
These pastern bands provided a continuous mechanical connection from the hoof and plantar aspect of the fetlock joint and digit, its associated ligaments, and the fascia over the plantar aspect of the metatarsus. The application of traction in different directions (proximal, distal, medial and lateral) by the forceps and following the tension lines on the ergot tissue demonstrated the transmission of traction along the mechanical pathway distributing widely among many connecting or linked structures such as ergot ligaments, distal digital annular ligament, hoof cartilage and digital cushion. Thus, the ergot tissue served as a central anchored point, through which tension forces distributed to the entire distal limb through the connections of the fascia and the skin.

A membranous sheet was observed spanning across the two extensions of the superficial digital flexor tendon (SDFT) at the level of the approximate distal end of the proximal

phalanx deep to the deep digital flexor tendon (DDFT). Both the DDFT and the SDFT were connected by small tendon-like connections termed the vincula tendinea that emerged from this membranous sheet (Fig. 9.34).



Figure 9. 1: Medial and lateral aspects of the left hind limb from crural region to distal limb (skin was removed).



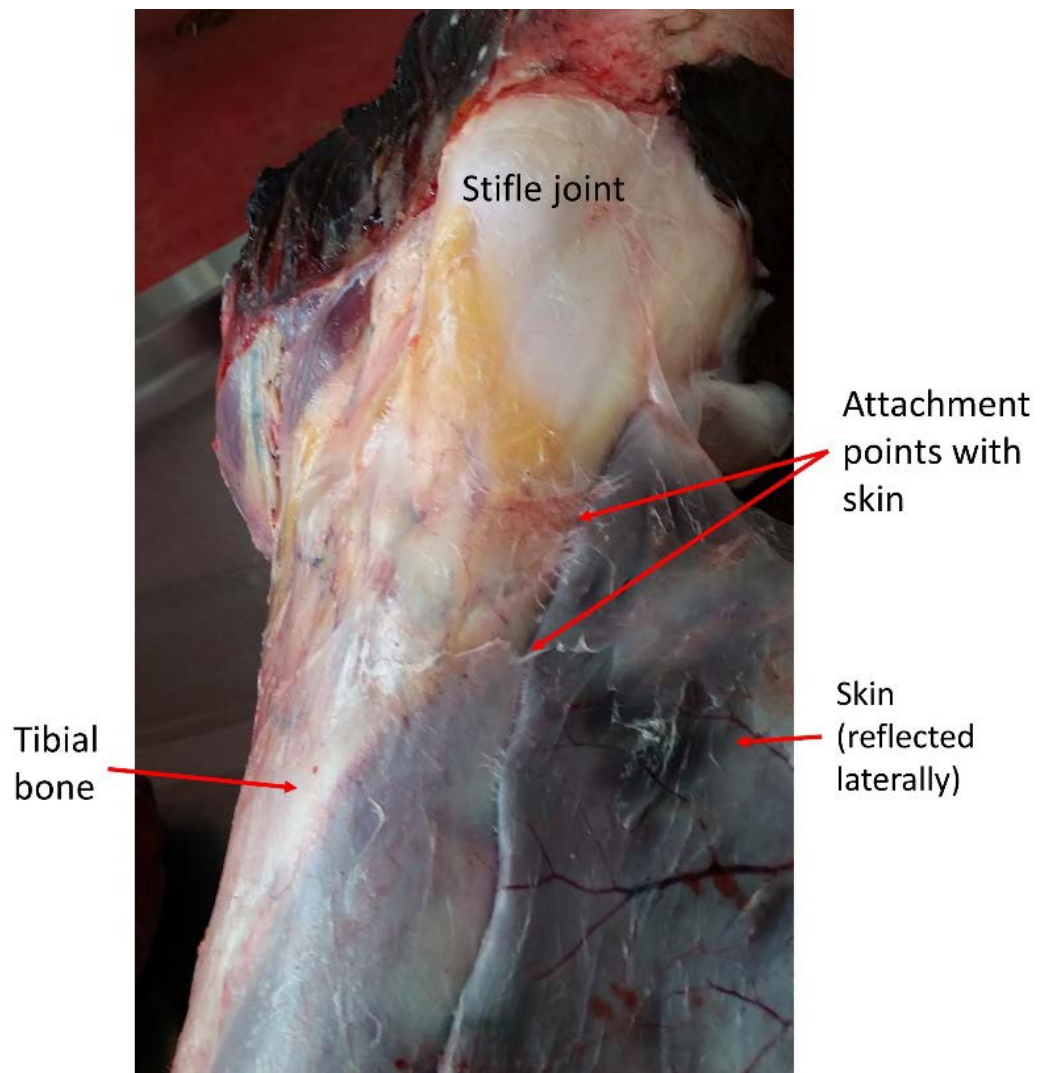


Figure 9. 4: Cranial aspect proximal two thirds of left crural region starting from the stifle joint showing superficial fascia attachments to the skin.

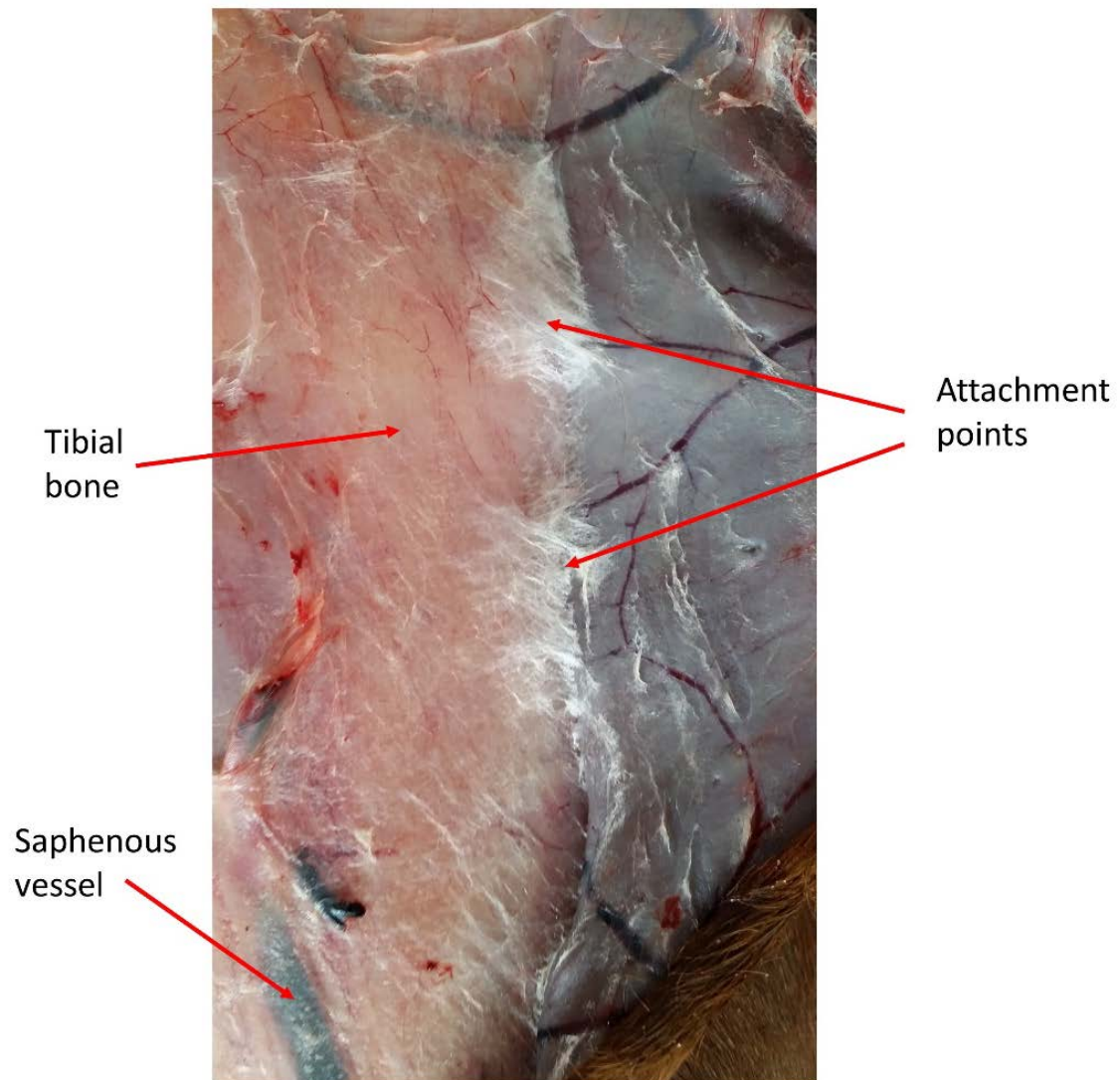


Figure 9. 5: Medial aspect of the left hind limb from proximal two third of crural region showing attachments of the superficial fascia to the skin.

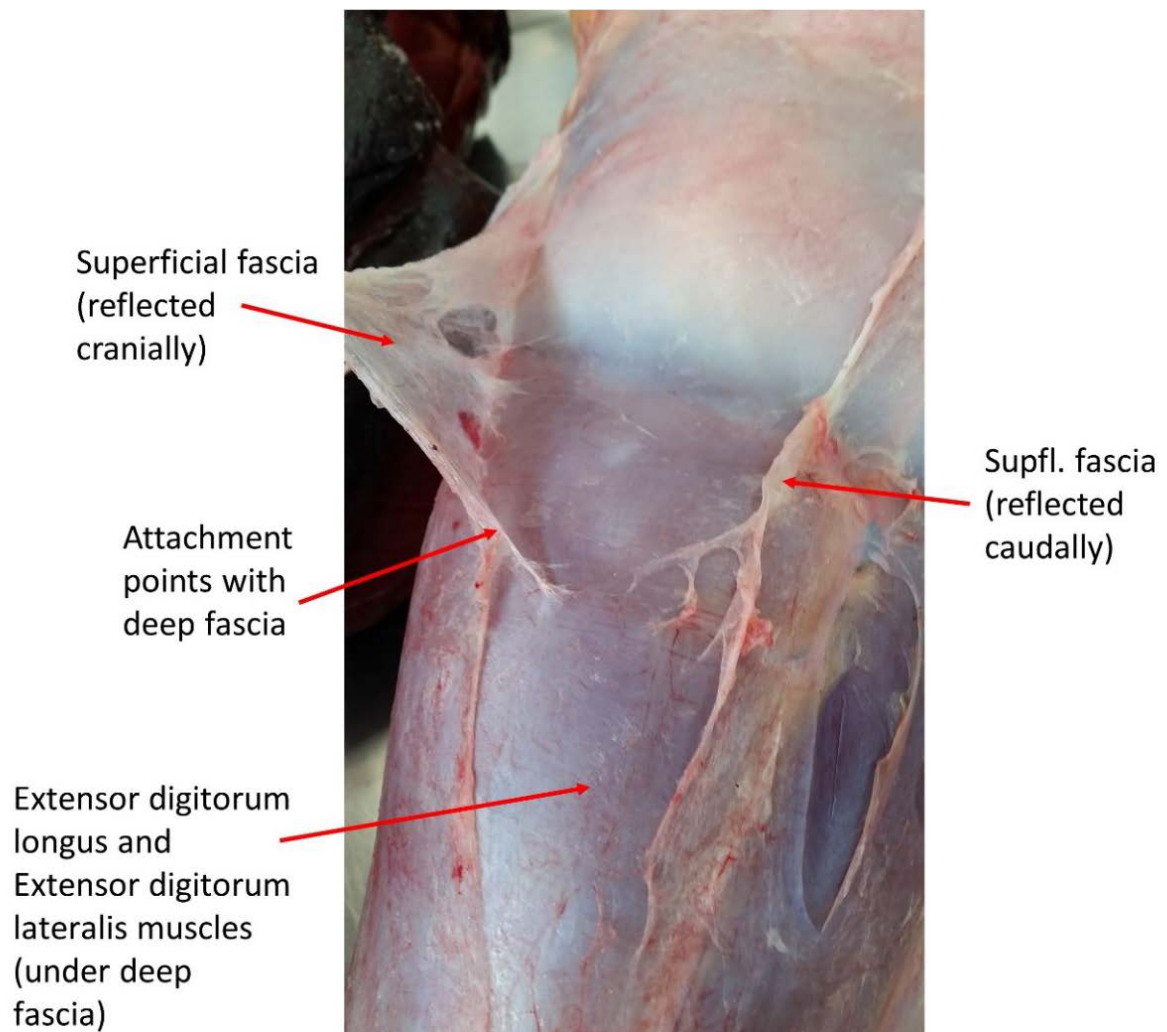


Figure 9. 6: Cranio-medial aspect of the proximal two thirds of crural region (left hind limb) showing connection of superficial and deep fascia (skin was removed).

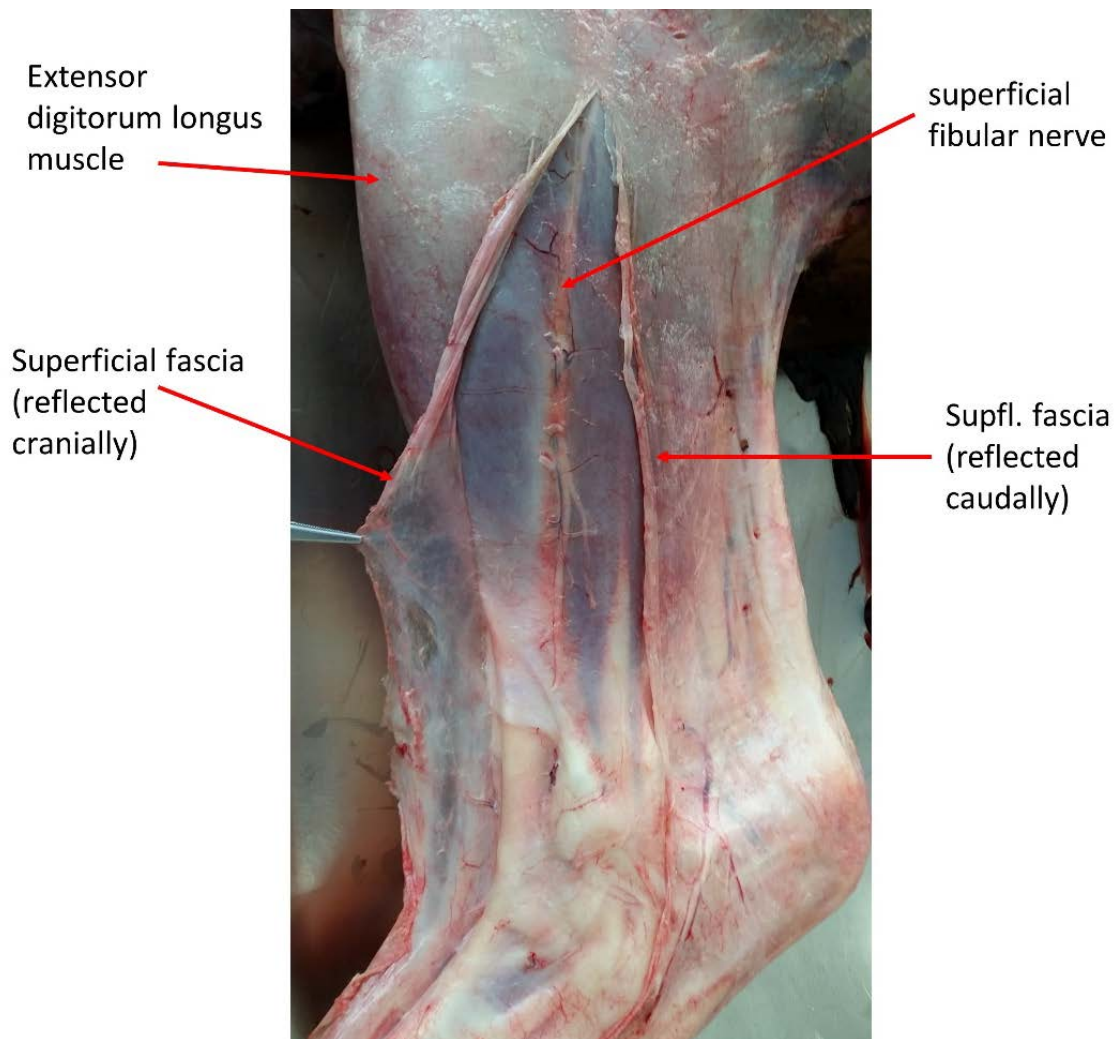


Figure 9. 7: Lateral aspect of the left hind limb showing reflection of superficial layer from the deep fascia and its continuity over different regions.

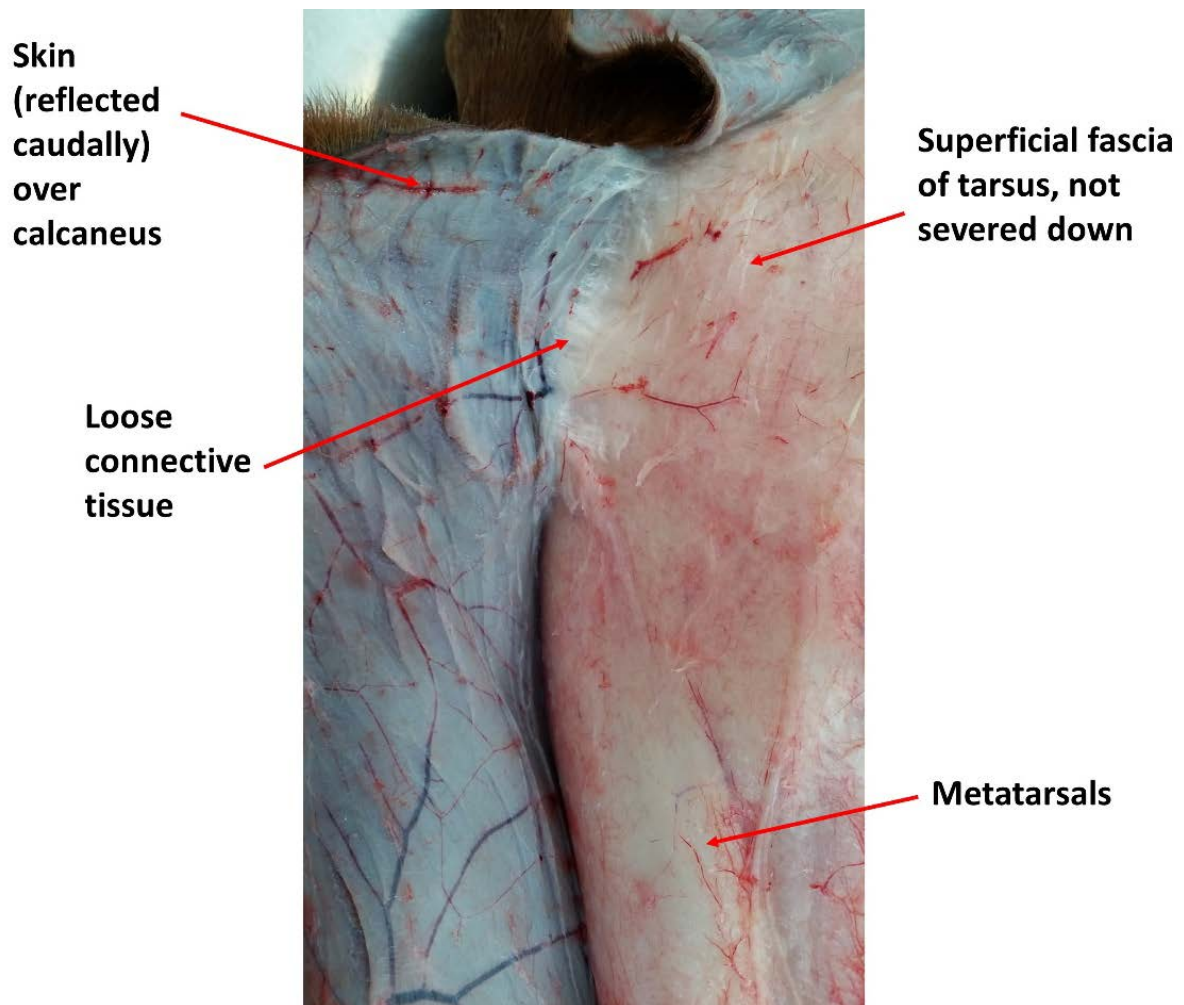


Figure 9. 8: Lateral aspect of the left tarsal joint showing the fibrous connectivity between the superficial fascia and the overlying skin and the increase over the tuber calcaneus.

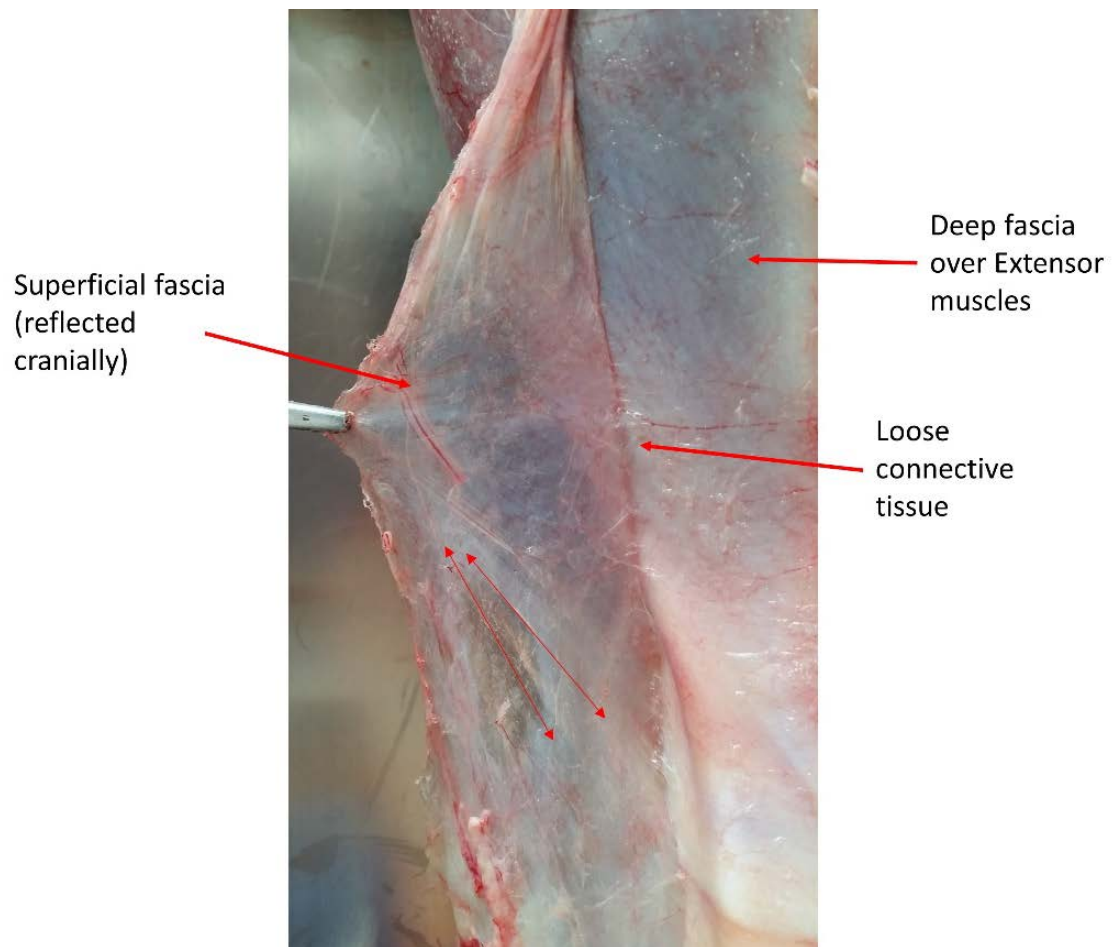


Figure 9. 9: Cranio-lateral aspect of the left distal crus with the extensor retinacula for extensor tendons, thin red arrows show the alignment and continuity of collagen fibres can be seen visually or using magnifying glass.

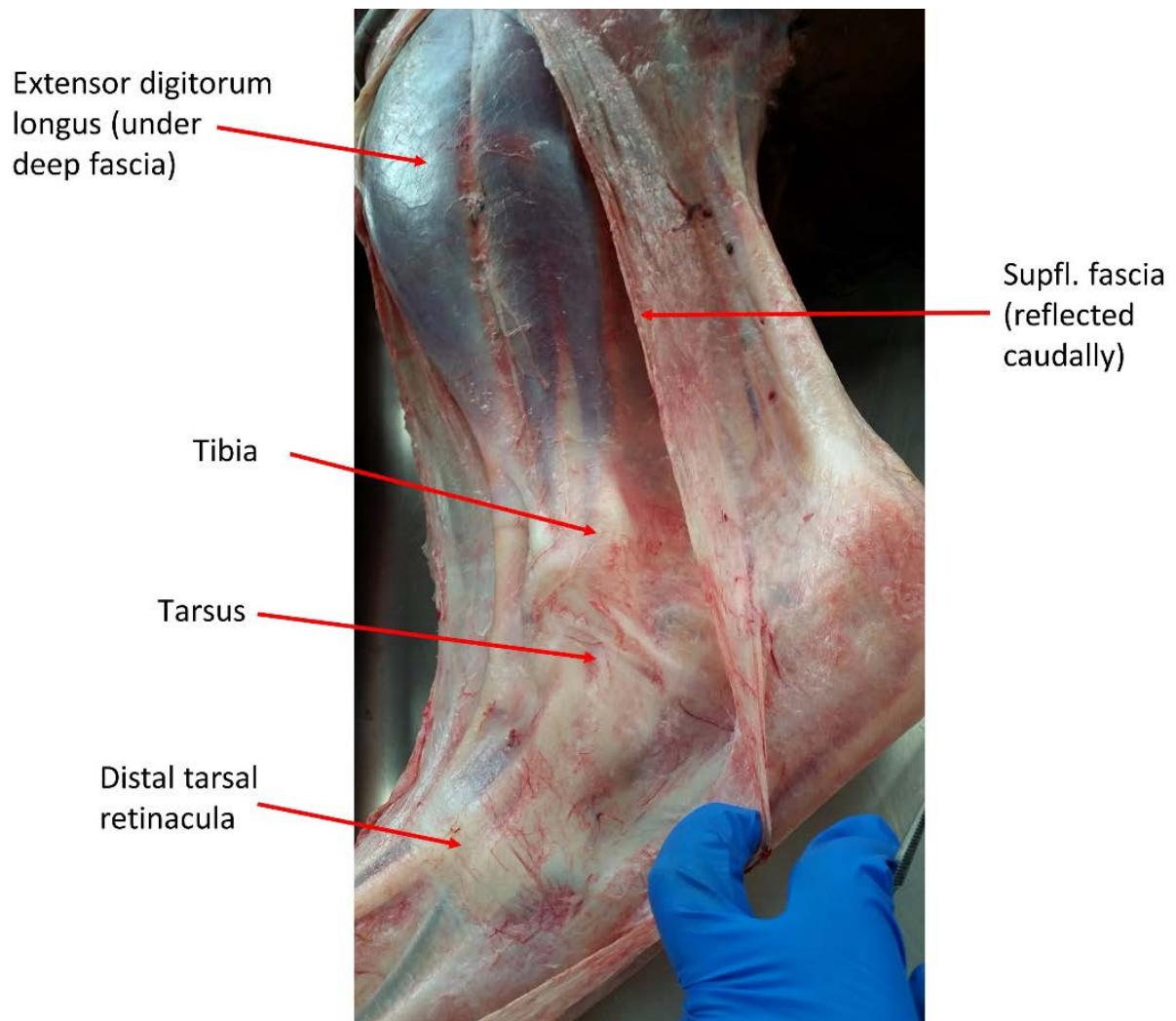


Figure 9. 10: Lateral aspect of tarsus and crural regions.

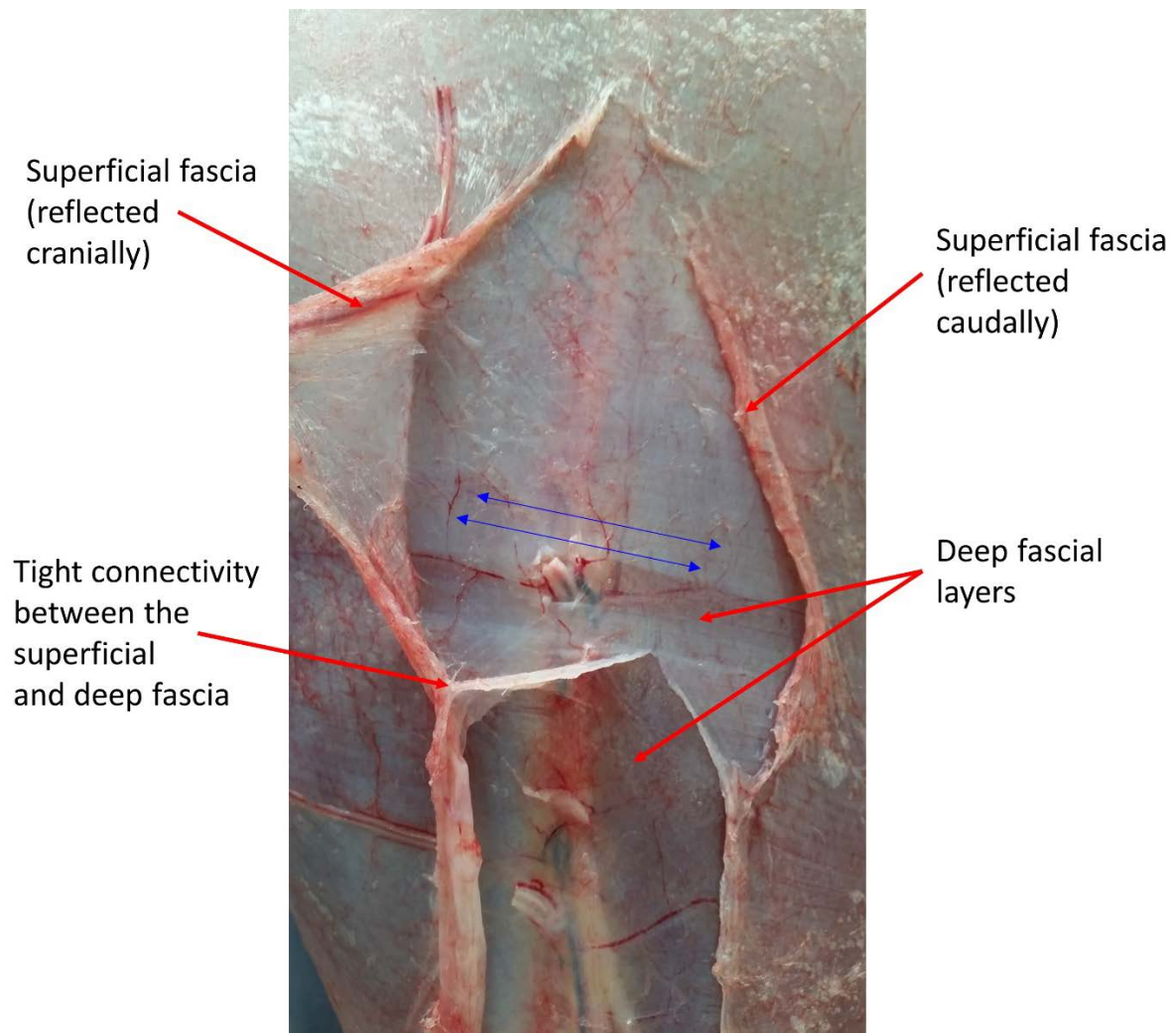


Figure 9. 11: Lateral aspect of proximal two thirds of crus, showing the interconnection of superficial and deep fascial layers, blue arrows. showing the orientation of collagen fibers can be seen visually or using of magnifying glass.

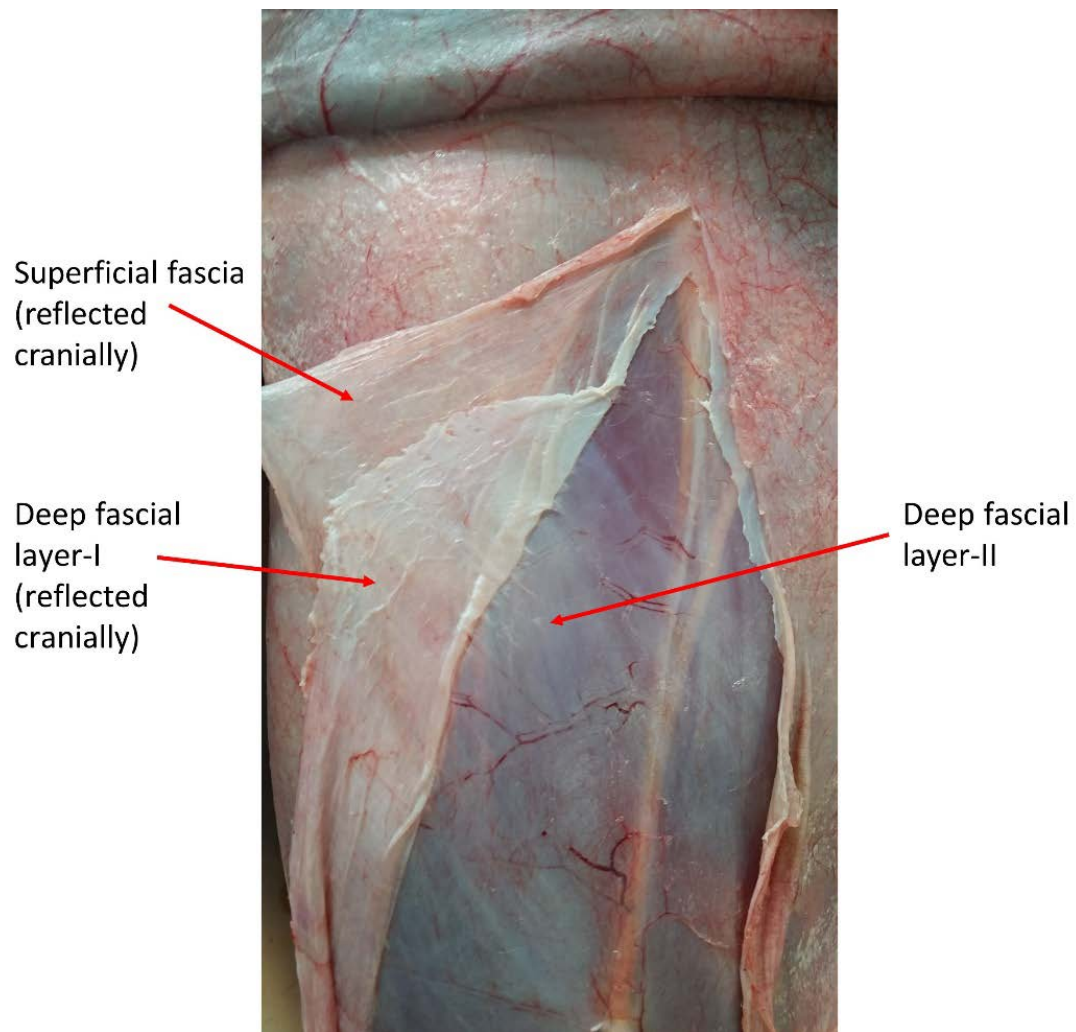


Figure 9. 12: Connectivity between the superficial and deep fascia over the craniolateral aspect of the left mid-crus (over the long lateral extensor muscle).

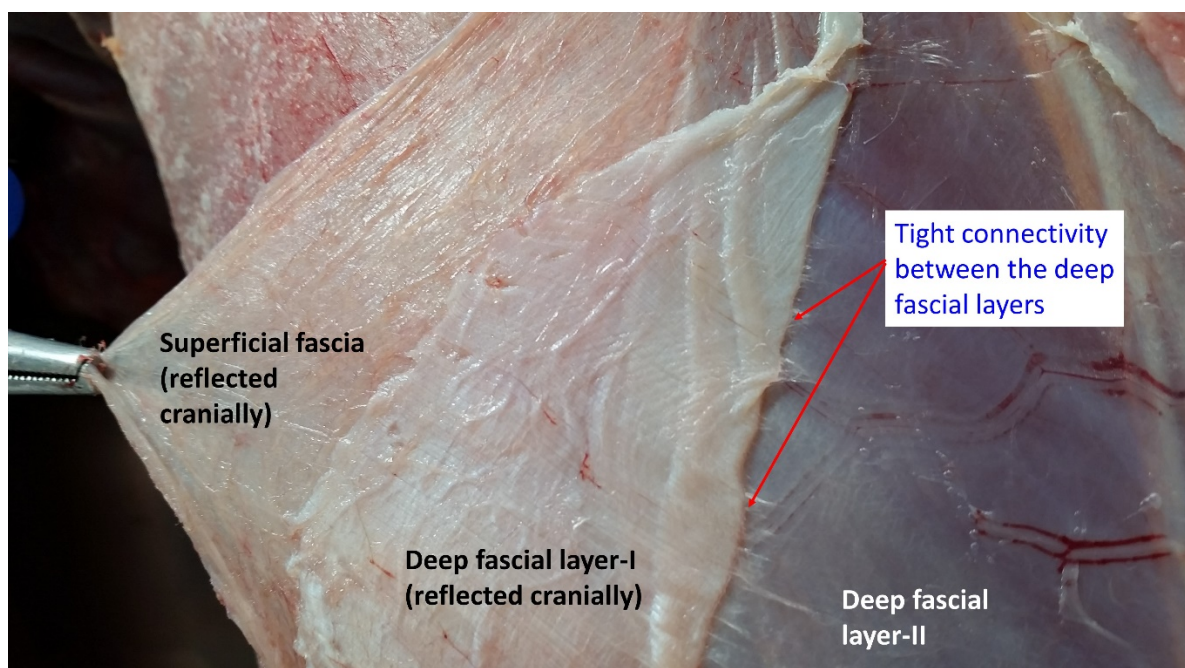


Figure 9. 13: Closer look at the connectivity between the superficial and deep fascia over the craniolateral aspect of the left mid-crus (over the long lateral extensor muscle).

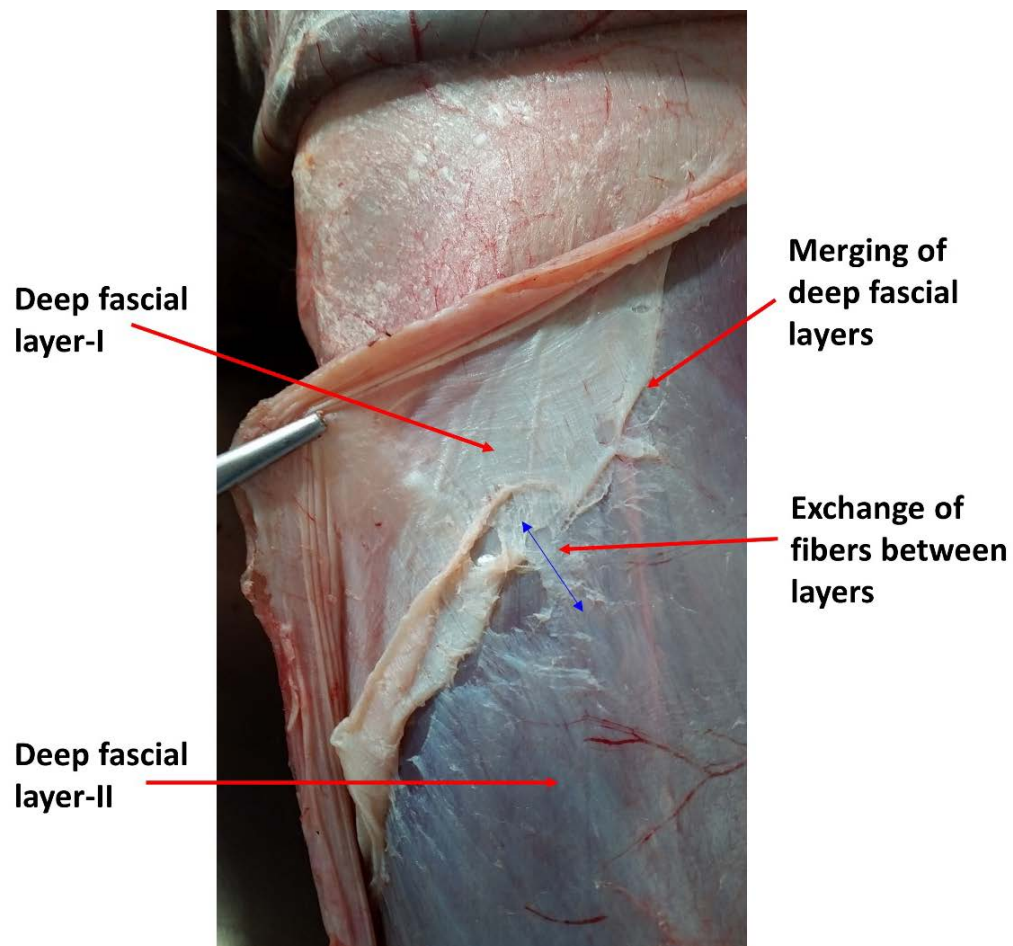


Figure 9. 14: Connectivity between the superficial and deep fascia with exchange of fibers between layers over the craniolateral aspect of the left mid-crus (over the long lateral extensor muscle).

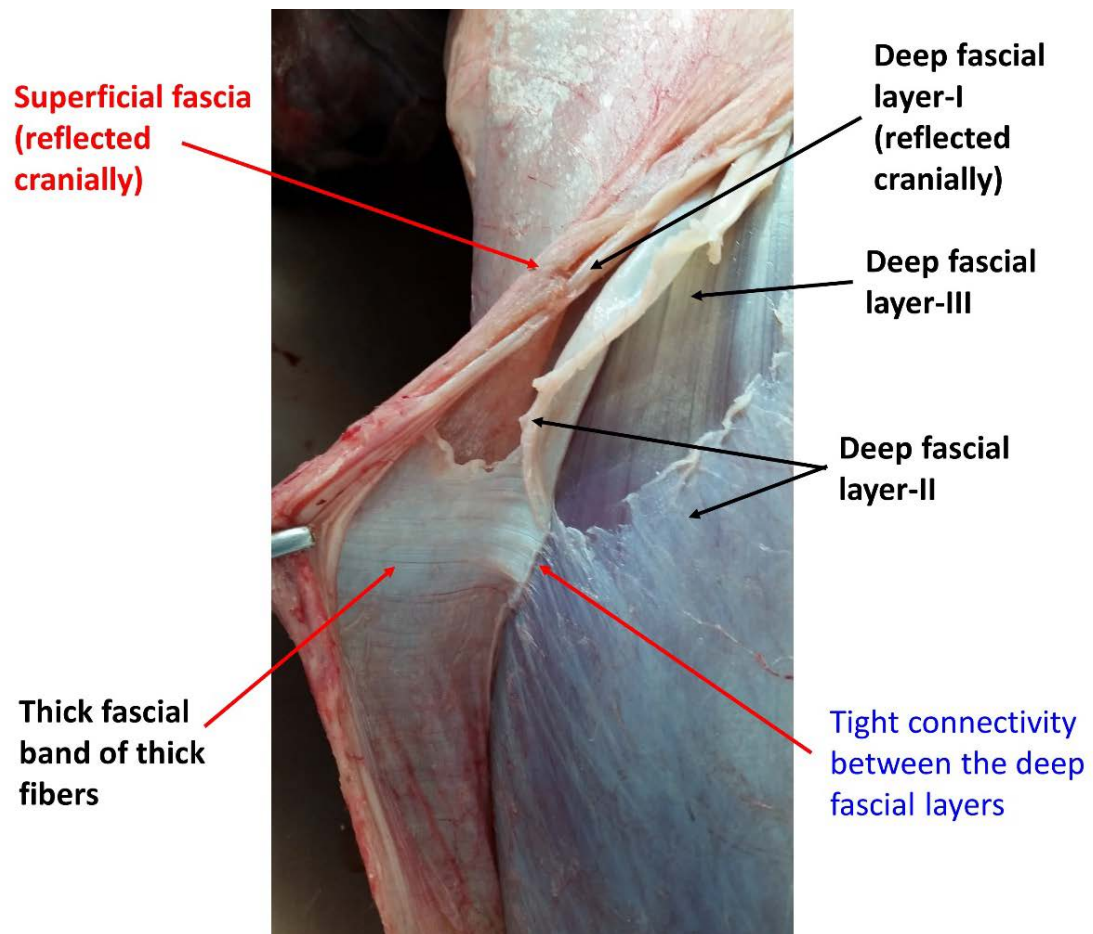


Figure 9. 15: Points of fusion with thick band (fascial reinforcement) of fibers between the superficial and deep fascia layers (deep fascial layer-I and deep fascial layer-II) over the craniolateral aspect of the left mid-crus (over the long lateral extensor muscle).

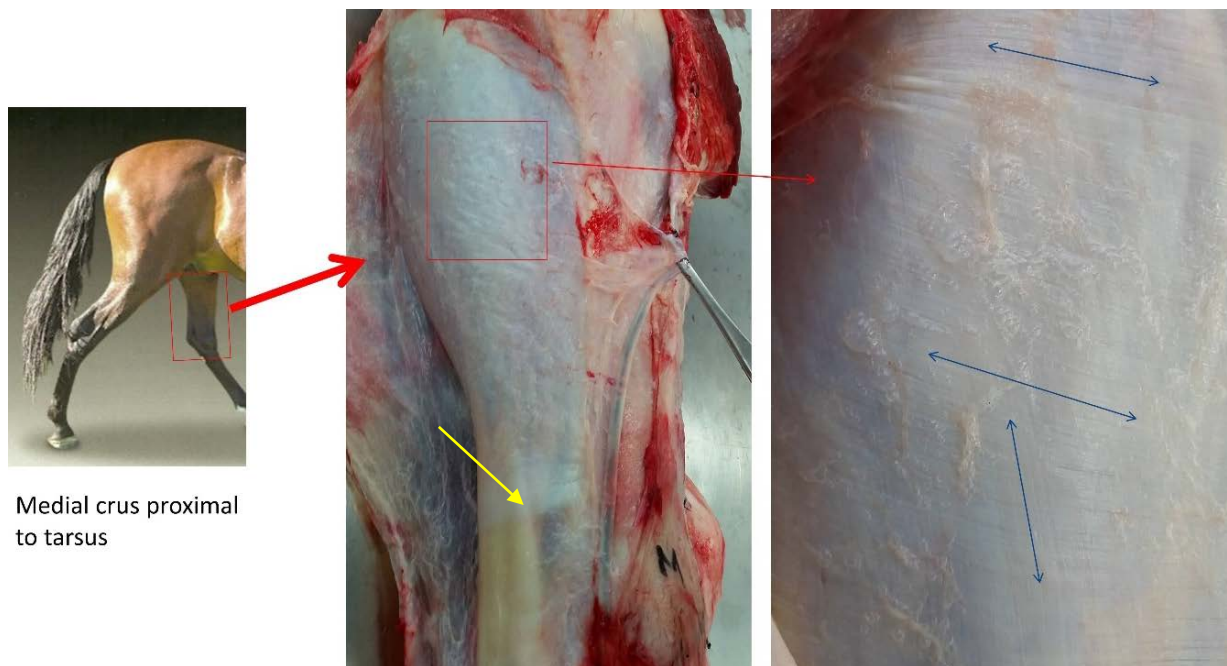


Figure 9. 16: Medial aspect of the left crural region with the superficial fascia severed and reflected caudally, blue arrows show the transverse, longitudinal and varying fibers directions of the deep fascia. Yellow arrow, thick distal band of fibers.

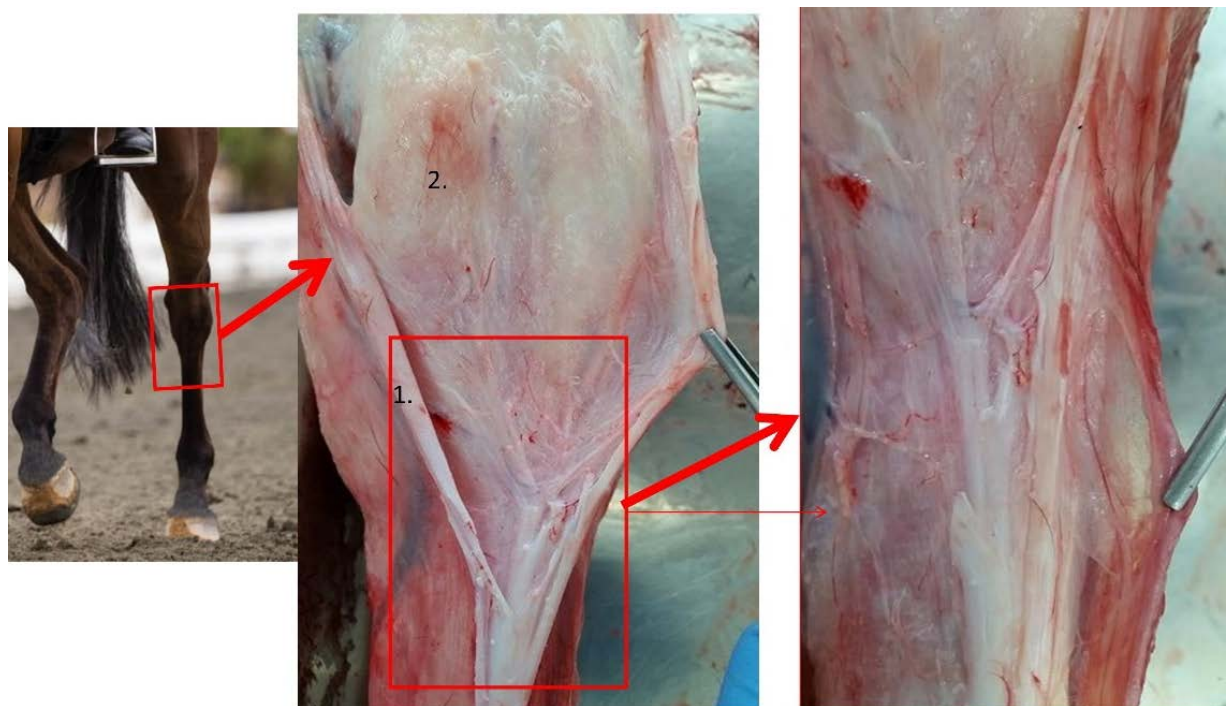


Figure 9. 17: Deep fascia over dorsal aspect of the left tarsus. Highlighted areas showing the direct attachments of fascial fibers into the CDET fibers (some fibers dissected for demonstration purpose), 1-2. Different layers of deep fascia with their own connections to CDET through their fibers insertions into the CDET.



Figure 9. 18: Deep fascial layers (layers II and III) over dorsal aspect of of the left tarsus. Showing the thicker sheath of fibers covering the dorsal aspect, Red arrows indicate the linkage of fibers (cut ends) of deep fascia with the CDET.

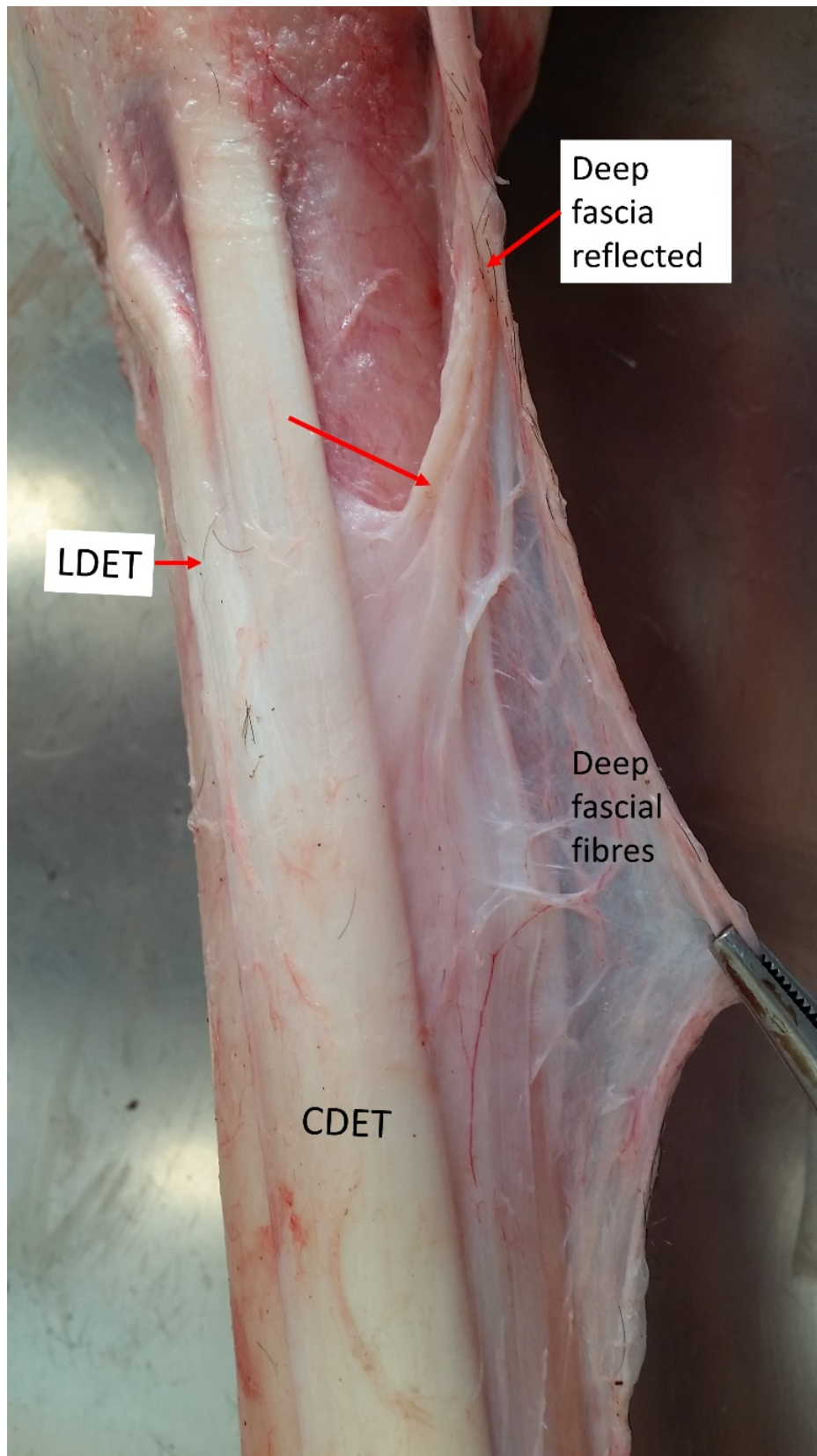


Figure 9. 19: Connectivity (direct link) between the deep fascial layers (layers II reflected laterally) and layer-III with exchange of fibers over CDET over the proximal metatarsus (red arrow). The superficial fascia was already removed.

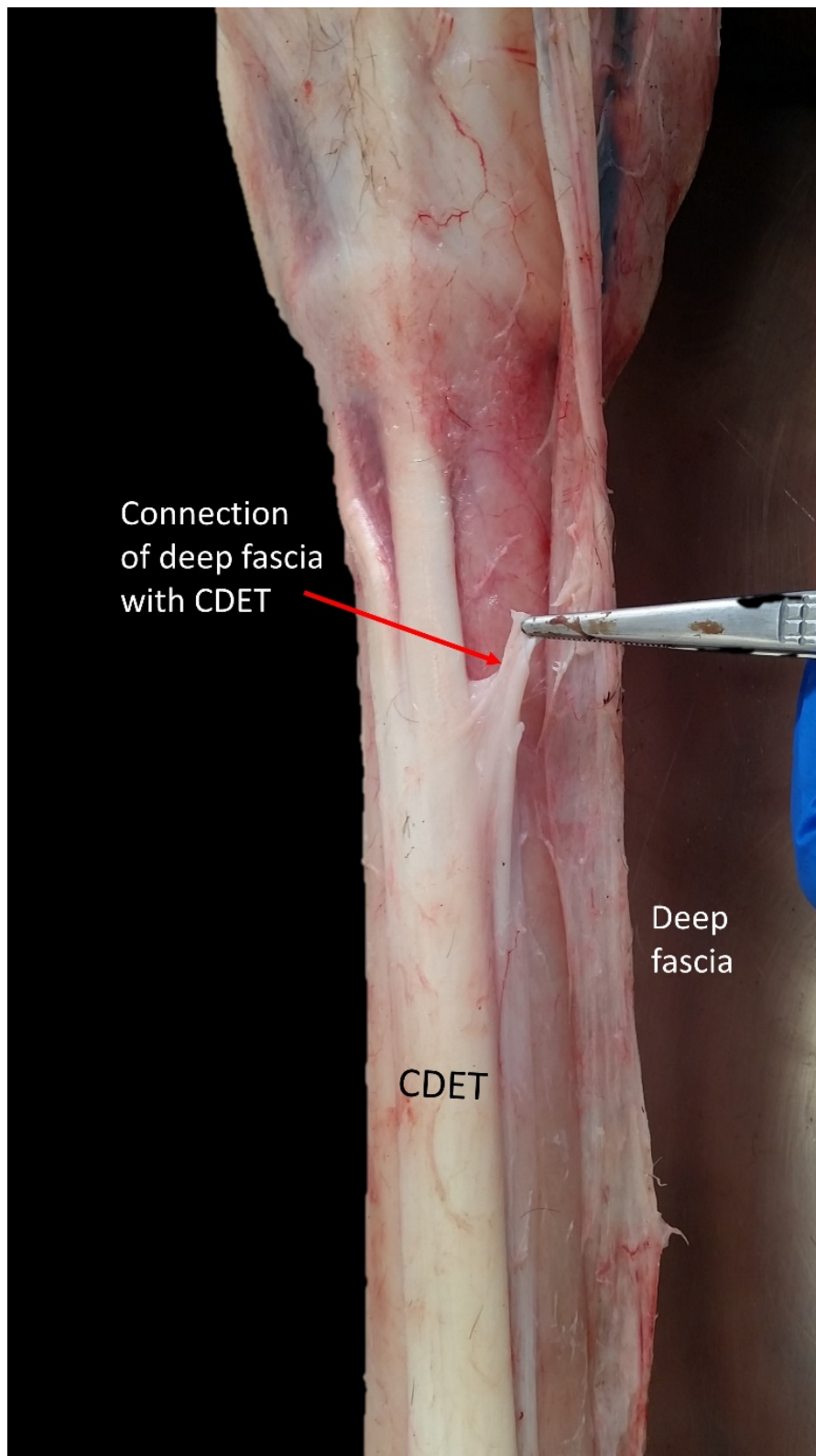


Figure 9. 20: Connectivity between the deep fascia with exchange of fibers over CDET over the proximal metatarsus. The fibers were cut and tension was applied to explore the strong connection and follow the directional pull. The fibers were difficult to isolate within CDET. The superficial fascia was already removed.

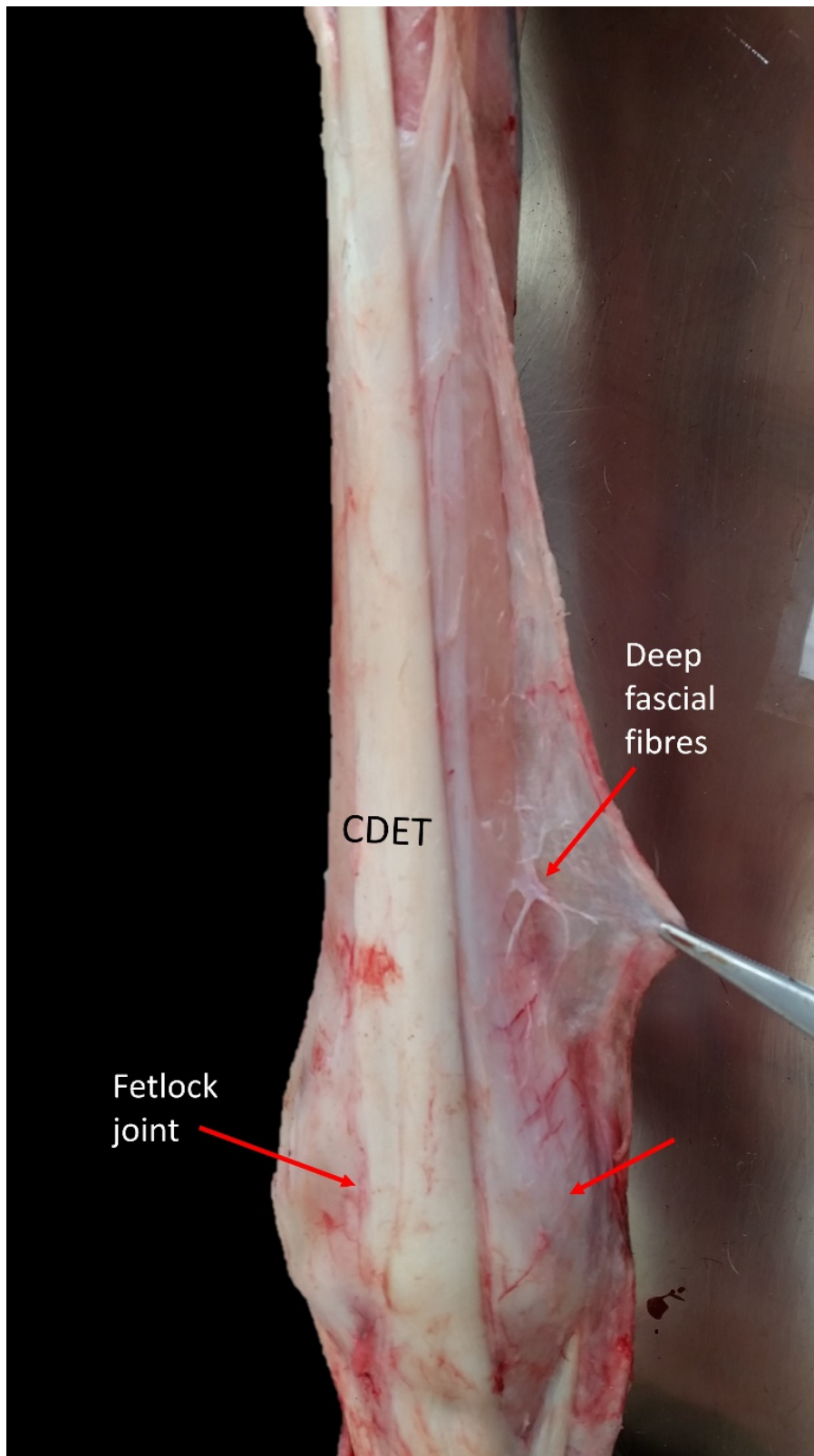


Figure 9. 21: Dorsomedial aspect of distal half of the metatarsus and fetlock (metatarsophalangeal) joint of right hindlimb. The superficial fascia was already removed. Arrow shows the fiber reinforcement.

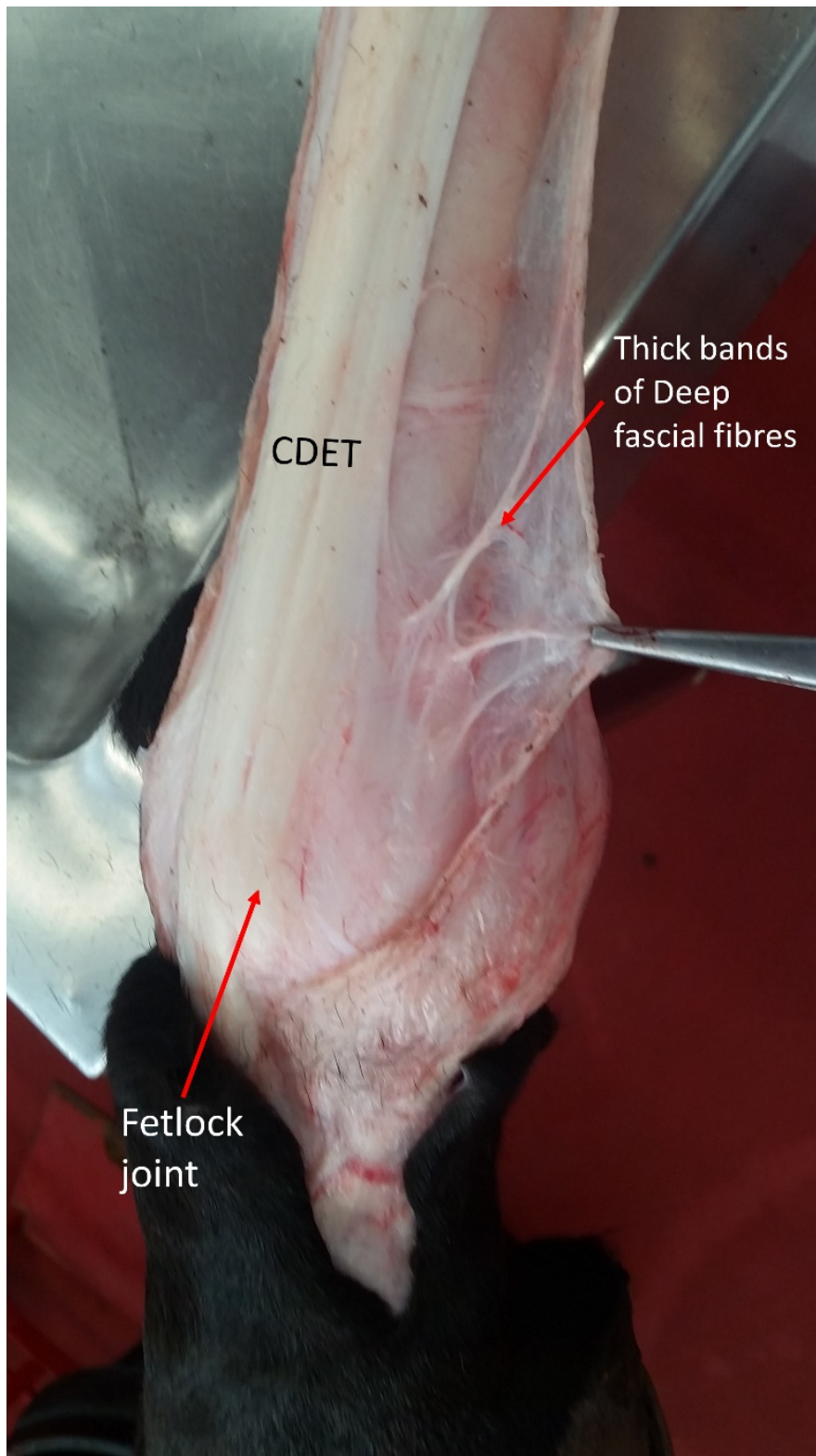


Figure 9. 22: Dorsolateral aspect of distal half of the metatarsus and fetlock (metatarsophalangeal) joint of left hindlimb. The superficial fascia was already removed. Arrow shows the thick fibers connections.

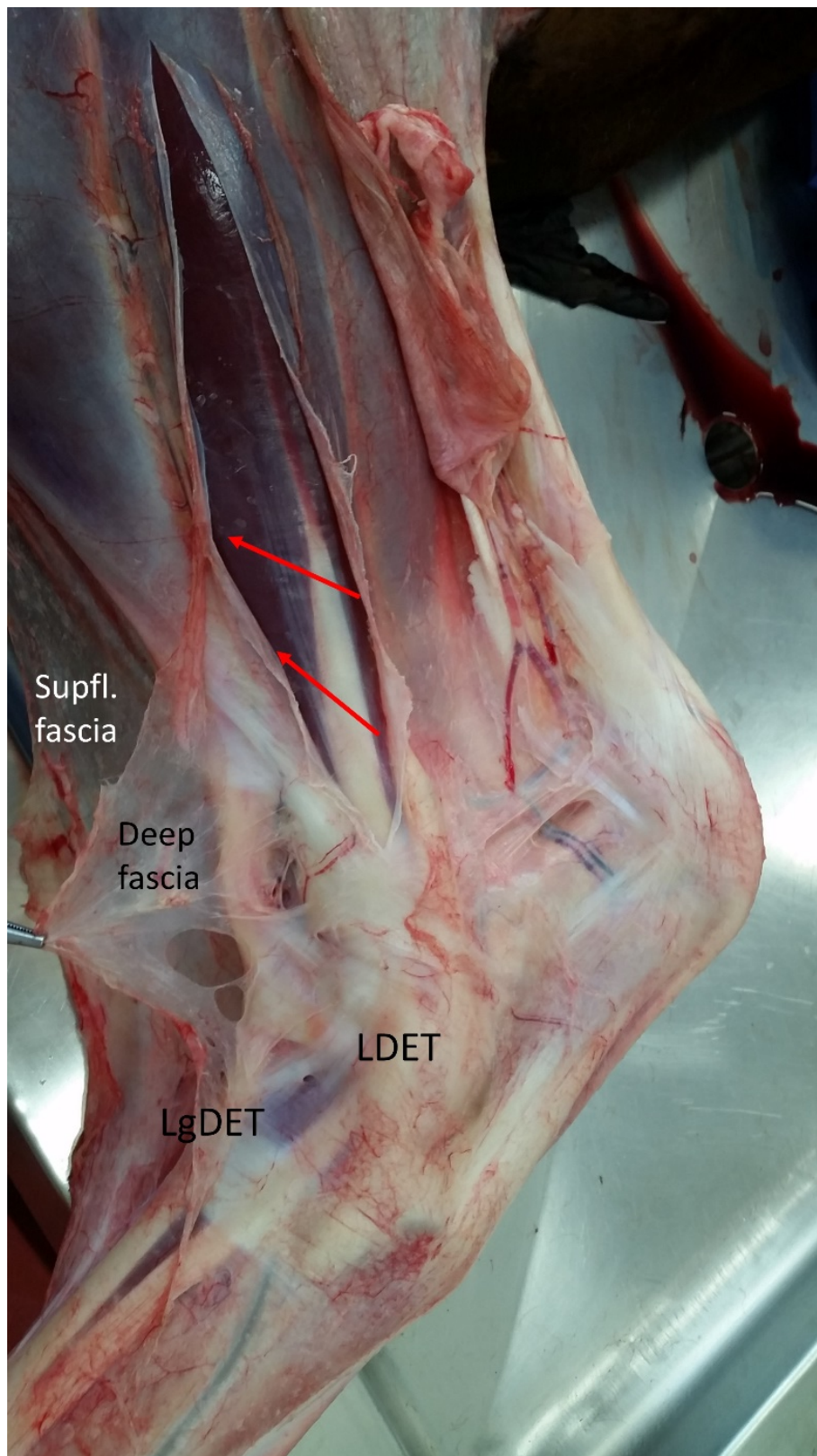


Figure 9. 23: Fascia over lateral aspect of left distal crus and proximal metatarsus. Arrows indicate the intermuscular septum highlighting the merging of aponeurotic and epimysial

fascia. The superficial fascia was already removed. LgDET. Long digital extensor tendon, LDET. Lateral digital extensor tendon.

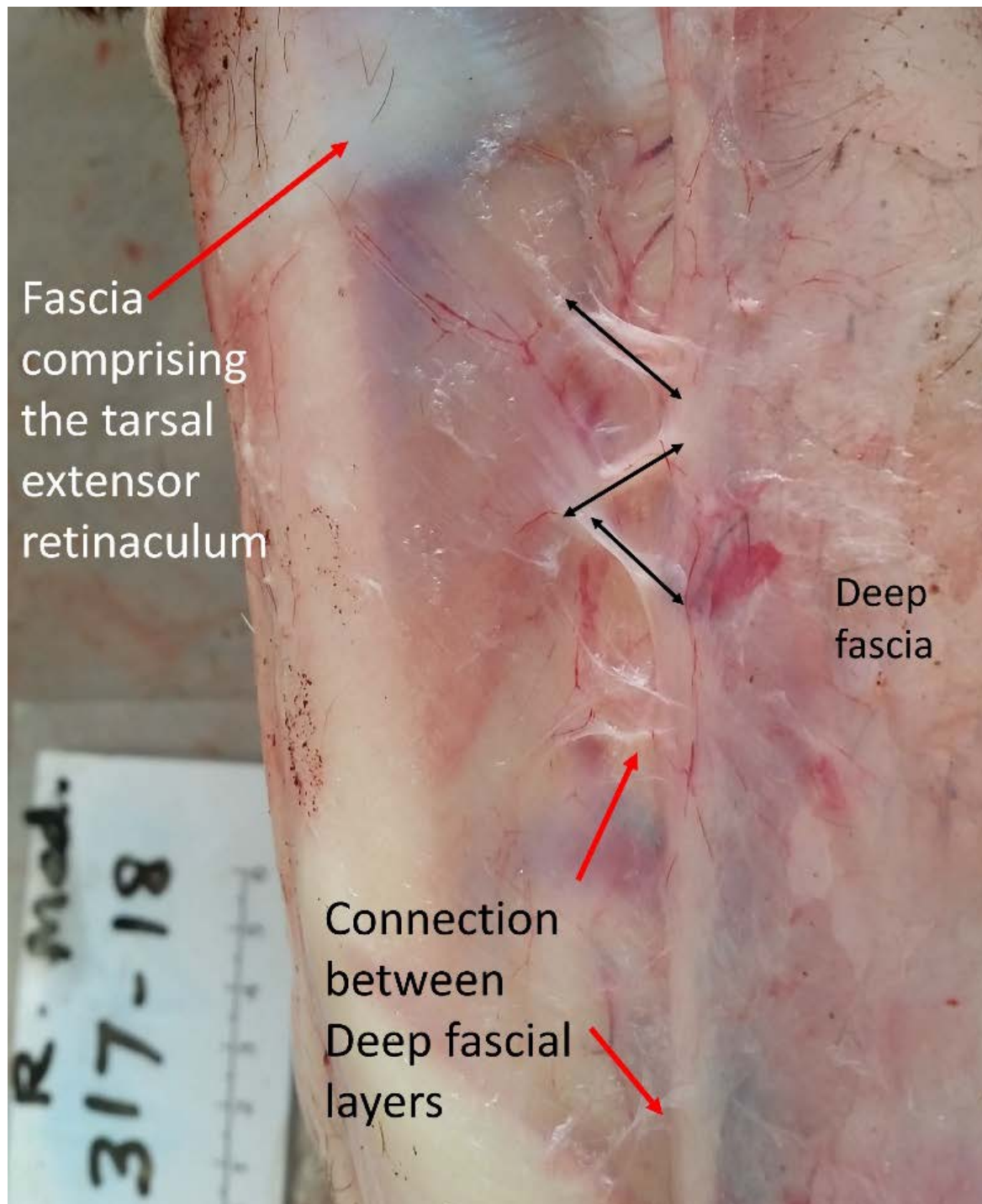


Figure 9. 24: Connectivity between the two layers of deep fascia (one layer being reflected caudally) with exchange of fibers over lateral aspect of distal third of crus. The superficial fascia was already removed.

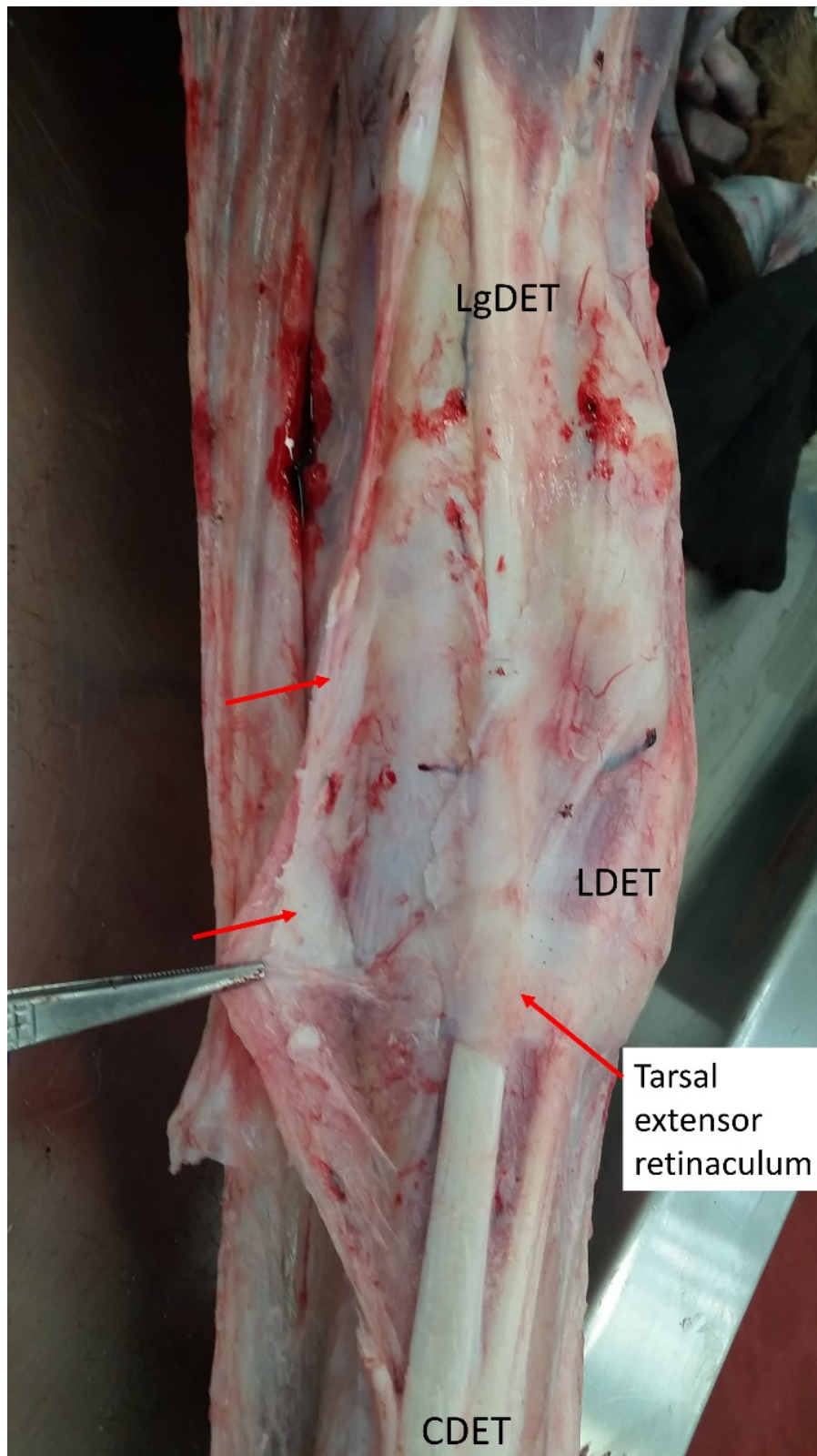


Figure 9. 25: Fascia over dorsal aspect of left distal crus and proximal metatarsus. Arrows, show the continuous connection of aponeurotic fascia with retinacular fibers. The superficial fascia was already removed. CDET. common digital extensor tendon, LDET. Lateral digital extensor tendon.

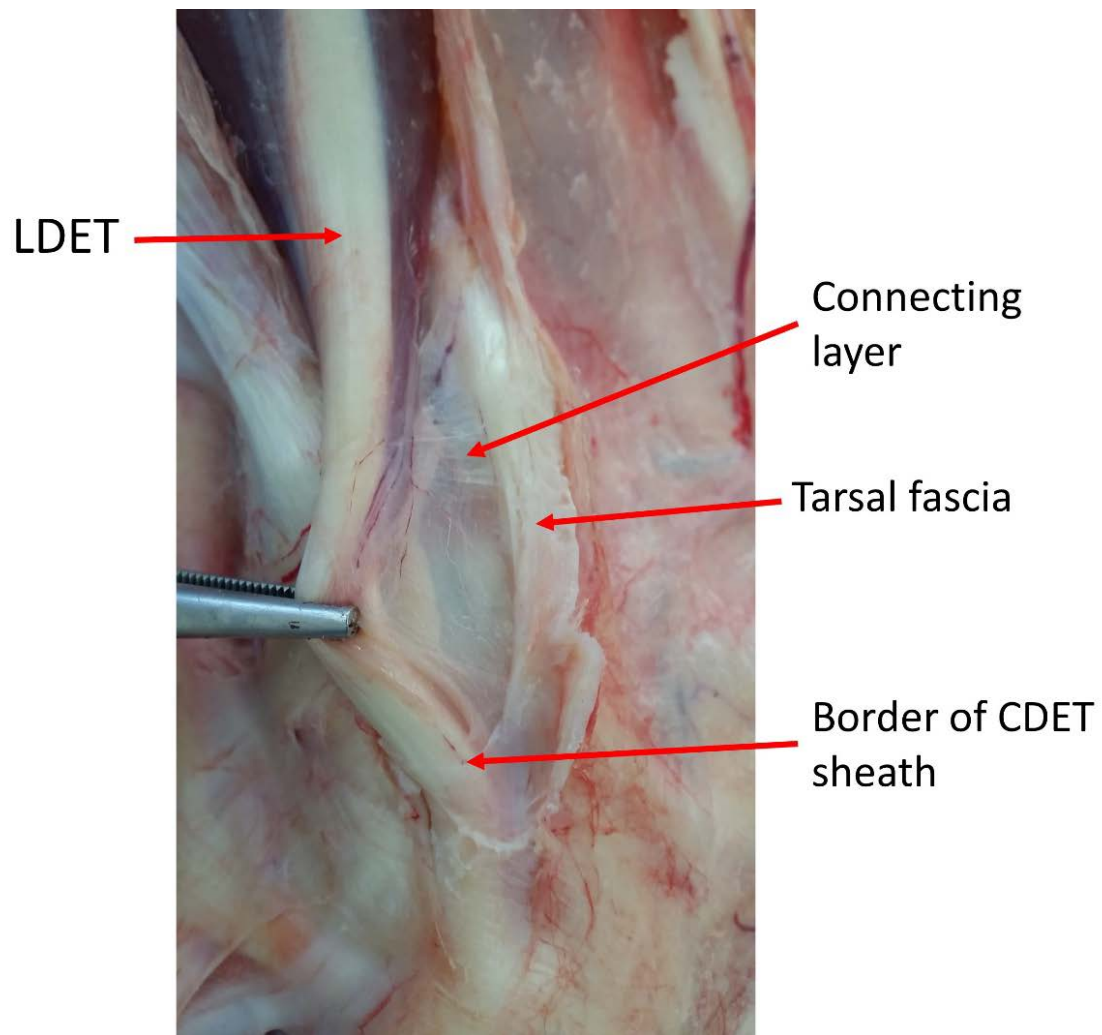


Figure 9. 26: Fascia over lateral aspect of left tarsus pertaining to the lateral digital extensor tendon sheath, string like fascial reinenforecment along the LDET sheath.

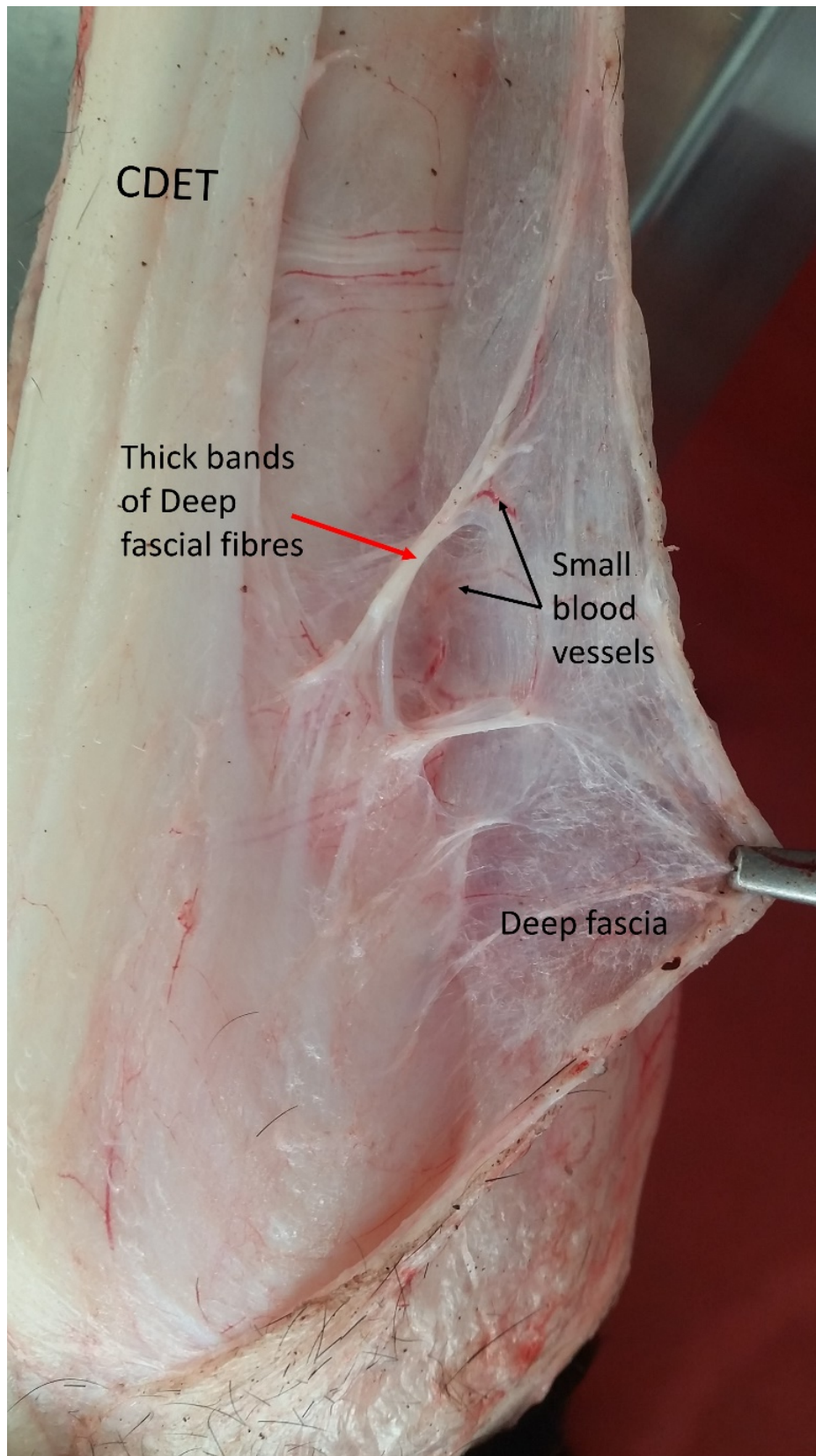


Figure 9. 27: Connectivity between the two layers of deep fascia with exchange of fibers over lateral aspect of distal third of metatarsus showing the passage of blood vessels along with thick fibers connectivity. The superficial fascia was already removed.

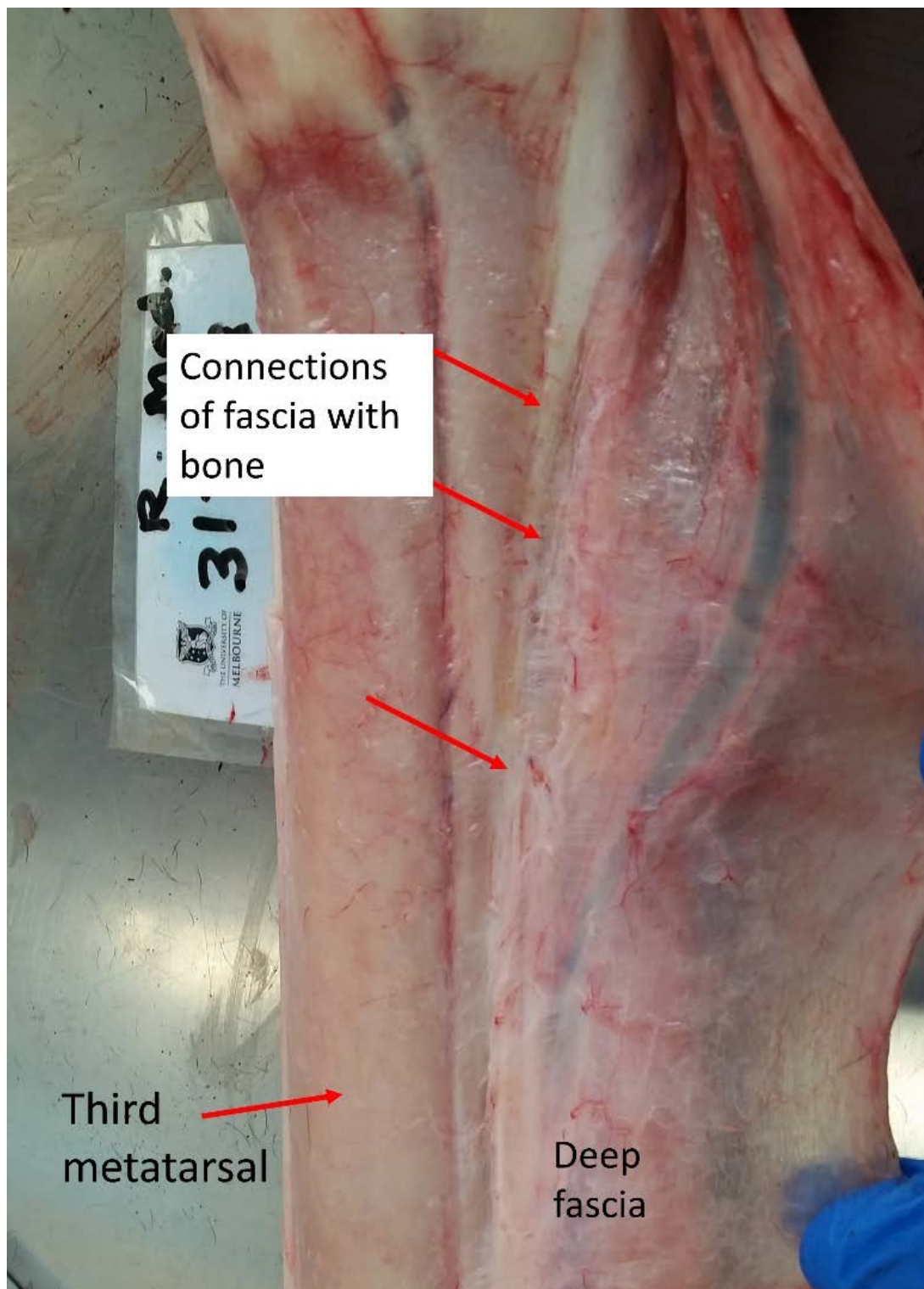


Figure 9. 28: Medial aspect of left metatarsus. The superficial fascia was already removed. The deep fascia layer-II reflected caudally, showing the connectivity of fascia with bone.

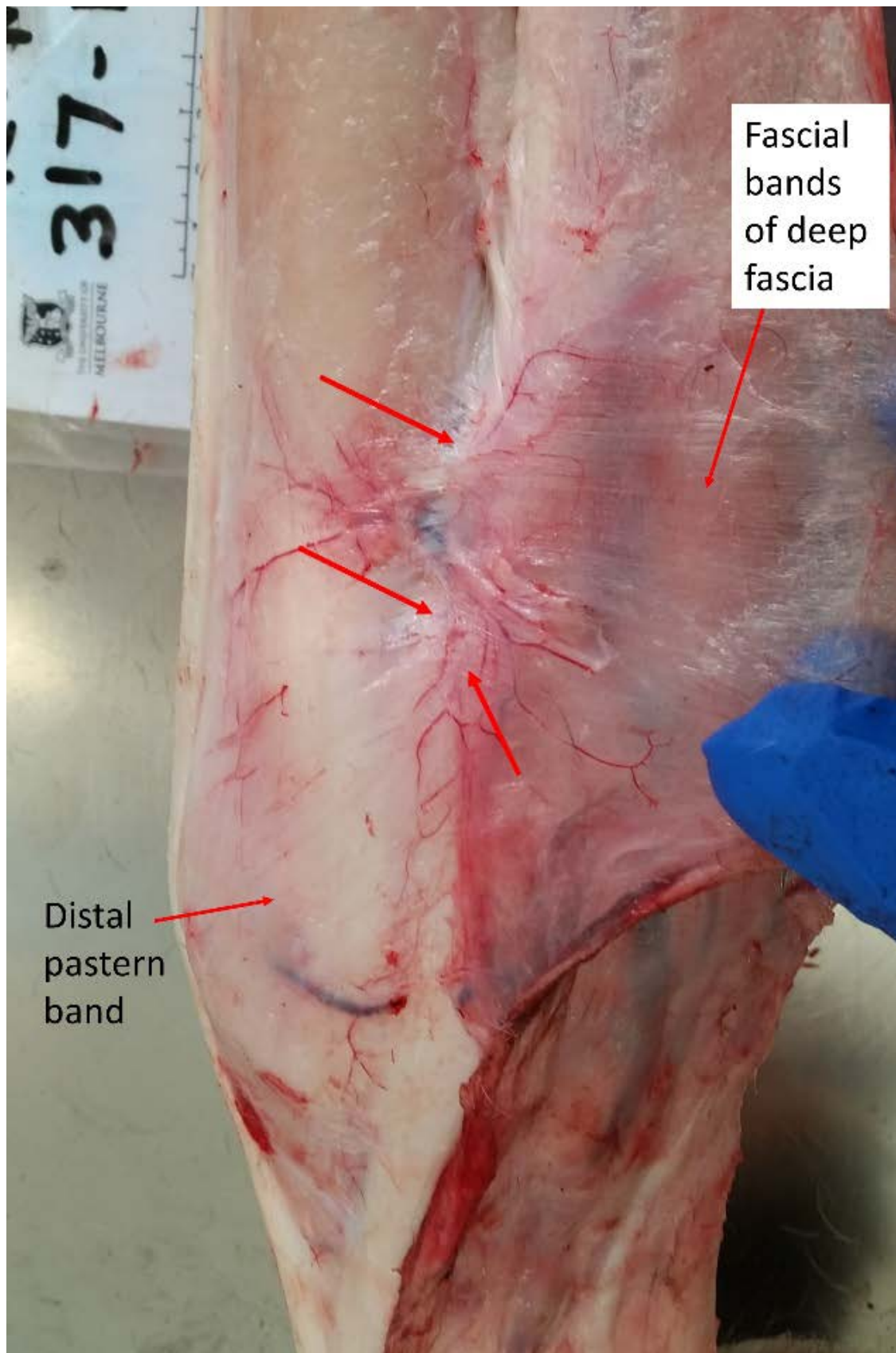


Figure 9. 29: Connectivity between the two layers of deep fascia with exchange of fibers over lateroplantar aspect of distal third of left metatarsus. The superficial fascia was already removed and deep fascial layer was reflected caudally.

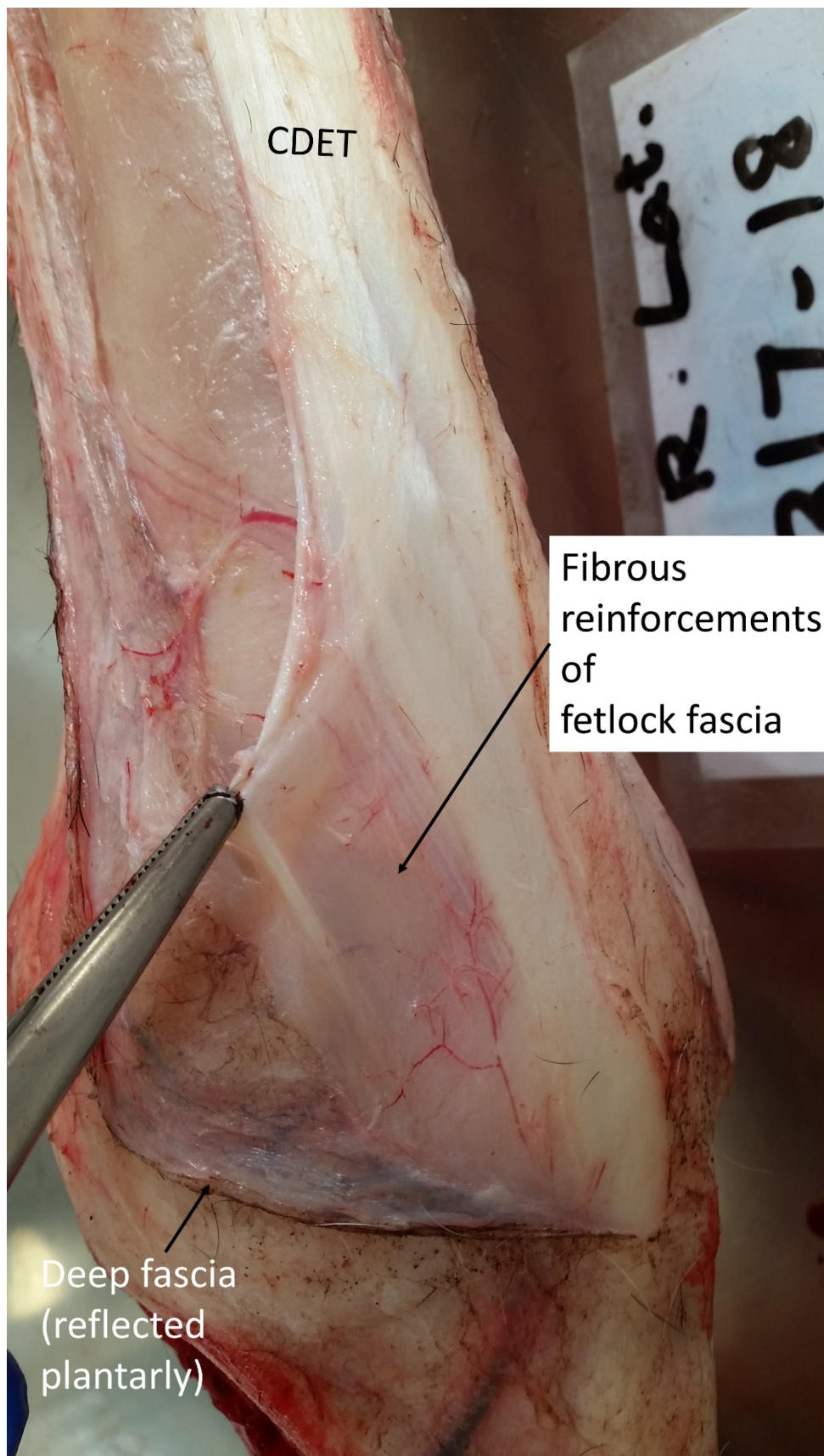


Figure 9. 30: Lateral aspect of distal half of the right metatarsus and metatarsophalangeal joint. The superficial fascia was already removed.

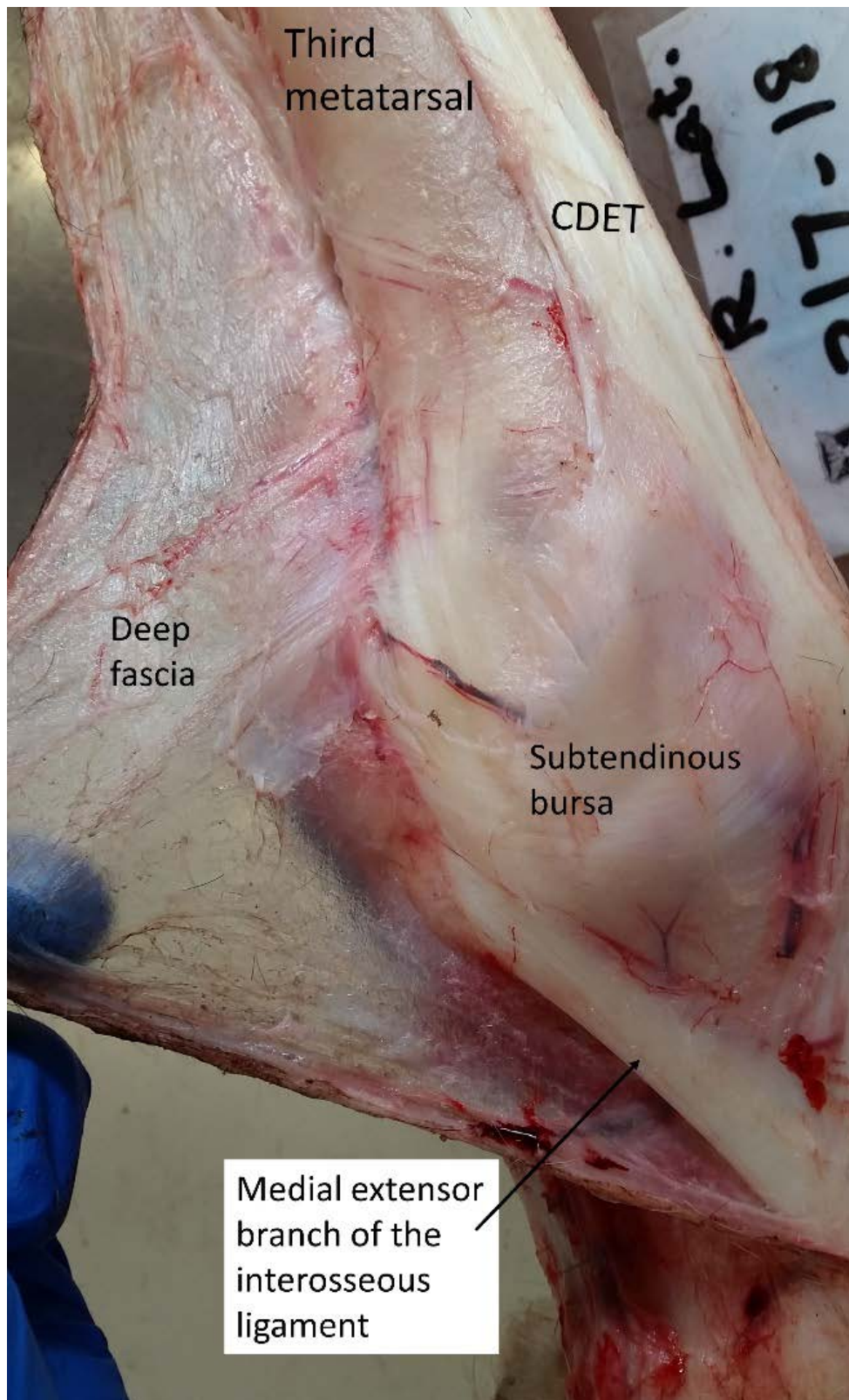


Figure 9. 31: Medial aspect of distal half of the right metatarsus and metatarsophalangeal joint. The superficial fascia was already removed.

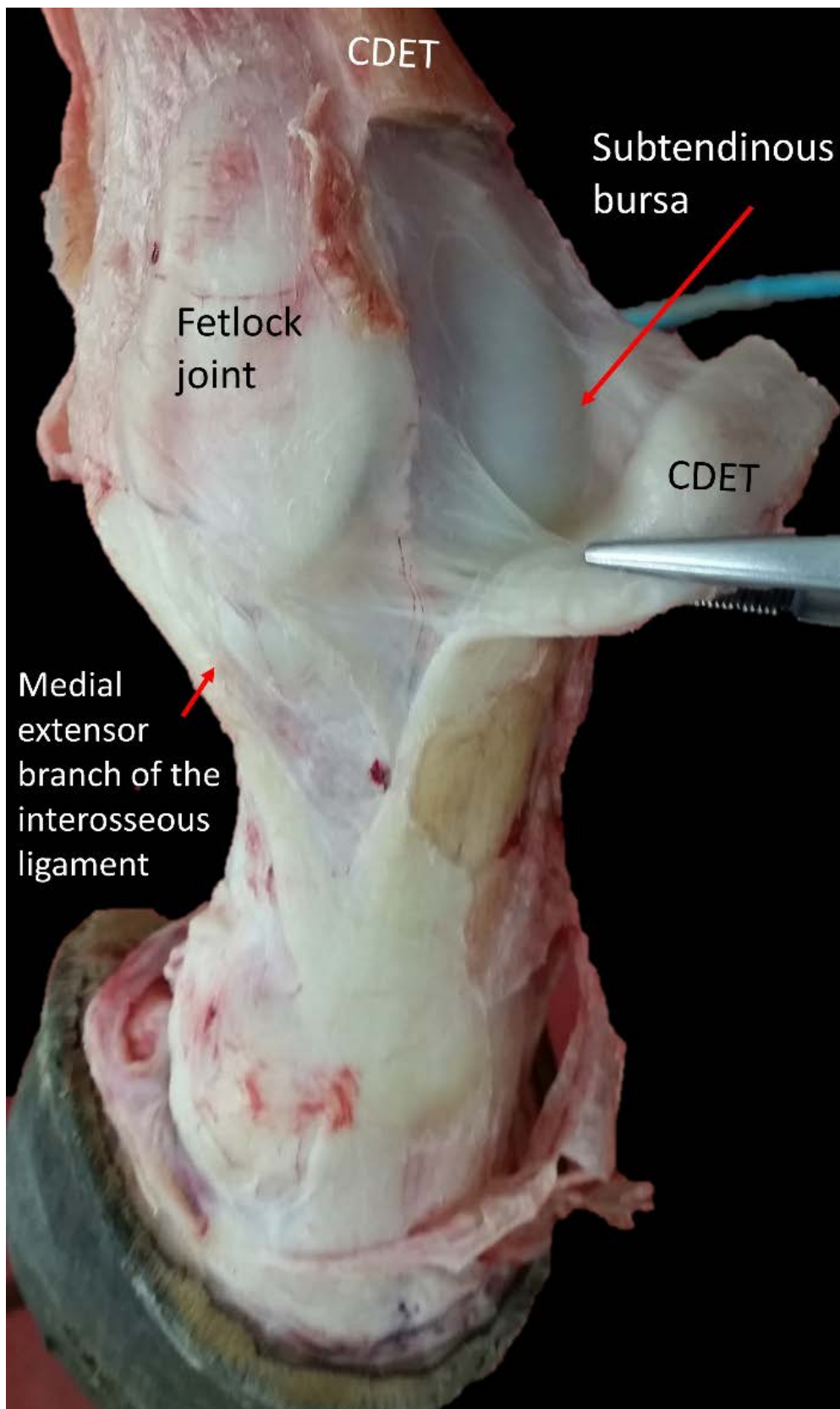


Figure 9. 32: Dorsomedial aspect of a left fetlock joint. The superficial fascia was already removed and CDET was transected and reflected.

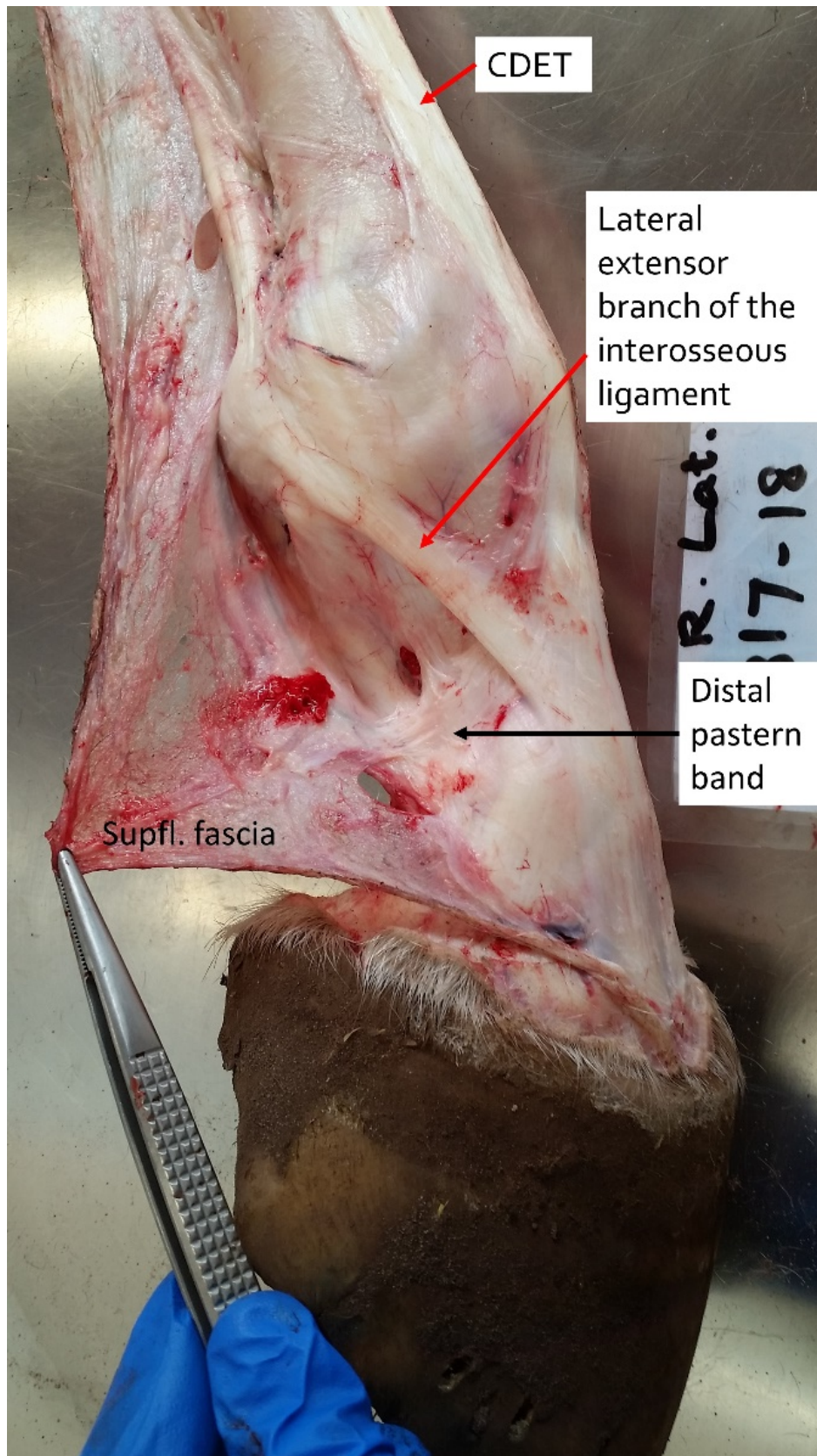


Figure 9. 33: Lateral aspect of a right fetlock joint.

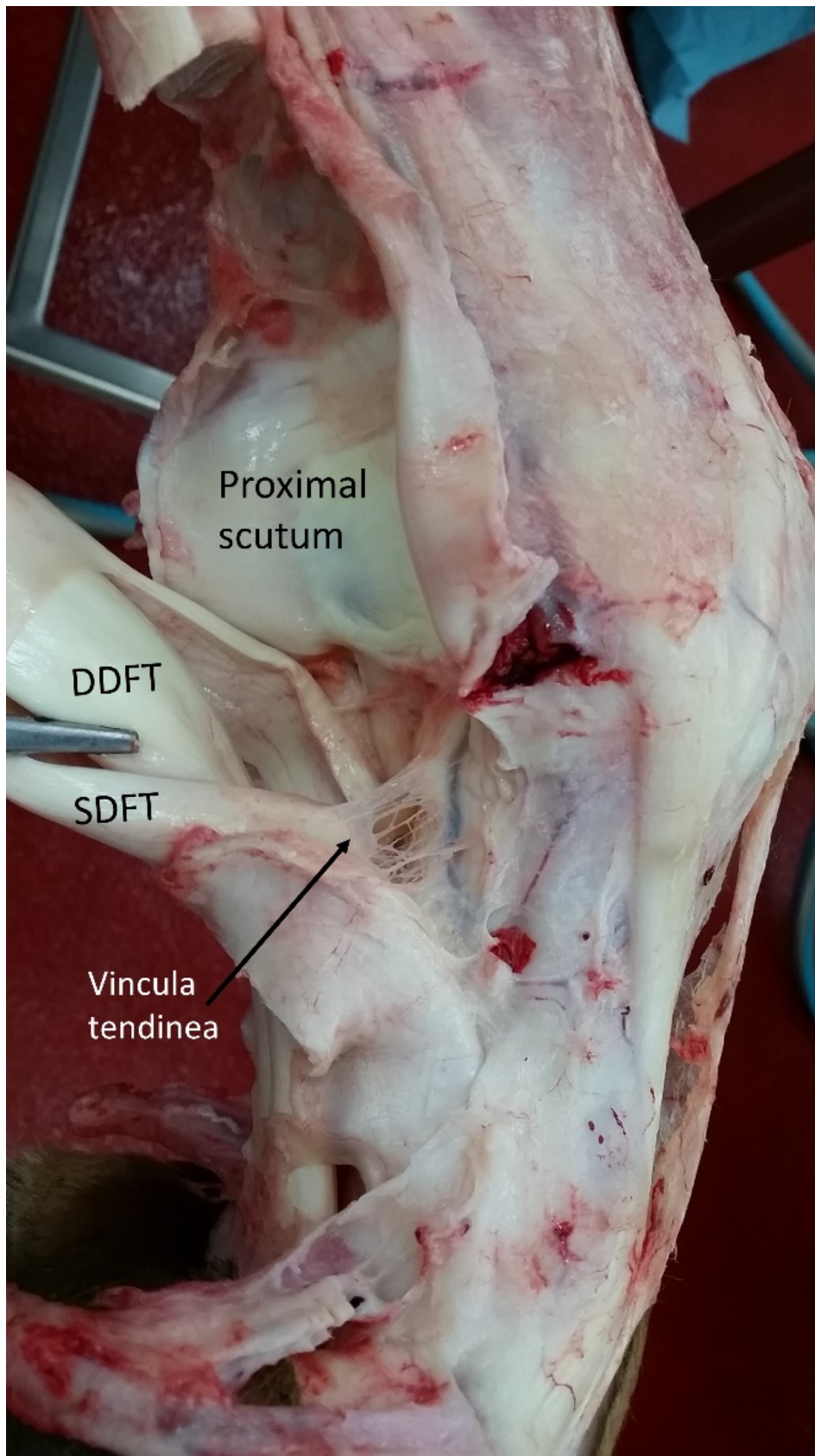


Figure 9. 34: Lateral view of the plantar aspect of a right fetlock joint.. The superficial fascia was already removed. DDFT and SDFT were transected and reflected caudally.

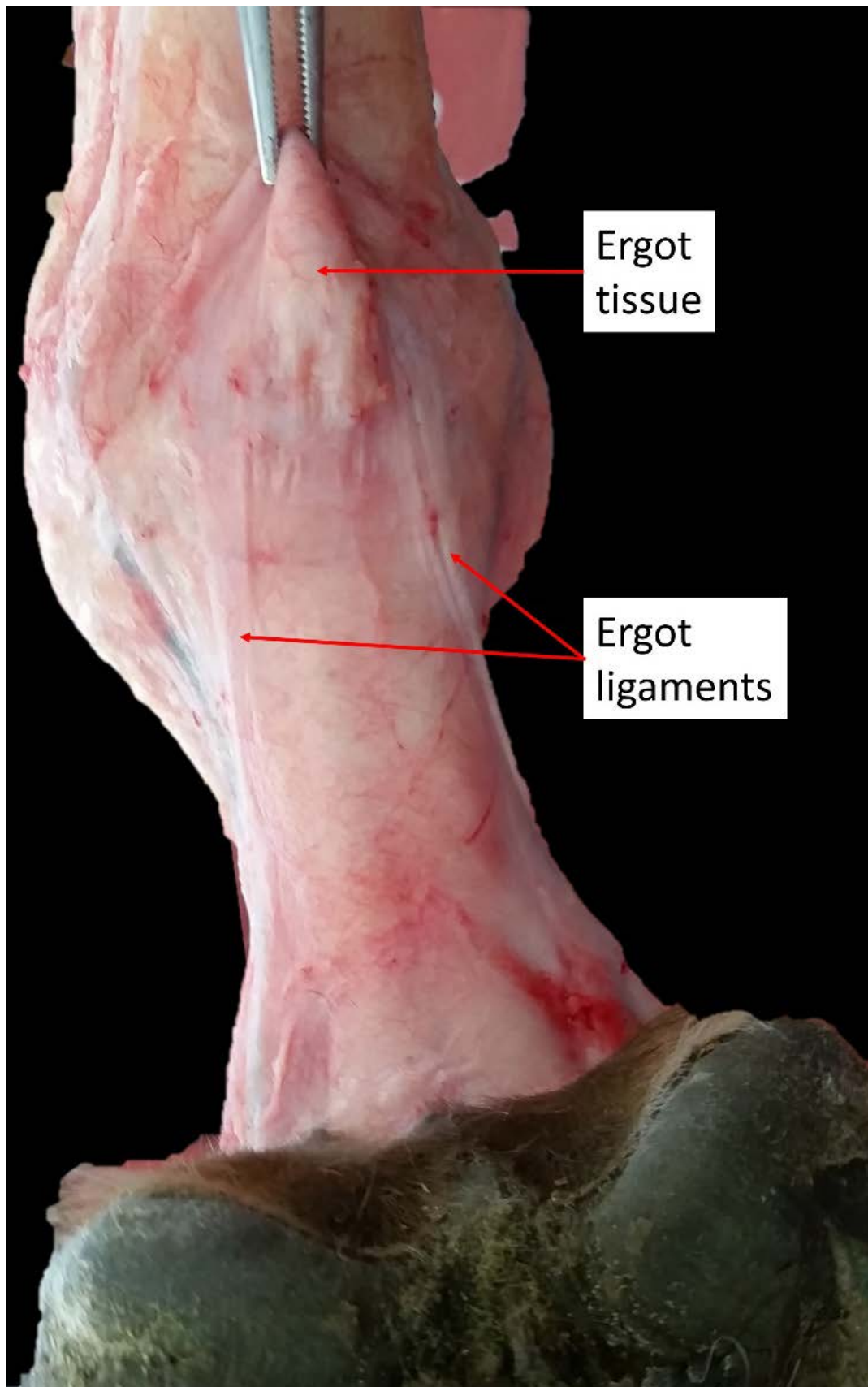


Figure 9. 35: Plantar aspect of fetlock joint and proximal digit.

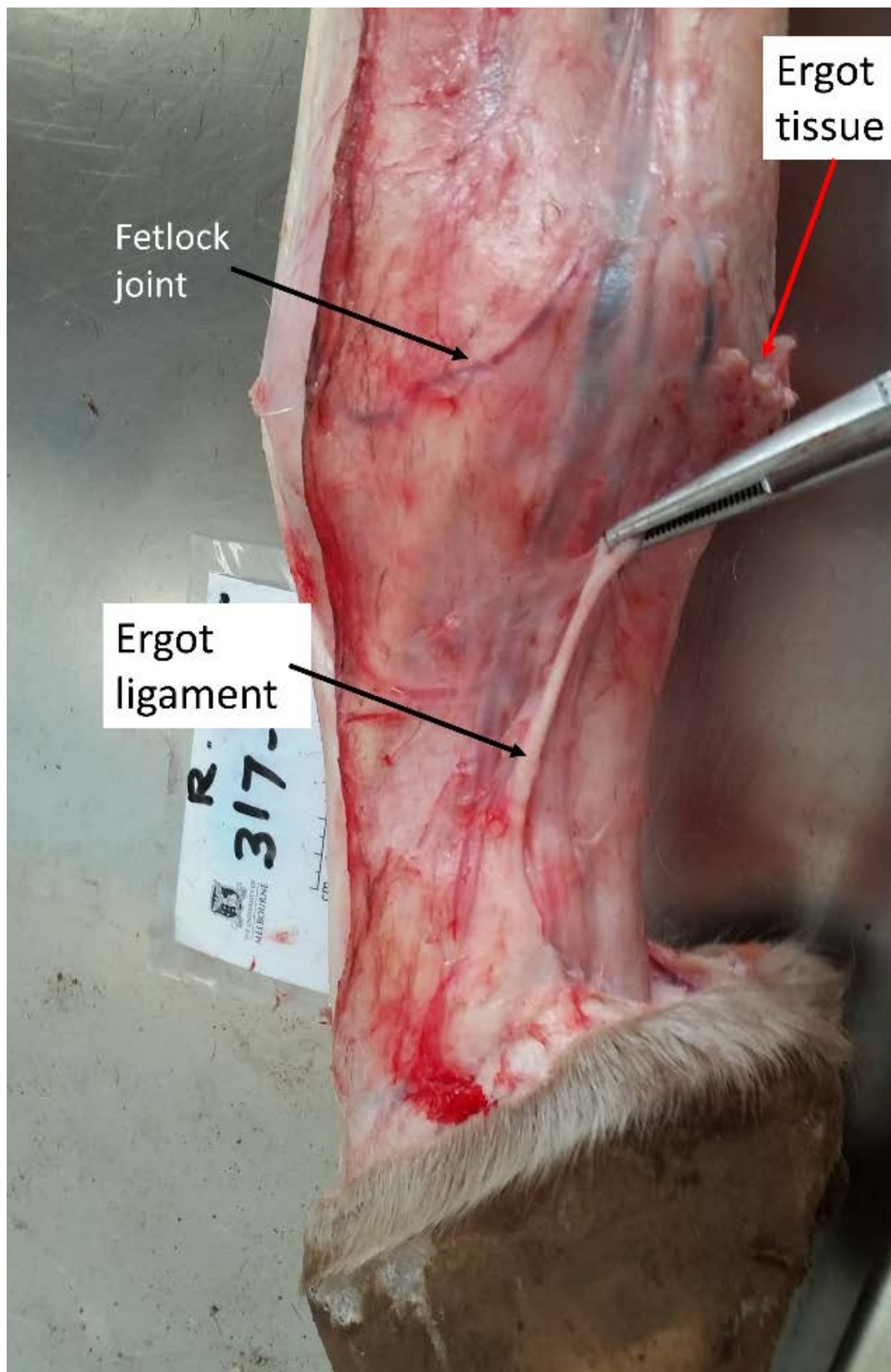


Figure 9. 36: Medial aspect of right hind fetlock joint and proximal digit.

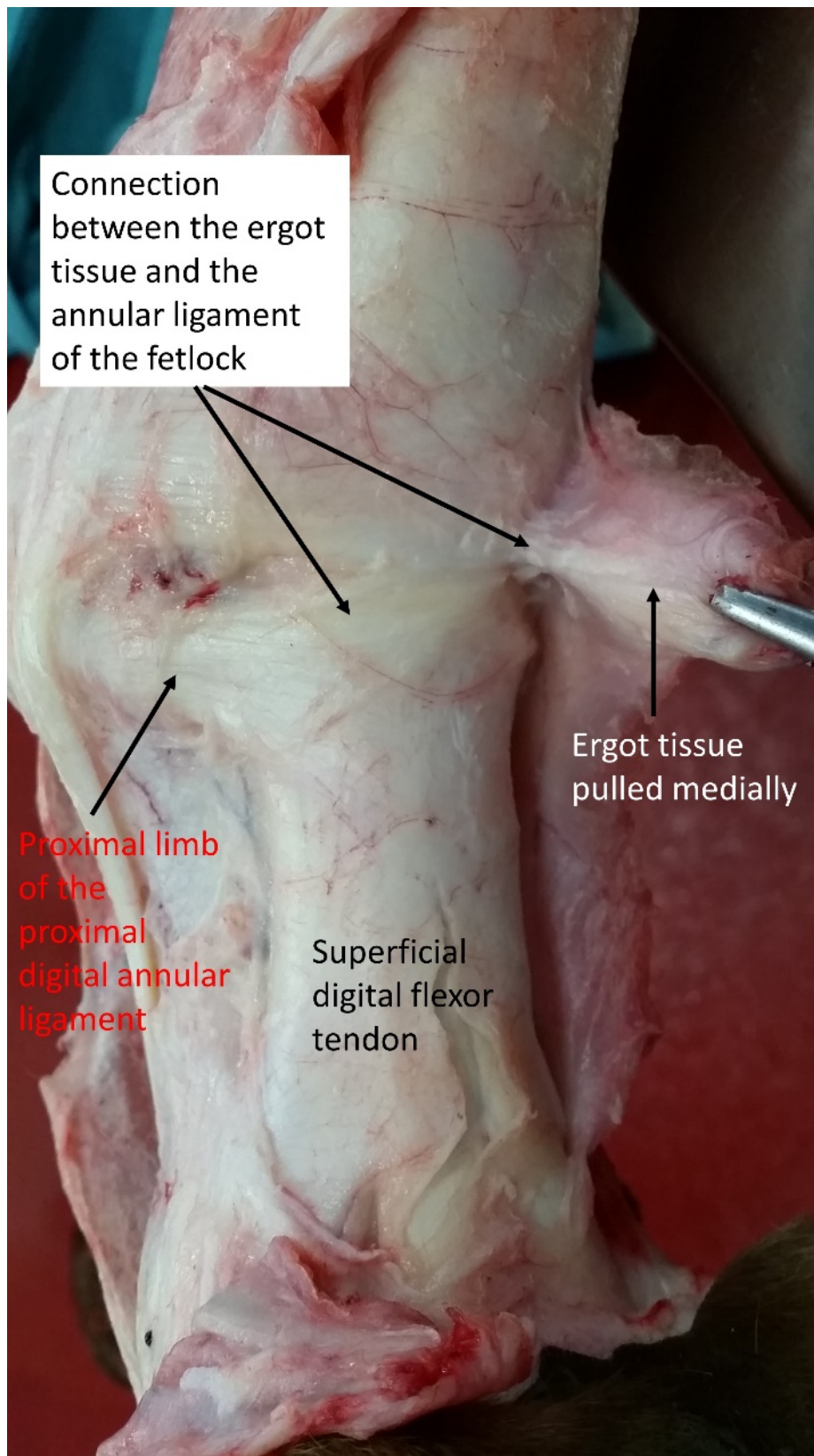


Figure 9. 37: Plantar aspect of right fetlock joint and proximal digit.

9.4 DISCUSSION:

This study investigated the fascial anatomy pertaining to the equine crural region to digital region of distal hindlimb, in order to identify its role in maintaining the mechanical connections throughout the entire hindlimb and their mechanical significance in coordination of the proximal and distal hindlimb regions around the tarsus.

There is an emerging area of research in providing inherent stability to joints during rapid locomotion, by investigating the role of mechanical connections across and between the joints. Many studies investigating this role, suggested that the mechanical connections of fascia may respond more rapidly than neural reflex mechanisms to impact-related perturbations (Moritz & Farley, 2004; Patla & Prentice, 1995). Hence, the fascial expansions over the tarsal joint may contribute to stability of the whole hind limb at fast speeds by providing mechanical linkages and coordination to proximal and distal limb regions by easy distribution of tensional and loading forces.

In general, it was found that the fascia of different regions of hind limb was anatomically complex because of the different integrated layers of fascia and also their relationship with the bones and musculotendinous units. The different layers of fascia not only provide mechanical connectivity between described structures of a particular regions but also between the structures of different regions comprising the crus (proximally), metatarsal (distally) of the tarsus.

The crural fascia of the tarsus holds the tendons as they pass over the joints of distal hind limb (König & Liebich, 2013). Observations from the current study describe, a clear identification of fascial connections and expansions, which serve to integrate function and extend the role

of these structures in providing the mechanical connectivity. The extent and functional characteristics of these fascial connections has not been available adequately in current literature despite their significance in understanding of the overall loading biomechanics and kinetics of the equine distal hind limb.

The irregular and multidirectional fibres pattern/orientation of the superficial fascia suggest that it provide strength and a degree of elasticity was multi directional rather a more specific point based which specialized and focused for load or strain distribution. In addition, the variation of attachment points with the overlying skin and also with the underlying deep fascia in different regions suggests its dual role in support of subcutaneous structures and the functional isolation of the tissue planes.

The dorsal and dorsomedial aspect of the metatarsal region allowed little sliding or loose attachments to occur between the overlying skin and the underlying structures. This finding was likely to be achieved through the role of lubrication of the ground substance (Stecco, 2014) as the loose connective tissue located between the superficial fascia and subcutaneous tissue or deep fascia. While in contrast, the areas where the superficial fascia tightly attached to either the subcutaneous or the deep fascia have suggested the importance of strong connection for not only maintaining the position but also directing or distribution of strain forces. For instance, the superficial layer has strong attachment at the distal level of first phalanx as it thinned and became difficult to separate as a distinct layer. It may reflect the extra support or stability required from the common digital extensor tendon and the extensor branches of the Interosseous ligament to maintain the support and management of impact related forces and strains of the digit.

The current study investigated two major aspects of the fascial architecture for the assessment of fascial contribution into management of load and locomotory efficiency. The first aspect related with the direction and arrangement of fascial fibres, whilst the second aspect related not only to the overall connectivity but also the continuity between the layers of fascia and with other associated structures.

Due to a misplacement of the centre of gravity in the leg of human, the femoral shaft was subjected to bending strains along the medial axis of its shaft while tensile strains were subjected along the lateral axis of the shaft (Pauwels, 1980). Later on, the strongest connections of the connective tissue system were located along the lateral axis of the femoral shaft in the lower limb connective tissue system in man (Gerlach & Lierse, 1990). Hence, it can be concluded that the longitudinal pattern of fibres in the iliotibial tract and also in the intermuscular septa creates a tensional band effect which help in ultimate reduction of the stress forces exerted on the lateral aspect of femur through bending over.

A careful consideration of the orientation of fascial fibres may provide an indication of management of strain forces during the weight bearing conditions and locomotion (Dyer, Lassila, Jokinen, & Vallittu, 2004; Rathnakar & Shivanand, 2013). In fact, in a previous study by Gerlach and Lierse (1990) there was a relationship between the bone strains and fascial structure which suggest a prime role of fascia in the management of functional strains in the functional construction of the fascia in the lower limb of humans (Gerlach & Lierse, 1990).

The longitudinal direction of fibres comprising the crural fascia covering the long digital extensor in the proximal and mid regions, over the craniolateral aspect suggested that the crural fascia might act like the lower limb in man by creating a tensional band effect which not only reduces the amount of radial bending but also the stress forces exerted on the bone.

The attachments of thick, inelastic sheets of intermuscular septa along the craniolateral aspect of the periosteal surface of the tibial bone create a strong rigid system between the fascia, bone and muscles.

Over the caudal aspect of the crural region, a much more varied fibre arrangement was observed which suggested more circumferential tensional forces around the crus and a resistance to torsional strains. In a similar context, the same reasoning can be applied to the fascia in the distal region to the crus. A paper reporting strains measured directly on the tibia of living horses showed that a small degree of compressive strain occurs in the distal region of the tibia, the principal axis of tensile strains was craniolateral, however the most prominent strains present were torsional (Schneider, Milne, Gabel, Groom, & Bramlage, 1982). In parallel with these findings, the fascial structure in the distal region of the tibia including all aspects had a very distinct cross hatched fibre arrangement with fibres running in an oblique and multi directions which suggested that such an arrangement provided stronger resistance to twisting forces acting on the crus by restricting the possible degree of stretching in more than one directions .

Distal to the tibia, the cross hatched arrangement of fibres continued into the tarsal region. The forces acting on the tarsus have not been clearly described in the literature, but the continuation of this arrangement of fibres implies a similar fascial role in support of the tarsus to resist torsional strains. The latero-medial collateral, dorsal and palmar ligaments of the equine carpus are together spirally arranged. These ligaments got tensed during axial rotation of the forelimb (Deane & Davies, 1995a). In a similar reasoning the oblique crosshatched arrangement of the fibres in the tarsal fascia would appear to provide the same additional

resistance and support to torsional strains along with ligaments (served as the major torsional support).

Another noteworthy contribution to this, is the continuation of the crural fascia further to the tarsal joint which provides further stability through the direct connections it forms with the tendons, retinacular bands, collateral ligaments and the joint sheath. Thus, the crural fascia and its connections play a vital role not only in coordination of different regions but also within the same region through their multiple mechanical connections within their overlying structures etc. muscles, tendons and bones.

The current study also demonstrated the intimate connectivity that existed between the tarsal ligaments and fascia and which prevented isolation of these ligaments. It was observed in this study that fascial fibres had strong connections with the joint sheath suggesting to provide extra support and stability to the joint. Despite these connections being present in all specimens including the foal, no such details are available in the standard textbooks which illustrate and describe clear and distinct tarsal ligaments without this connectivity (Getty, 1975, Barone, 2010; Dyce et al., 2002; Nickel et al., 1986).

The continuation of connectivity was also found between the tarsal fascia and the metatarsal fascia. A connection through the direct continuation of fibres into the CDET is worth mentioning here. This introduces an additional consideration of fascia function which this study has begun to explore. The continuity of fascia across the limb segments suggested that there is an aspect of coordination and control in the limb which is widely based on the passive transmission of the forces rather than being controlled by the nervous system exclusively.

In another prospective, the interlocking wedge arrangement of the equine carpus enables them to transfer sudden forces from the carpal bones to the elastic intercarpal ligaments

supporting the bones (Deane & Davies, 1995a). Although it's worth pointing out that the anatomy, function, and loading of the carpus is different than the tarsus, there is more motion between carpal bones in the carpus than between the bones in the tarsus, which would intuitively mean that the intercarpal ligaments have to play a vital role. In an extension of this idea, it can be proposed that the tensional forces acting upon on the tarsal bones are redirected not only through the tarsal ligaments securing them while avoiding displacements during loading of the joint, but also through their connecting fascia which extends proximally into the crus, and distally into the metatarsal.

The above-mentioned continuity between the tarsal ligaments and the fascia is one example of this, the other examples can be listed here as the continuation of the crural fascia to the tarsal extensor and flexor retinaculum fibres. Another example is the tendon-like flange associated with the CDET. The fascial fibres blend distally with the CDET in the proximal third of the metatarsus and proximally with the deep fascia involved in the architecture of retinacular fibres, providing a direct mechanical pathway from the dorsal aspect of the crural region to the proximal phalanx (to which the LDET partially attaches). The fascia provides additional support to maintain the tendons in their proper orientation allowing them to function appropriately. In this way, fascial tension over the dorsal aspect of the crus may contribute in a small way to the extension of the distal limb and vice versa but this needs further investigation.

The areas of fusion between the superficial and deep fascial layers were described in relation to the vessels and nerves on the lateral and medial aspects. This connectivity may serve many purposes. The likely major advantage of fascia surrounding vessels and nerves is the provision of a protective sheath to prevent any damage that can occur as a result of compressional or

frictional forces and this further highlight the significance of fascia (Mike Benjamin, 2009). However, at the same time any stiffness or limited stretching ability of fascia can lead to rupture or damage of these vessels and nerves, particularly with an extreme range of joint movement (C. W. McIlwraith, Frisbie, Kawcak, & Van Weeren, 2015).

In addition, a clear fascial bursa was observed on the caudal side of the calcaneus tuberosity. This finding seems to confirm what is presented by many authors (Post, Singer, & Clegg, 2007; Post, Singer, Clegg, Smith, & Cripps, 2003; Virginia B. Reef, 1998). Also, in relation to our results, descriptions and illustrations of the subcutaneous calcaneal bursa have been reported as a sac that overlies the calcaneus tuberosity and covers a wide part of the superficial digital flexor tendon (Julia E. Tomlinson, Redding, Berry, & Smallwood, 2003; J. M. Vilar, Rivero, Arencibia, Morales, & Pinedo, 2008). The calcaneal bursa may communicate with the intertendinous calcaneal bursa at the point of the calcaneus tuberosity (Post et al., 2007), thus, direct trauma from a hard object can cause a capped hock (Veenendaal, Speirs, & Harrison, 1981) that is common in horses. This architecture suggests that abnormalities of the subcutaneous bursa may mechanically reduce the range of motion of the tarsal joint.

The deep fascia also had many other noteworthy characteristics throughout the equine hind limb. For instance, it is involved in forming a number of bursae at the fetlock joint such as the subfascial bursae on the dorsomedial aspect. The bursa was inconsistently observed among the few specimens examined in the current study or simply overlooked in earlier dissections. The previous studies, such as Ottoway and Worden (1940) have not mentioned such bursae at this location, so it might be possible that it is present in exceptional occasions or it develop in response to mechanical loads.

The deep fascia extending between the CDET and IL extensor branches looked to have a role in functional integration of these structures. The extensor branches run obliquely across the fetlock joint on the dorsal aspect of the proximal phalanx to combine with the CDET (Nickel et al., 1986). Therefore, it can be suggested that the gross vertical and oblique orientation of collagen fibres play a role in diversion of tensions between the dorsal aspect of the digit and the plantar aspect of the metatarsus because the fascia extends between the extensor tendons and the IL extensor branches. The continuity between the structures, may further suggest a role in decreasing the peak tensions that may be applied to the CDET and IL. In this way, it might facilitate the proximal and distal distribution of load during weight bearing by proper handling or redistribution of impact related forces and ground reaction forces.

The strong connectivity of the ergot tissue at the plantar aspect of the fetlock provides further indication of proximal distribution of forces concentrated in the digit and digital cushion. The findings of the present study demonstrated an intimate relationship between the skin and the ergot (underlying dense connective tissue mass). As the continuity of the skin throughout the body suggested that it has an integrative role in the joint stability and providing assistance in distribution of the forces in body locomotion.

Due to the higher degree of flexion capability in the fetlock joint, the skin on the plantar aspect of the joint is often subjected to a higher degree of stretching (Back et al., 1995; Butcher & Ashley-Ross, 2002; Holmström & Philipsson, 1993). The strong attachment of ergot tissue with the skin suggested that any tensile forces applied on the skin can have a direct influence on the connective tissue of the ergot along with its fascial and ergot ligamentous connections and vice versa.

The ergot tissue serves as a mechanical continuity between the skin, metatarsal fascia proximally and digital fascia distally, thus serving as a junction point and link, along with connection of superficial and deep fascial layers. In addition, the ergot tissue also has strong attachment to the plantar annular ligament of the fetlock joint. Thus, traction applied to the ergot tissue in the proximal direction (Figure 9.35) demonstrated the distribution of tension to the digital annular ligament, digital cushion and hoof cartilage through the ergot ligaments and their connections to the distal pastern bands. Similar findings were observed in ergot ligaments of equine forelimb when traction was applied in a previous anatomical study suggestive their identical function and joint stability in both fore and hind limbs (Lusi & Davies, 2017).

Hence, this connectivity of the ergot tissue and its derived ergot ligaments suggested that it has many advantages like its role in maintaining the functional relationship between the different structures and the mechanical connectivity throughout the entire distal limb during movement. The ergot tissue may also have a role in the absorption and dissipation of the ground reaction forces by providing a stable elastic foundation, although relative smaller size. Similar findings were observed in the heel pad of the human foot, where the heel pad provided stability to resist shear forces and helped in shock absorption (Safran, Garrett JR, Seaber, Glisson, & Ribbeck, 1988). In addition to this, the ergot connectivity suggested that the flexibility of the ergot ligaments and skin influenced the degree of hyperextension of the fetlock joint.

From a mechanical point of view, hyperextension of the fetlock is mostly limited by the flexibility of the deep digital flexor tendon (DDFT), superficial digital flexor tendon (SDFT) and IL extending along the plantar aspect (Budras, Sack, & Rock, 2003; Hermanson & Cobb, 1992).

Limited information is available in the current literature regarding the functional importance of the thin fibrous connections between the DDFT, SDFT and the straight sesamoid ligament as these connections, termed vincula tendinea, are commonly present in the human hand and have been concluded to be important in the vascular supply of the SDFT and deep digital flexor tendons (DDFT) (Stewart et al., 2007). Vincula tendinea were considered to be a means of uniting parietal and visceral layers of the tendinous sheath, with their major function being to carry vessels and nerves (Nickel et al., 1986a).

In conclusion, the fascia of the equine hind limb is expansive in its connections between the other limb structures. Investigation of fascial connectivity and fibre orientations have provided insight into possible tensional lines, load distribution and the overall support of the hind limb. The above-mentioned continuity between the fascia and other constitutive structures appear to serve as the mechanical linkages for better coordination and support around the joint. These linkages also seem to influence the function by playing a role in maintaining the tendons in their proper orientation allowing them to function appropriately, distribution of tensional forces during loading conditions as well as mechanically restricting the movements (over-flexion or extension) of joint within a normal range of motion.

***Chapter 10:* Exploration of the relevance of histological features and fascial thickness in maintaining the normal range of joint motion and proprioception around the complex tarsal region.**

10.1 INTRODUCTION:

Recent research has revealed an association between increased fascial thickness and reduced joint flexibility in patients with chronic pain in humans (Wilke, Macchi, De Caro, & Stecco, 2019). Other factors such as hydration status of soft tissue, contractile cell activity, and the presence of elastic (elastin) and collagen fibers contribute to the tensile stiffness. However, to the author's knowledge despite the increasing interest in fascia in different diagnostic, treatment and physiotherapy approaches, the exploration of mechanical connections, normal variation in thickness, its role in proprioception and internal composition of fascia have not been fully understood. Thus, morphology and internal composition of the connective tissue may play an important role in locomotor mechanics.

The term fascia has been derived from the Latin term for bandage or band (Benjamin, 2009). The fascia has been described as the bag that holds the body together. The fascia is generally composed of two sub-types including the superficial (or subcutaneous) fascia and deep fascia. The fascial connective tissue is composed of collagen and elastin fibers. The superficial fascia has a smaller number of these fibers while the deep fascia has a larger number of fibers which are densely packed together (Mike Benjamin, 2009).

Deep fascia has been classified into aponeurotic and epimysial fasciae, depending on the thickness and relationship with underlying and surrounding muscles. The aponeurotic fascia is the fibrous sheet that covers a group of muscles, keeping them in place, or provides

the insertion for several muscles Stedman (1995). All the deep fascia in the equine hindlimb, including fascia lata, crural fascia, and plantar fascia, are examples of aponeurotic fascia by this definition. The epimysial fascia is a thin layer, strongly attached to muscles allowing sliding during muscle contraction (Carla, Veronica, Andrea, Fabrice, & Raffaele, 2011; C. Stecco, Hammer, Vleeming, & De Caro, 2015). It envelops a single muscle, giving insertion to muscle fibres and providing fibres that permeate the muscle; thus, it is transmitting muscle forces and providing a more localized range of action (C. Stecco et al., 2015). In general, the epimysial fascia is thinner than the aponeurotic fascia, but they both have the same structural features as demonstrated by (Purslow, 2010).

The deep fasciae of different topographical regions have diversified structures which seem to be a reflection of specific functions and the connection of the lower and upper limbs to the trunk in the human body (Maas & Sandercock, 2010; C. Stecco & Day, 2010; Yucesoy, Maas, Koopman, Grootenboer, & Huijing, 2006). The fasciae may also play important roles in tensional stress distribution, pain etiology, and interactions among the muscles of the limb due to their significant mechanical properties and rich innervation (M Benjamin, Kaiser, & Milz, 2008; Helene M Langevin, 2006; Vleeming et al., 1996).

More recent studies have investigated the potential of fascia for initiating or at least mediating movement, demonstrating it to have both contractile (Schleip, Klingler, & Lehmann-Horn, 2005) and proprioceptive (Stecco et al., 2010) properties in differing regions of the body. These findings have identified the likely significance of fascia in coordinating joints and maintaining them within physiologically safe ranges.

Based on these studies which showed variations in the methodology in their description of fascia and its role in maintaining the normal range of joint motion, and loading

biomechanics, it was deemed necessary to evaluate the basic anatomical fascial characteristics like fibers directions, orientations, points of attachments, histological composition and thickness surrounding the equine tarsus. These findings could be quite helpful in defining the role of hind limb fascia in the joint's range of motion, coordination, movement perception and directions of tension distribution. Thus, the present study aimed to investigate the possible association of fascial features, such as thickness, with the macroscopic and microscopic architecture around the tarsal joint.

10.2 MATERIALS AND METHODS:

10.2.1 Microscopic analysis:

10.2.1.1 Subjects:

The microscopic analysis of superficial and deep fascia was conducted using 6 fresh equine (3 right, 3 left) hind limbs from three Thoroughbreds. The limbs were not associated with any apparent pathology, collected from horses euthanized for reasons not related to current study. The skin was not removed in order to include it with the superficial and deep fasciae as full-thickness specimens from the skin through to the muscle layers were collected from different sites as described below. Tissues were sampled for histological study.

10.2.1.2 Anatomical regions:

The sites for collection of tissue samples and positioning of the ultrasound probes were selected (Figure 10.1) by landmarks. The limb was divided into 3 regions, the proximal and distal ends of tibia for crural region and proximal and distal end of third metatarsal for metatarsal region while the rest was tarsal region in-between of these two regions.

The tissue samples were obtained from six regions in each hindlimb after locally shaving the skin (if required). The tissue samples (of 2 cm length, width) included the layers from the epidermis and through to a depth of 2–5 cm although there was some variation in this depth related to the sampling sites. The following sites were selected for sample collection.

S1 and S4: Mid level of crural region from lateral and medial aspects respectively.

S2 and S5: Mid level of tarsal region from lateral and medial aspects respectively.

S3 and S6: Mid level of metatarsal region from lateral and medial aspects respectively.

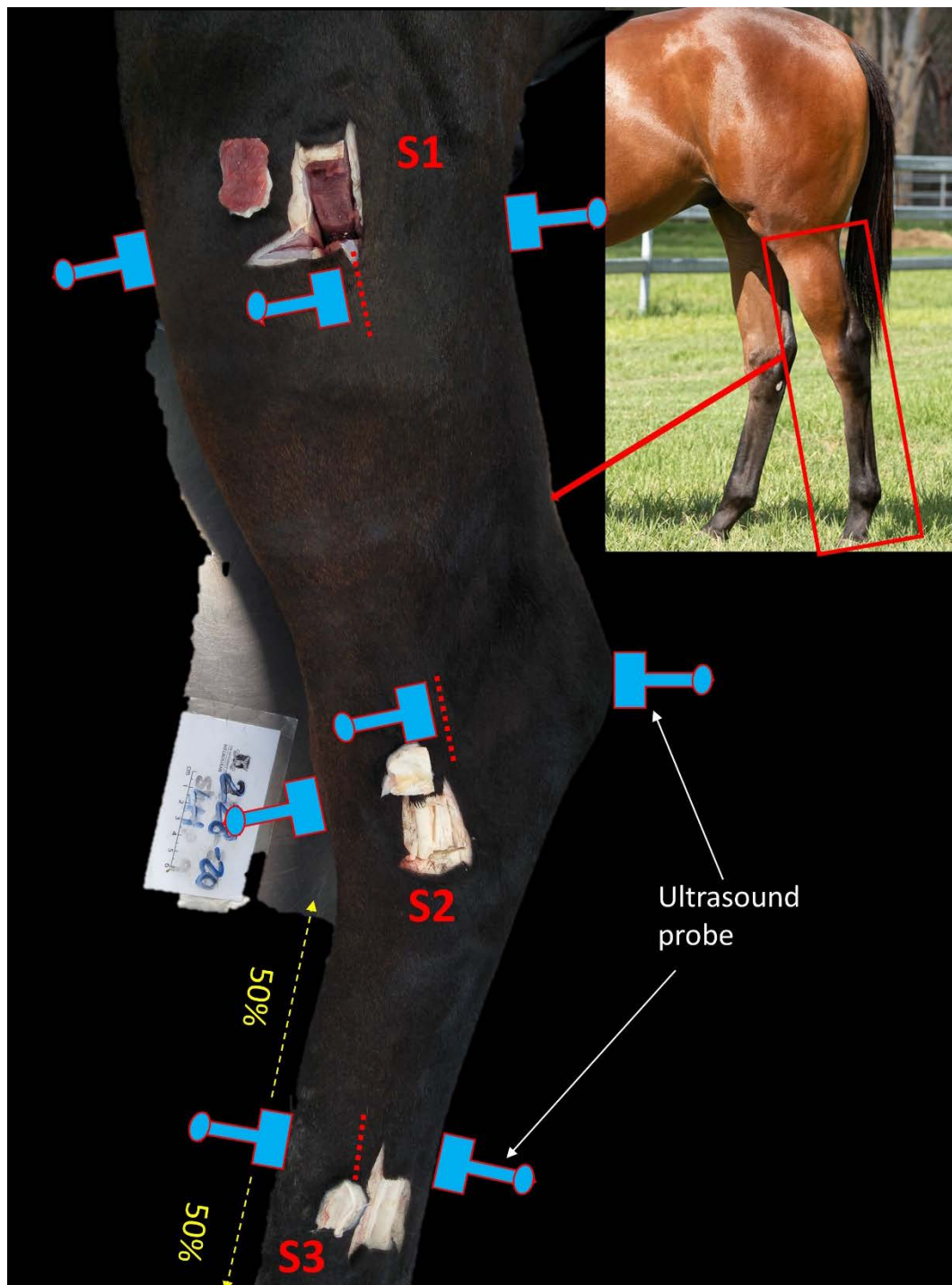


Figure 10.1: Lateral aspect of left hindlimb showing three of the sites for collection of tissue samples and positioning of the ultrasound probes placed in opposite directions near the collection sites, S1, S2, S3. Sites for the medial aspect were placed at the same level. Probe positions (for transverse and longitudinal orientations), probe placements sites were proximal one third of the crural length, the mid length of the tarsal region (between crural and metatarsal regions) and similarly metatarsal lengths (50% of length).

10.2.1.3 Tissue processing for histology of collected specimens:

From the skin to the level of any underlying muscles, full-thickness specimens (about 2 to 3 cm²) were excised from the six sites in each hindlimb. The collected tissue specimens were fixed with 10% buffered formalin until further processing which occurred within 48 hrs. For histological evaluations, the longitudinal and transverse sections were collected from specimens. For the longitudinal sections, all sections were prepared to see all layers of the tissues from skin surface to muscle.

For the transverse sections, the superficial and deep fascial layers were isolated for detailed morphology and cassettes were prepared with the help of spongy pads to avoid deformation of the layers. Each section was prepared by embedding in paraffin and sectioned at a thickness of 3-5 μm . The sections were prepared by using the following stains: hematoxylin and eosin for general description. For the identification of collagen fibers Masson's Trichrome was used. Van Gieson stain was used for the detection of elastic fibers.

Immunohistochemistry was also performed using a specific antibody to identify the nerve endings in the superficial and deep fascia for determination of any mechanoreceptors and free nerve endings. Staining with the antibody S100 (Agilent AUST, Polyclonal Rabbit Anti-S100, cat Z0311) was performed using a 1:200 dilution, Dako Envision+ Dual Link System HRP (Agilent AUST, cat K4061) and VECTOR Novared peroxidase Substrate Kit (Vector Laboratories, AUST, cat SK-4800) with a light hematoxylin background for differentiation. Antigen retrieval was not required for this technique and Fetal Calf serum was used to aid in blocking of non-specific staining.

10.2.2 Fascial thickness analysis:

This pilot study examined the thickness of the deep fascia from five horse hind limbs of thoroughbred. These cadaver hindlimbs were taken from horses that were euthanized for reasons not related to this study. They included two males (four limbs) and one female (left limb) with an average age of 5 years (range of 3 to 12 years). The specimens were not frozen but kept in cold room for less than 12 hours after collection until scanning was completed. Before using ultrasound, CT and MRI scanning was used but the scan images acquired were not good enough for fascial thickness measurements.

The hind limbs were scanned using a high-resolution ultrasonographic device machine (Philips Epiq5G. Year of Manufacture 2016-02. Philips Ultrasound Bothell Washington, USA). The probe used was: L18-5 (frequency range of 5-18 MHz and the frequency was adjusted as per area of interest) in the veterinary hospital Werribee campus, (U-Vet) University of Melbourne. The thickness of the deep fascia was determined from different aspects (Dorsal, caudal, lateral and medial) of various regions including the crural region, tarsal, metatarsal (as shown in figure 10.1) and (position of US probe was set at the level of dorsal aspect of first phalanx) of digital regions.

The ultrasound probe was stabilized longitudinally over the long axis of investigated areas during the examination time. The images were acquired and fascial layers (superficial and deep) were identified between the skin and muscles and the measurements of deep fascia were performed. The thickness measurements were calculated through the MicroDicom software program. Fascial layer thickness was measured in pixels and then converted to mm by the help of a scale (1cm) in the acquired images. To eliminate the impact of fascial thickness variations, five random points per image were selected for repeat measurements

with similar distances (average around 5-8 mm) between two points, and then the average values of repeat measurements were calculated and analyzed (Figure 10.2).

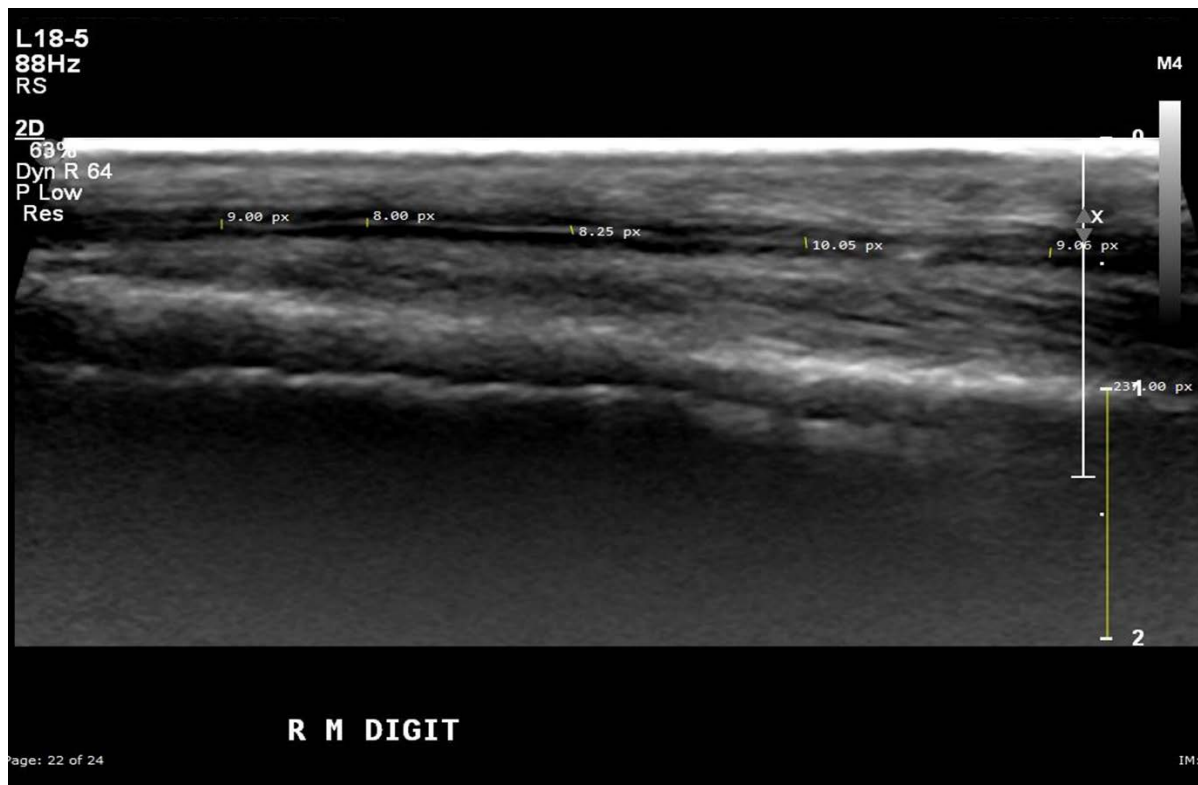


Figure 10.2: Long-axis sonograms of the digital fascia at the proximal aspects of first phalanx below fetlock joint showing the measurement points and scale.

10.2.3 Statistical Analysis:

Mean $\pm$ standard deviation of the thickness deep fascia was calculated for five repeated measurements in GraphPad Prism 8.1 software. Comparisons between fascial thickness in different regions were achieved with a matched one-way ANOVA. Tukey comparison test was used to compare each group with others.

10.3 RESULTS:

10.3.1 Histological description of superficial fascia

The longitudinal section from skin to muscle for evaluation of different layers arrangement and transverse sections were used for particular fascial layer for its composition. The prepared sections were stained both with Hematoxylin and eosin and Van Gieson stains. The superficial fascia was composed of connective tissue made up of collagen and elastic fibers. A network of fibrous connective tissue was visible linking the superficial fascia to the skin layer. This was composed of collagen fibers along with scattered elastic fibers (Figure 10.3). A thin sheet of striated muscle (panniculus carnosus) was observed to be present between two leaves of superficial fascia in the proximal part of the crural region. It was found to be embedded inside sublayers of superficial fascia and not attached directly to the skin. The superficial fascia was composed of two leaves having similar features in the arrangement of the collagen and elastic fibers in both, but the thickness of the two layers was slightly different. The layer opposite the skin appeared thinner than the layer towards the deep fascia. The superficial fascia also enveloped some blood vessels, nerve fibers and some fatty tissue (Figures 10.6 and 10.10). These structures were spread inside the superficial fascia. Elastic fibers were present in the periphery of blood vessels, whereas collagen fibers surrounded the nerve fibers.

These sections illustrated that the elastic fibers were distributed throughout the superficial fascia as shown in Figures 10.4(A), 10.5 (A-B), and 10.7 (A-B). The Mason's trichrome staining revealed the superficial connective tissue was comprised of more collagen fibers than elastin fibers. The arrangement of bundles of collagen fibers was multidirectional as shown in Figure 10.12 (A-F). This gave an irregular shape to this connective tissue. The

arrangement of the elastic fibers was observed to be diffuse within the superficial fascial layer as shown in Figure 10.14 (A-F).

10.3.2 Nerve findings in superficial fascia

The anti-S100 immunohistochemical stain was used for the fascial samples to identify the mechanoreceptors and free nerve endings. Both were observed among connective tissues in the superficial fascia at high magnification (200 x to 400 x).

Ruffini's corpuscles were present as clusters composed of three to six ramifications, and they existed in small numbers in lateral and medial aspects of the crural and metatarsal regions (Figure 10.15 A-D). Vater–Pacini corpuscles were detected as encapsulated receptors with an oval or circular form, arranged in two to four clusters in different regions (Figure 10.16 A-D). Small nerve ending corpuscles appeared the most frequent among the sections of superficial fascia. They were without a specific shape and difficult to distinguish from other mechanoreceptors as shown in Figure 10.17 (A-D).

10.3.3 Histological description of deep fascia

The deep fascia attached to the superficial fascia and underlying muscles by loose connective tissue, which allowed sliding of the superficial fascia and movement of muscle fibers during contraction of the hindlimb (Figure 10.3). It was a continuous sheath of connective tissue, enveloped muscles through their epimysium, and consisted of an irregular mesh of collagen and a few elastic fibers. In general, the different regions had the same features in the structure of deep fascia, but there were some differences in its organization in different parts of the hind limb. Histological analysis of the deep fascia in the crural region showed that the crural fascia was a thick sheet of connective tissue, composed of three layers separated by loose connective tissue, permitting gliding between layers as shown in Figures 10.2, 10.3(B) and 10.4 (A-B). These layers were composed of bundles of collagen fibers as shown in Figure 10.10 (A-B). Crural fascia enveloped the muscles in the leg through the epimysium, and many muscles had a fascial insertion. The alignment of the collagen fibers of the deep fascia was organized in different directions (longitudinal, transverse, and oblique) towards the muscle fibers as shown in Figure 10.10 (A-F). The alignment of the elastin fibers of the deep fascia was also organized in different directions as shown in the figures (Figure 10.12 (A-F)).

Nerve findings in deep fascia

Mechanoreceptors were found in the deep fascia with the S-100 protein antibody. Among the connective tissue of the fascia, Ruffini's corpuscles appeared as clusters of three to six ramifications in deep fascia as shown figure 10.19, whereas Pacini corpuscles found as ovoid or circular structures as shown figure 10.18 (A-D). The free nerve endings were fine and long without a definite shape as shown in figures 10.20 (A-D).

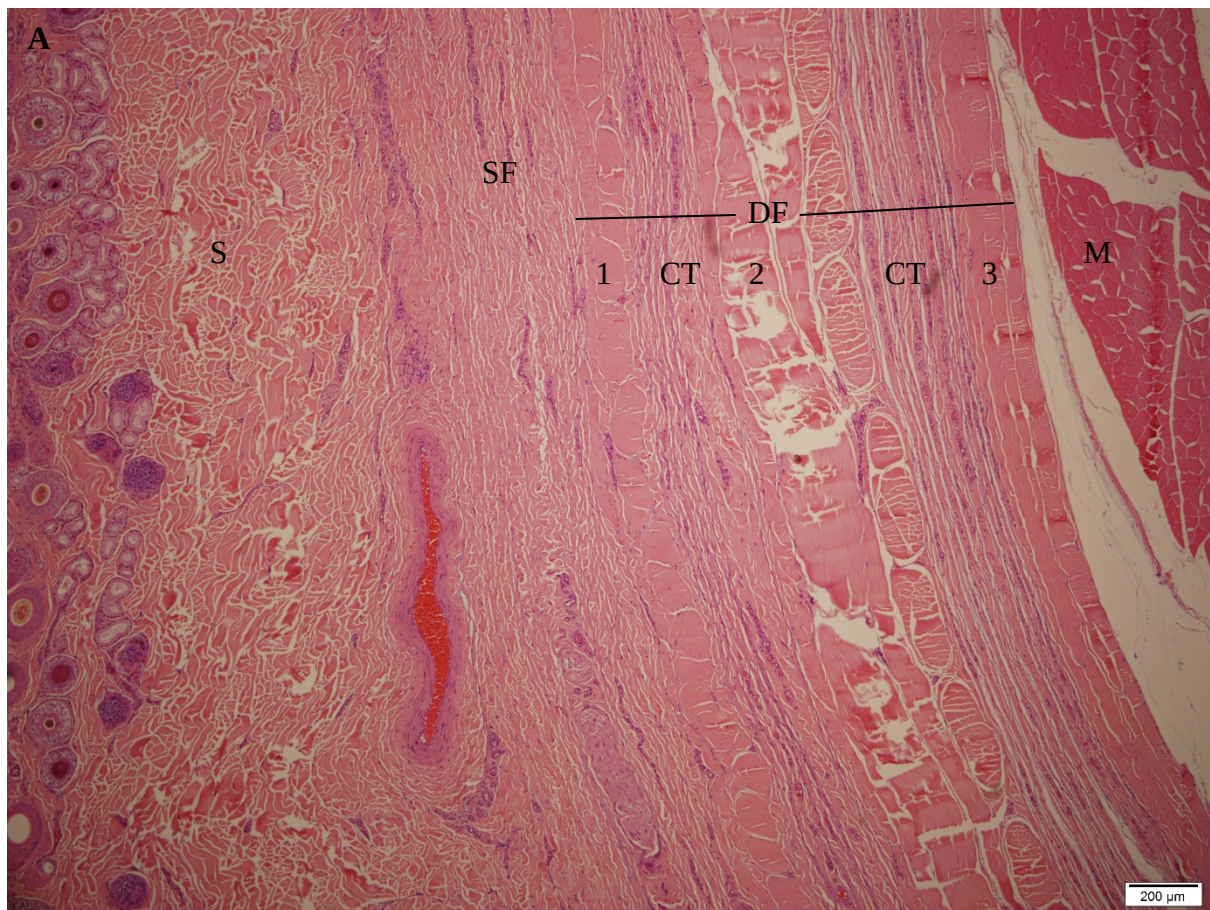


Figure 10.3: A longitudinal section of the lateral crural region (S1), Skin(S), Superficial fascia (SF), Deep fascia (DF) including layers 1,2,3, (CT). Connective tissue, Muscle (M). Haematoxylin & eosin and Van Gieson stains.

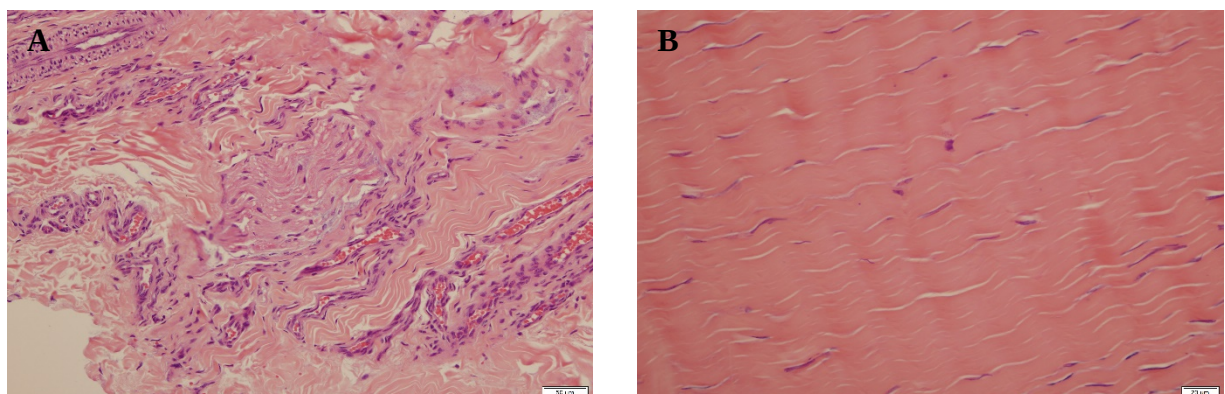


Figure 10.4: A transverse section of the superficial fascia, B Deep fascia in the lateral crural region. Haematoxylin & eosin and Van Gieson stains.

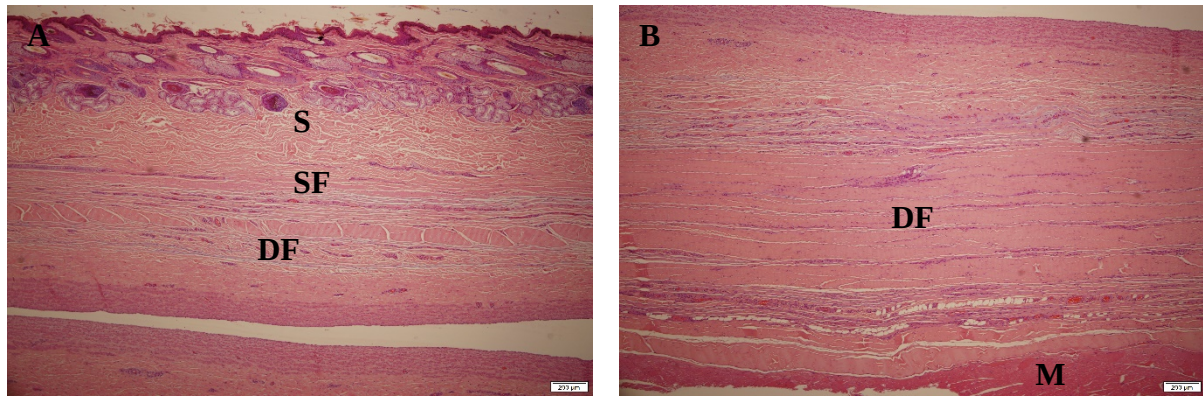


Figure 10.5: A longitudinal section of the medial crural region (S2), Skin(S), Superficial fascia (SF), Deep fascia (DF), Muscle (M). Haematoxylin & eosin and Van Gieson stains.

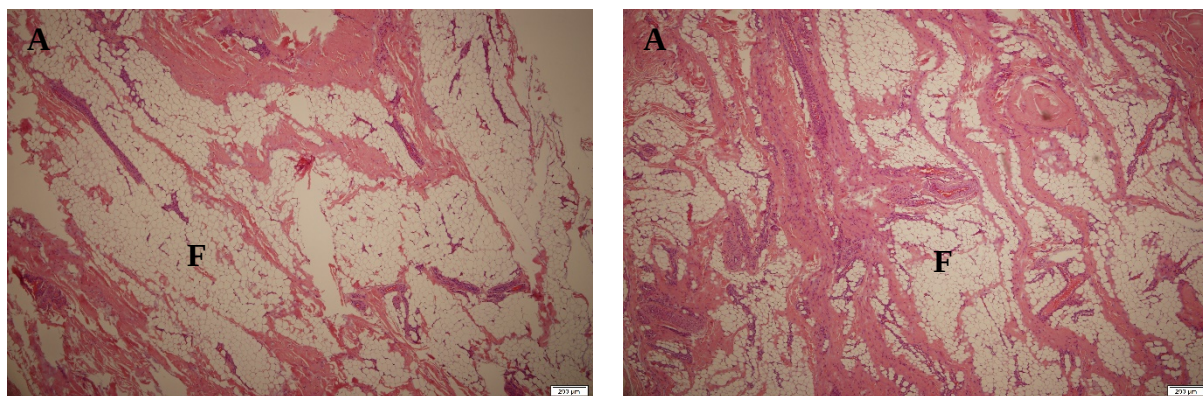


Figure 10.6: A transverse section of the superficial fascia, B Deep fascia in the medial crural region, Fat (F). Haematoxylin & eosin and Van Gieson stains.

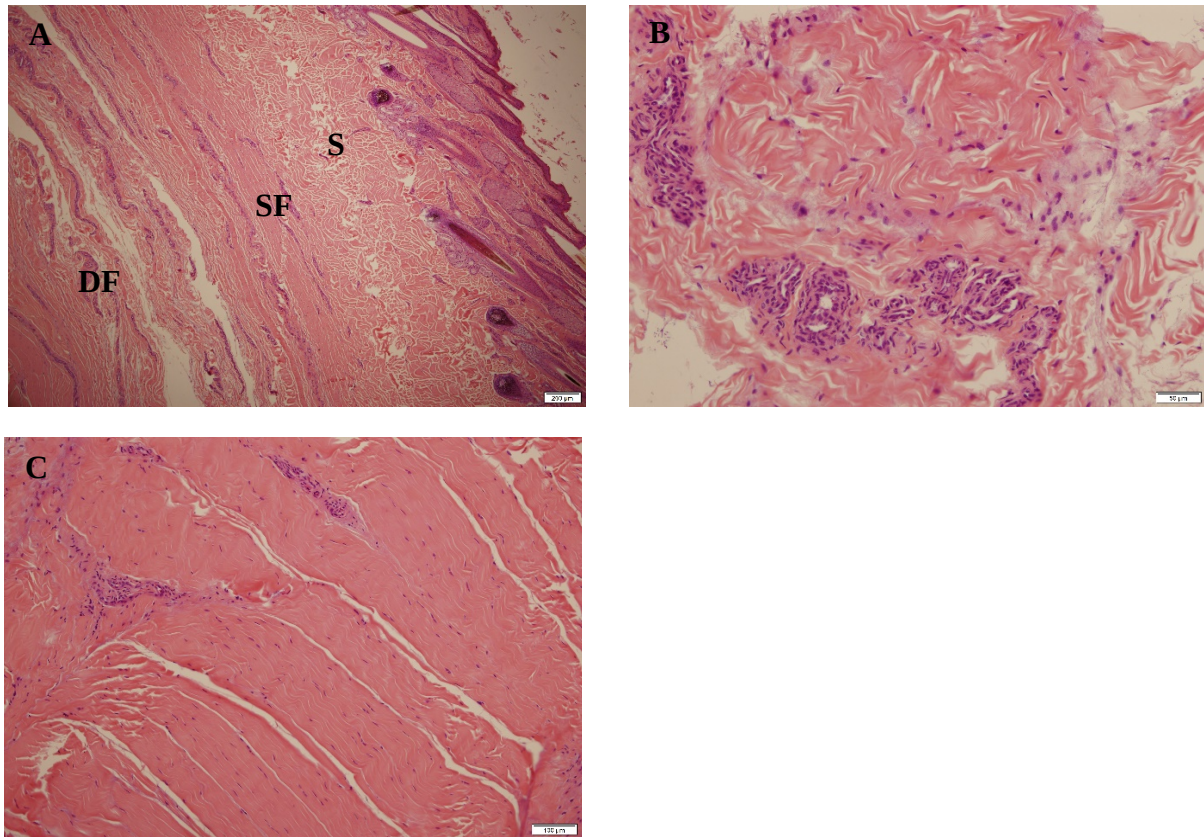


Figure 10.7: A longitudinal section of the lateral tarsal region (S3), Skin(S), Superficial fascia (SF), Deep fascia (DF), Muscle (M), B transverse section of the superficial fascia (LT). C transverse section of the Deep fascia (LT). Haematoxylin & eosin and Van Gieson stains.

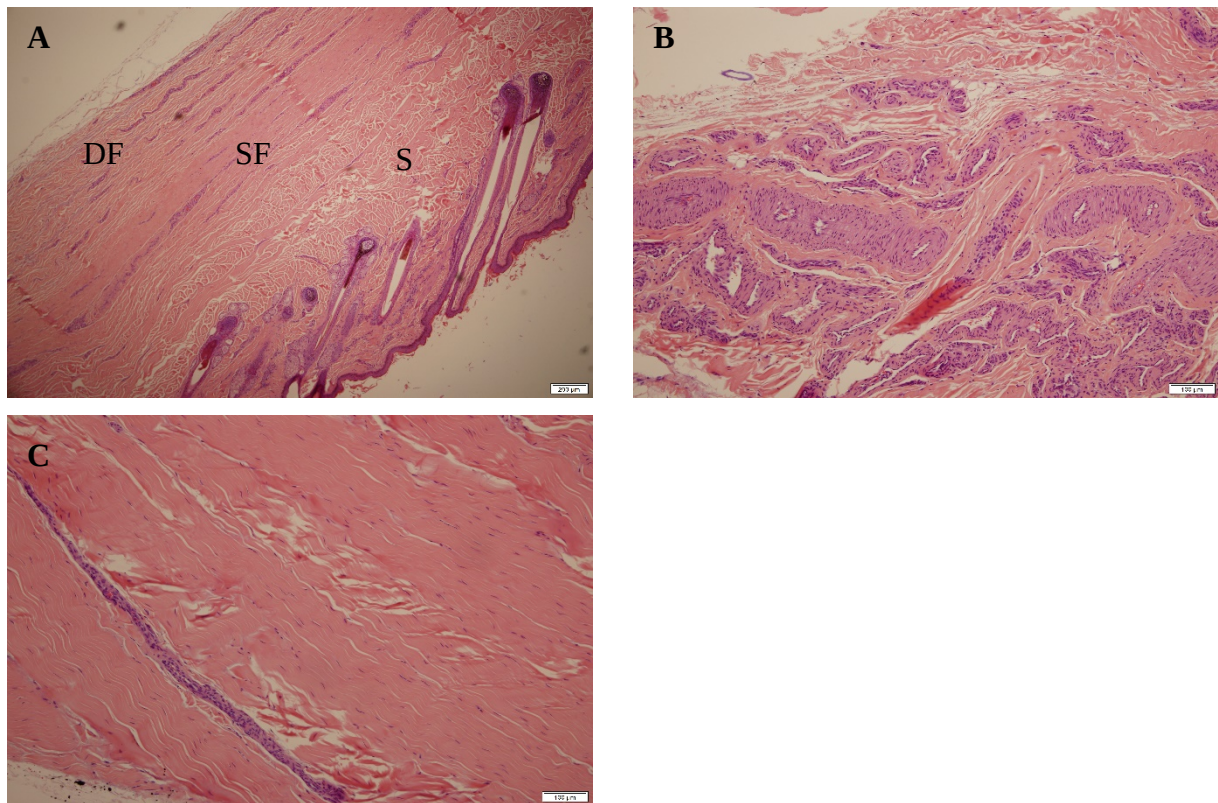


Figure 10.8: A longitudinal section of the medial tarsal (S4), Skin(S), Superficial fascia (SF), Deep fascia (DF), Muscle (M), B transverse section of the superficial fascia (MT). C transverse section of the Deep fascia (MT). Haematoxylin & eosin and Van Gieson stains.

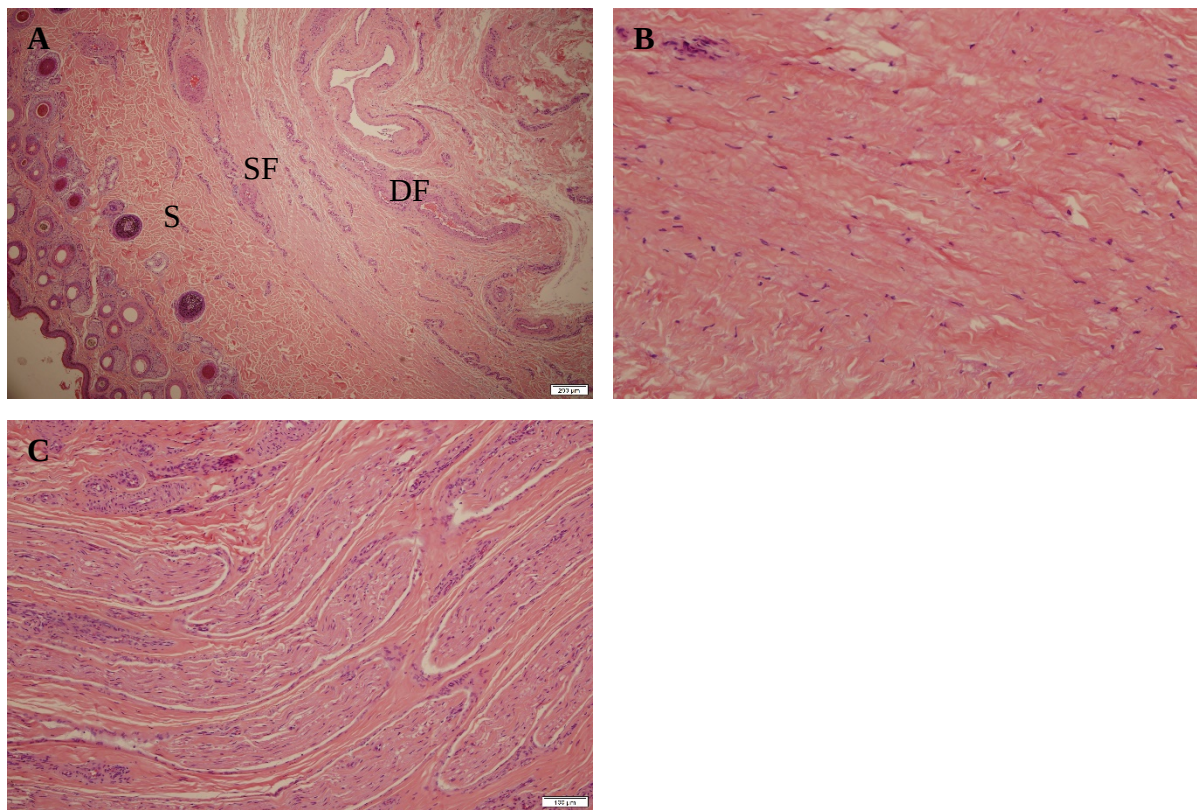


Figure 10.9: A longitudinal section of the lateral metatarsal region (S5), Skin(S), Superficial fascia (SF), Deep fascia (DF), Muscle (M), Nerve (N). B transverse section of the superficial fascia (LMT). C, transverse section of the Deep fascia (LMT). Haematoxylin & eosin and Van Gieson stains.

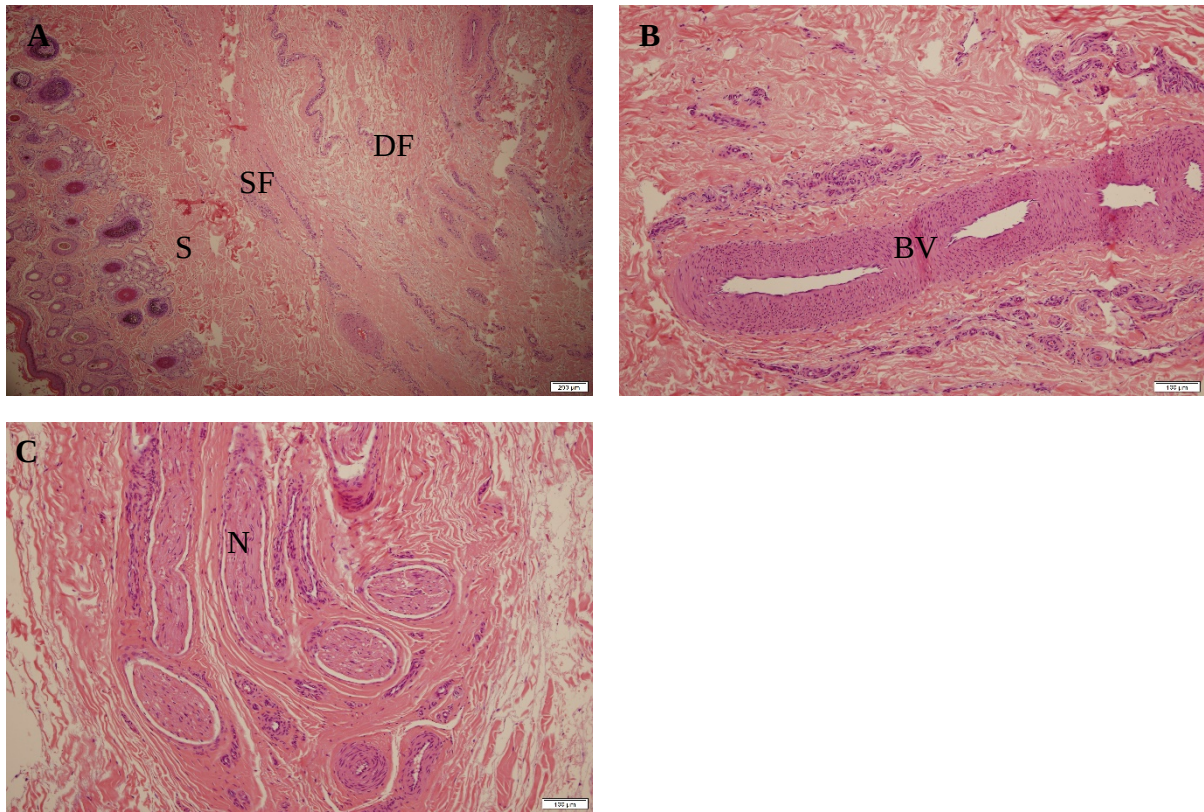


Figure 10.10: A longitudinal section of the medial metatarsal region (S6), Skin(S), Superficial fascia (SF), Deep fascia (DF), Muscle (M), Blood vessels (BV), Nerve (N). B transverse section of the superficial fascia (MMT). C, transverse section of the Deep fascia (MMT). Haematoxylin & eosin and Van Gieson stains.

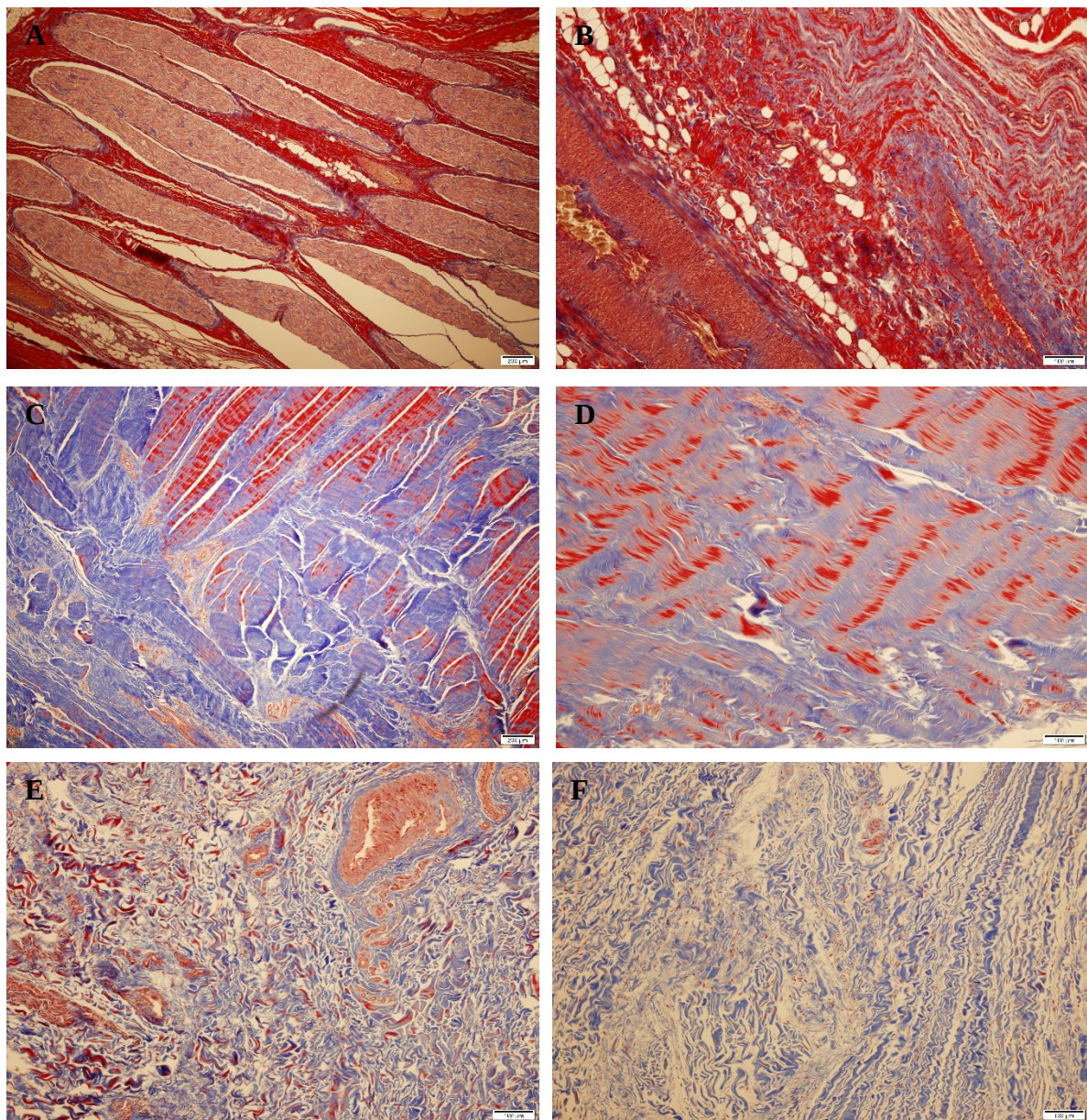


Figure 10.11: Transverse sections showing collagen fibers of the Deep fascia; A lateral crural, B medial crural, C lateral tarsal, D medial tarsal, E lateral metatarsal, F medial metatarsal. Masson's trichrome stain Magnification: (A) 4x; (B) 10x; (C) 4x;(D) 10x; (E) 10x; (F) 20x

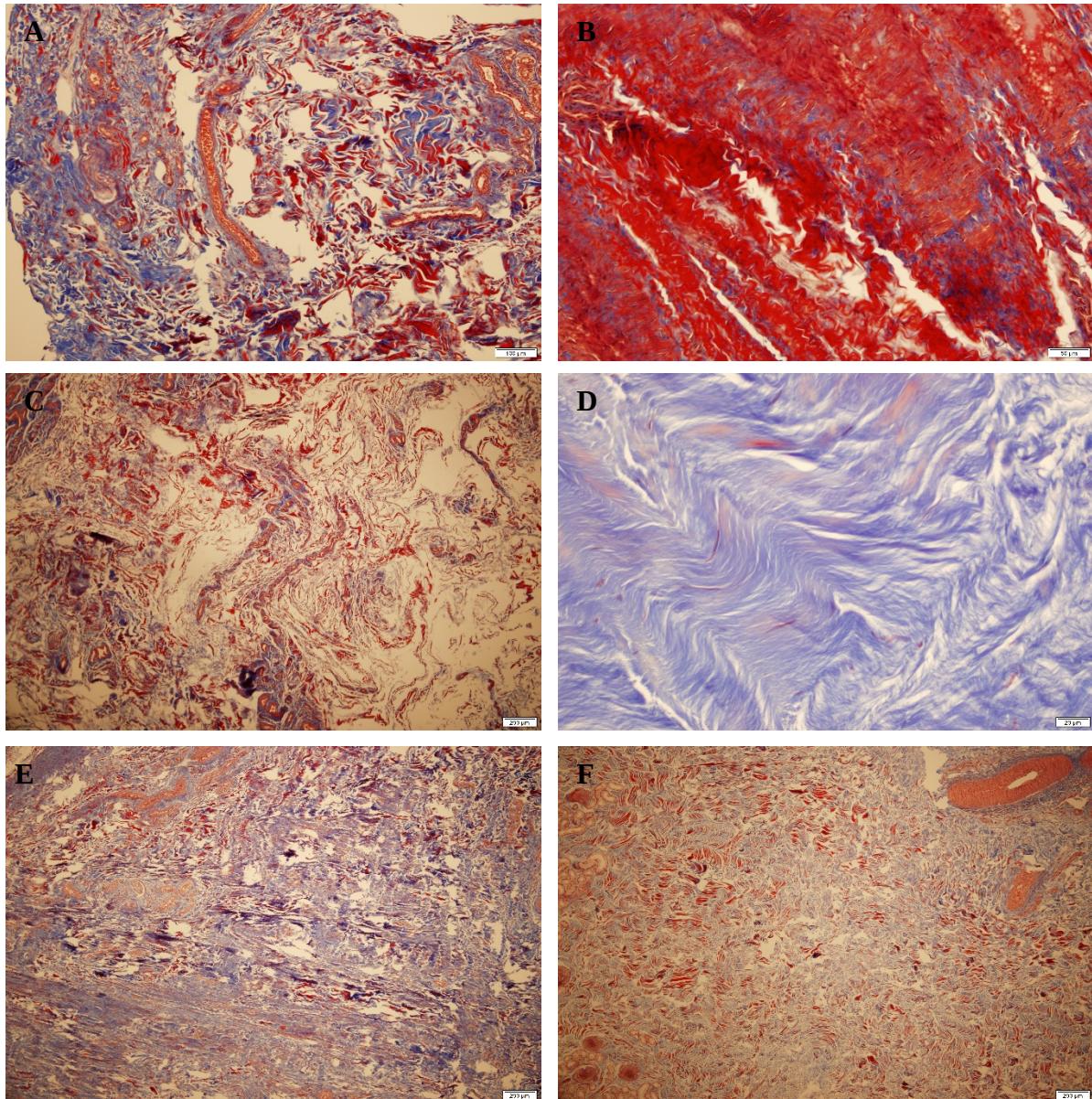


Figure 10.12: transverse section showing collagen fibers of the Superficial fascia; A lateral crural, B medial crural, C lateral tarsal, D medial tarsal, E lateral metatarsal, F medial metatarsal. Masson's trichrome stain Magnification: (A) 10×; (B) 20×; (C) 4×;(D) 40×; (E) 4×; (F) 4×

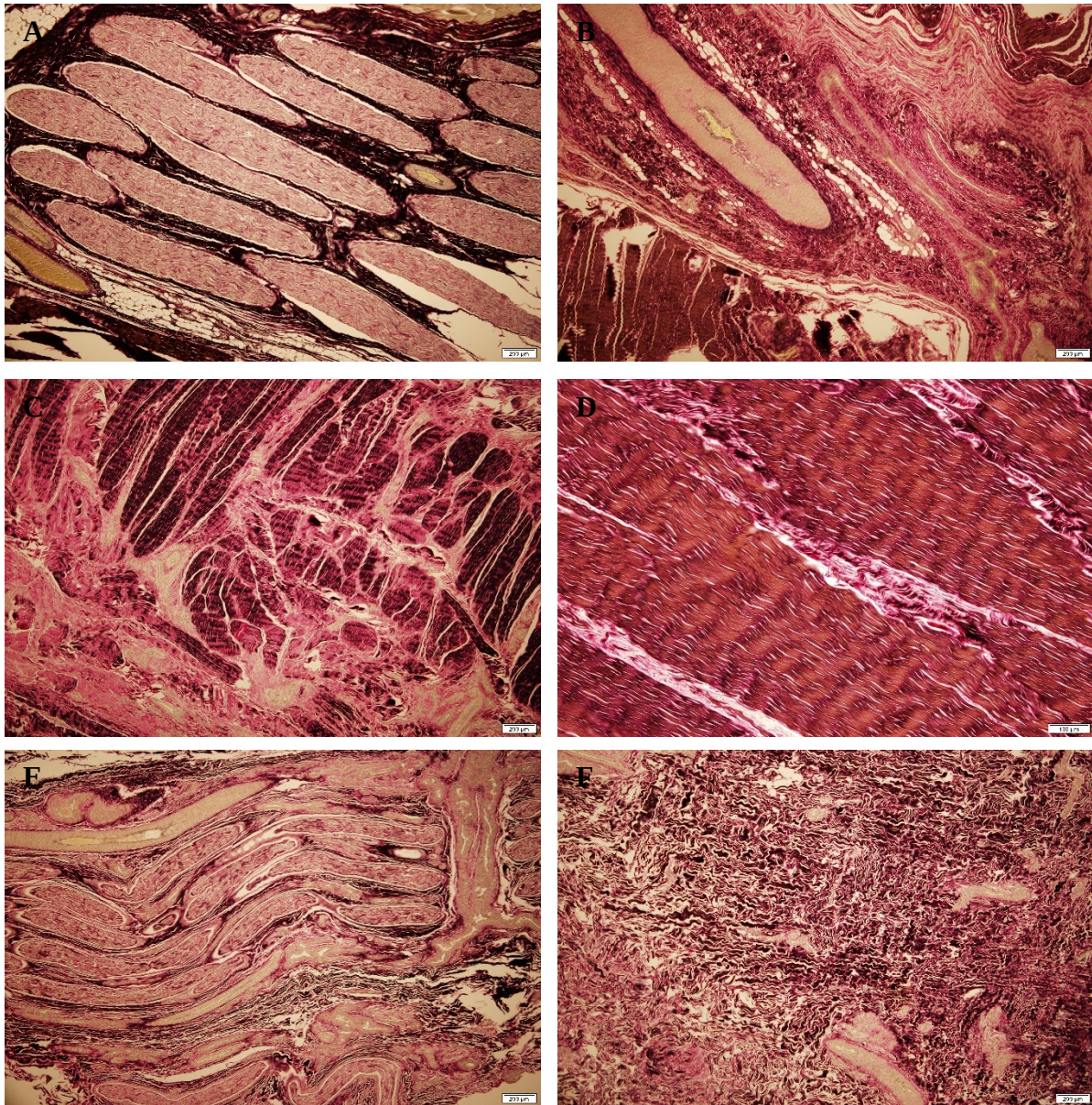


Figure 10.13: Transverse section showing elastic fibers of the Deep fascia; A lateral crural, B medial crural, C lateral tarsal, D medial tarsal, E latera metatarsal, F medial metatarsal. Van Gieson stain Magnification: (A) 4x; (B) 4x; (C) 4x;(D) 100x; (E) 4x; (F) 4x

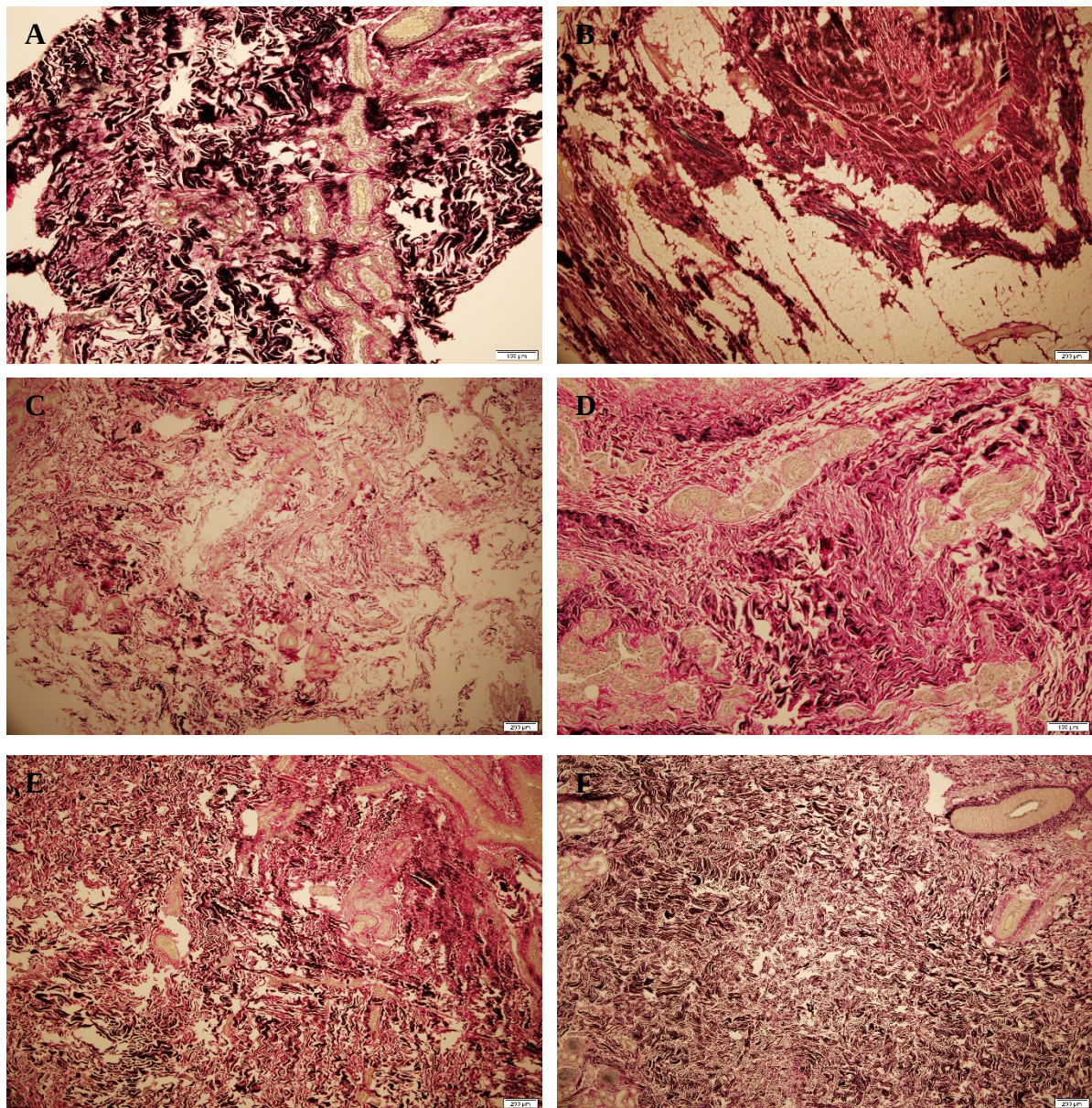


Figure 10.14: transverse section showing elastic fibers of the Superficial fascia; A lateral crural, B medial crural, C lateral tarsal, D medial tarsal, E latera metatarsal, F medial metatarsal. Van Gieson stain Magnification: (A) 10×; (B) 4×; (C) 4×;(D) 10×; (E) 4×; (F) 4×

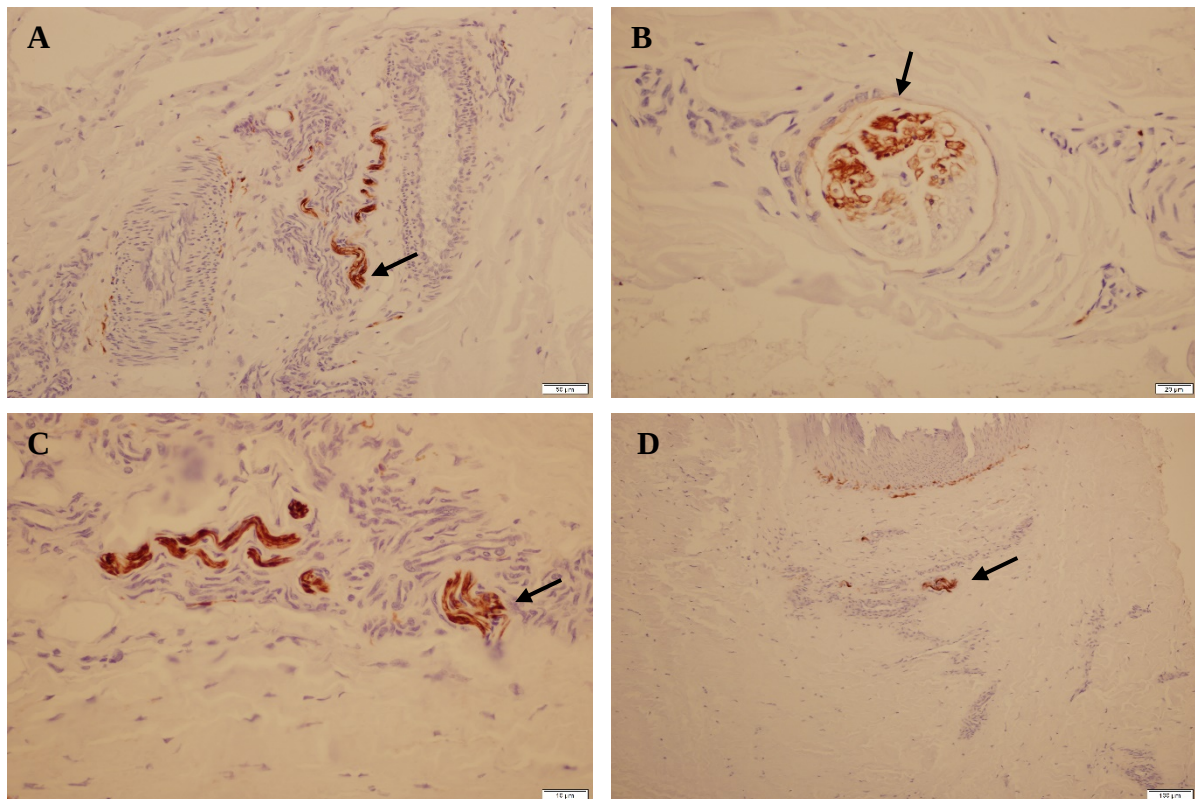


Figure 10.15: Images of the superficial fascia stained with anti-S100 antibodies showing Ruffini's corpuscles in the lateral crural region (A), in the lateral metatarsal (B), in the medial crural (C) , in medial metatarsal (D), Magnification A 20×, B 40×, C 10×, D 10×.

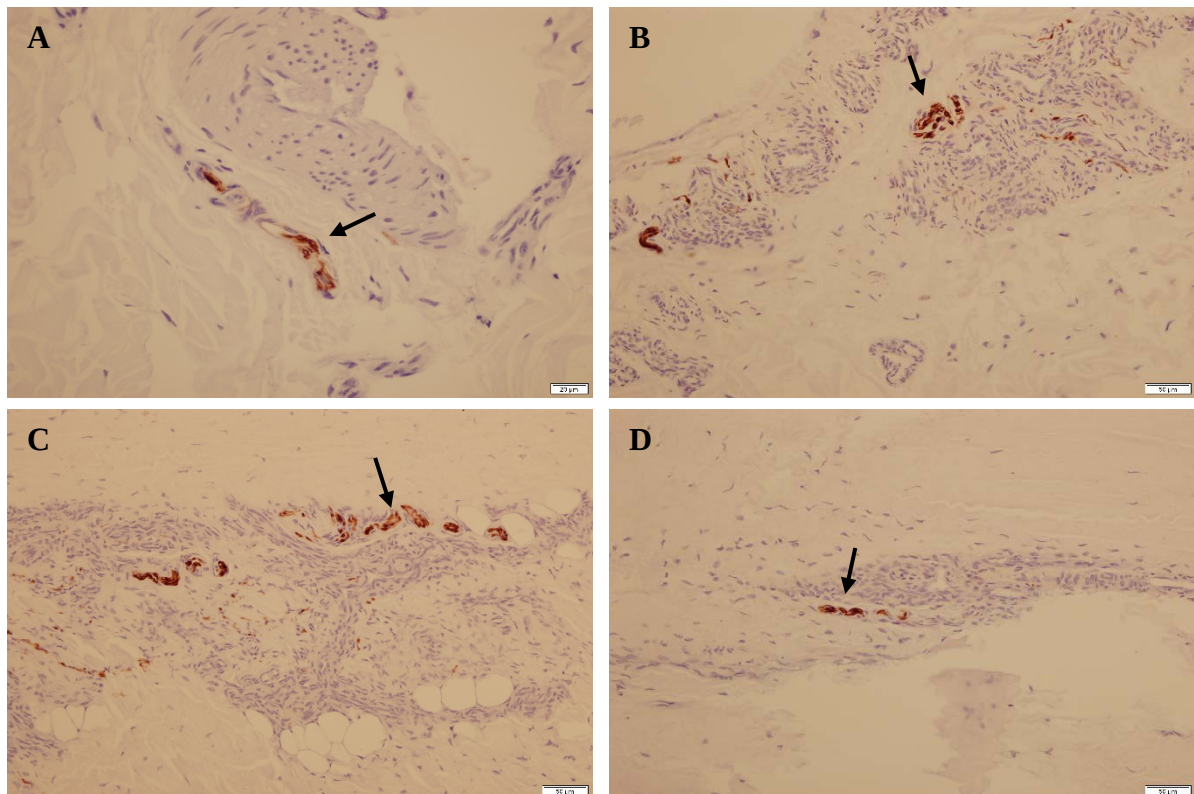


Figure 10.16: Images of the superficial fascia staining with anti-S100 antibodies showing Pacini corpuscles, in the latera metatarsal (A), in the lateral tarsal (B), in the medial crural (C) and in the medial metatarsal (D). Magnification A 40×, B-D 20×.

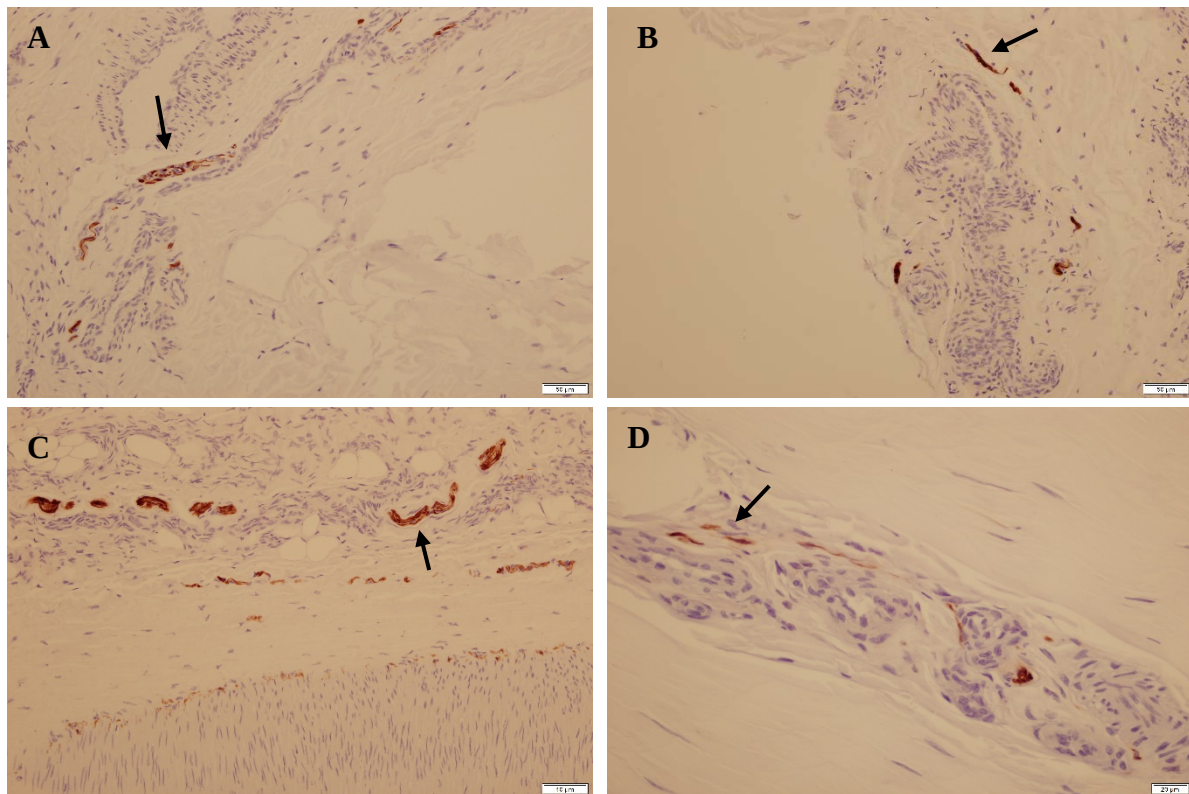


Figure 10.17: Images of the superficial fascia staining with anti-S100 antibodies showing free nerve ending, in the lateral crural (A), in the latera tarsal (B), in the medial crural (C) and in the medial tarsal (D) Magnification A & B 20×, C & D 40×.

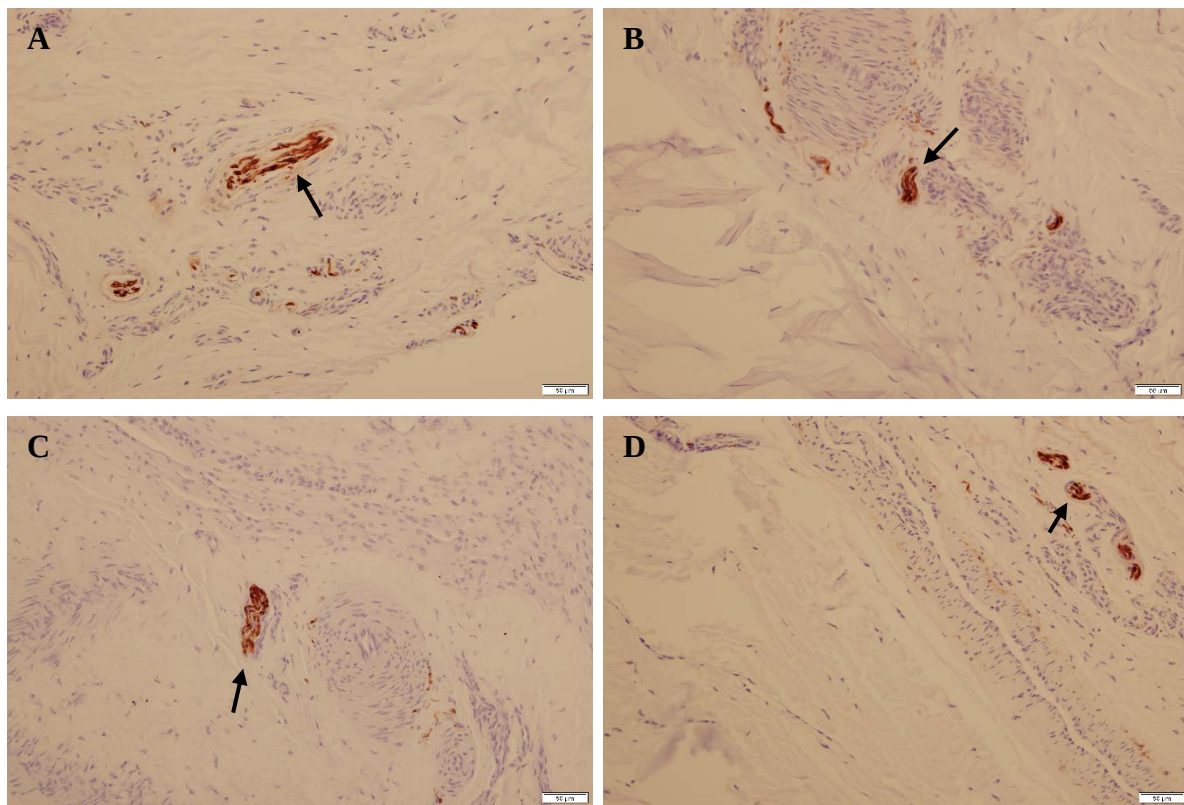


Figure 10.18: Images of the deep fascia staining with anti-S100 antibodies showing Pacini corpuscles, in the latera metatarsal (A), in the lateral tarsal (B), in the medial crural (C) and in the medial metatarsal (D) Magnification A-D 20×.

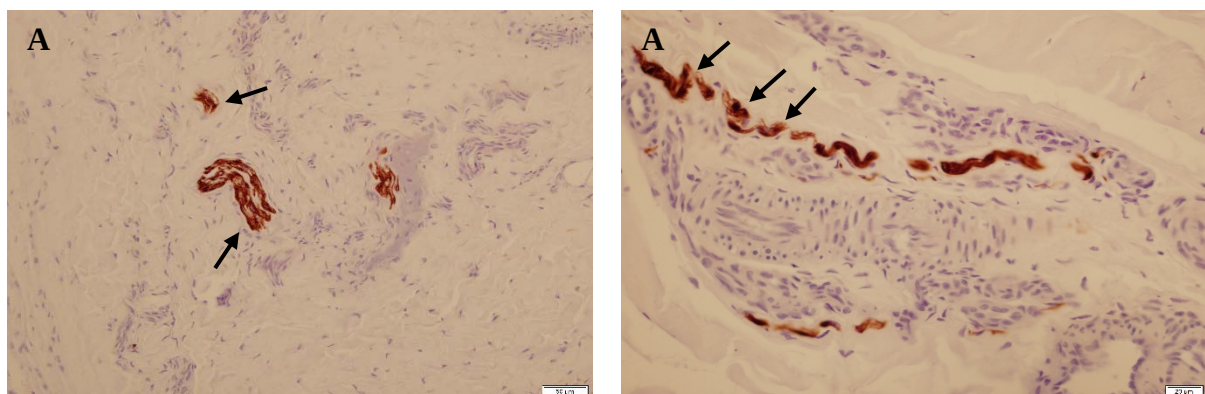


Figure 10.19: Images of the deep fascia staining with anti-S100 antibodies showing Ruffini's corpuscles, in the medial crural (A) and in the lateral tarsal (D) Magnification A 20×B 40×.

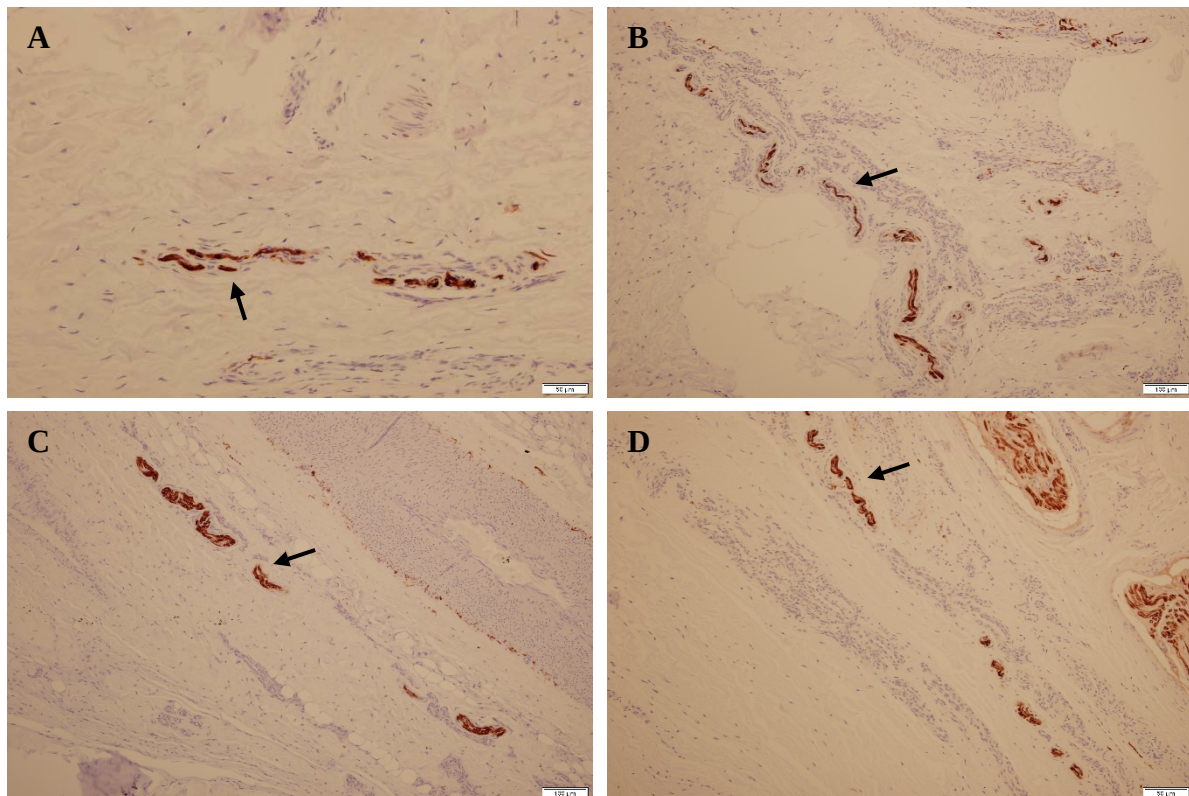


Figure 10.20: Images of the deep fascia staining with anti-S100 antibodies showing free nerve ending, in the latera metatarsal (A), in the lateral tarsal (B), in the medial crural (C) and in the medial metatarsal (D). Magnification A-D 20×.

10.3.4 Fascial thickness

The ultrasonographic measurements of the deep fascia in the current study showed differences between the thickness in different aspects of the same region, for instance the mean thickness of the lateral aspect of the crural fascia was thickest at 0.39 ± 0.01 mm as compared to the cranial aspect with a mean thickness of 0.36 ± 0.01 mm (Table 10.1 and 10.2), (Figure 10.21). The overall mean thickness of the crural region from all 4 aspects of this region (cranial, caudal, medial, lateral) was noted as 0.37 ± 0.01 mm, forming the highest thickness of all the investigated regions of the hindlimb. The tarsal fascia presented a mean thickness ($\pm$ SD) of 0.31 ± 0.01 mm. The mean thickness of the lateral aspect of tarsal fascia was thickest at 0.32 ± 0.01 mm as compared to the medial aspect with minimal mean thickness of 0.31 ± 0.01 mm (Table 10.3 and 10.4), (Figure 10.22). The mean thickness ($\pm$ SD) of the metatarsal fascia was 0.36 ± 0.02 mm, whereas, the fascial thickness was 0.34 ± 0.01 mm for the cranial digital fascia (Table 10.7). The mean thickness of the caudal aspect of the metatarsal fascia was thicker at 0.37 ± 0.01 mm compared to the medial aspect with a minimal mean thickness of 0.32 ± 0.01 mm (Table 10.5 and 10.6), (Figure 10.23).

In all selected sites (crural fascia, tarsal fascia, metatarsal fascia and digital fascia), there were no observed significant differences between the left and right sides. However, there were found clear significant differences ($P \leq 0.05$) between the deep fascia in different aspects of the same regions (within the same region) but also between different selected regions of the individual limb (Figures 10.25).

The maximum difference in the thickness was between the crural fascia and the deep fascia in the tarsal region, while the metatarsal and crural regions showed minimum differences in fascial thickness. The overall thickness of fascia was thicker in lateral aspects as

compared to medial aspects in proximal parts of limb such as the crural and tarsal regions followed by the caudo-lateral aspects of the metatarsal region as shown in figures 10.25-10.28. The caudal aspect of the metatarsal region exhibited the maximum thickness (Table 10.5).

Table 10. 1: Deep fascia thickness data for crural fascia (CF) in cranial, caudal, lateral and medial aspects with analysis of statistical significance.

Deep fascia	Mean thickness ± SD (mm)	SEM of differences (mm)	confidence interval (95%) of mean thickness (mm)
Cranial	0.364 ± 0.01	0.004336	0.352 to 0.376
Caudal	0.3696 ± 0.01	0.004996	0.3557 to 0.384
Lateral	0.398 ± 0.00	0.003286	0.3889 to 0.407
Medial	0.3668 ± 0.01	0.008015	0.3445 to 0.389

Table 10. 2: Mean $\pm$ standard deviation (SD) and standard error of means (SEM) of the difference in the thickness of crural deep fascia in horse hindlimbs (n = 5) for comparison of the cranial, caudal, lateral and medial aspects. The changes in the fascial thickness are shown as an increase (+) or decrease (-) in measurement.

Group comparison	Mean Difference $\pm$ SD (mm)	Confidence Interval (95%)	P value
Cranial crural region vs. Caudal crural region	-0.0056 $\pm$ 0.01	-0.02765 to 0.01645	0.885
Cranial crural region vs. Lateral crural region	-0.034 $\pm$ 0.01	-0.05605 to -0.01195	0.0022**
Cranial crural region vs. Medial crural region	-0.0028 $\pm$ 0.01	-0.02485 to 0.01925	0.983
Caudal crural region vs. Lateral crural region	-0.0284 $\pm$ 0.01	-0.05045 to -0.006350	0.0097**
Caudal crural region vs. Medial crural region	0.0028 $\pm$ 0.01	-0.01925 to 0.02485	0.983
Lateral crural region vs. Medial crural region	0.0312 $\pm$ 0.01	0.009150 to 0.05325	0.0046**

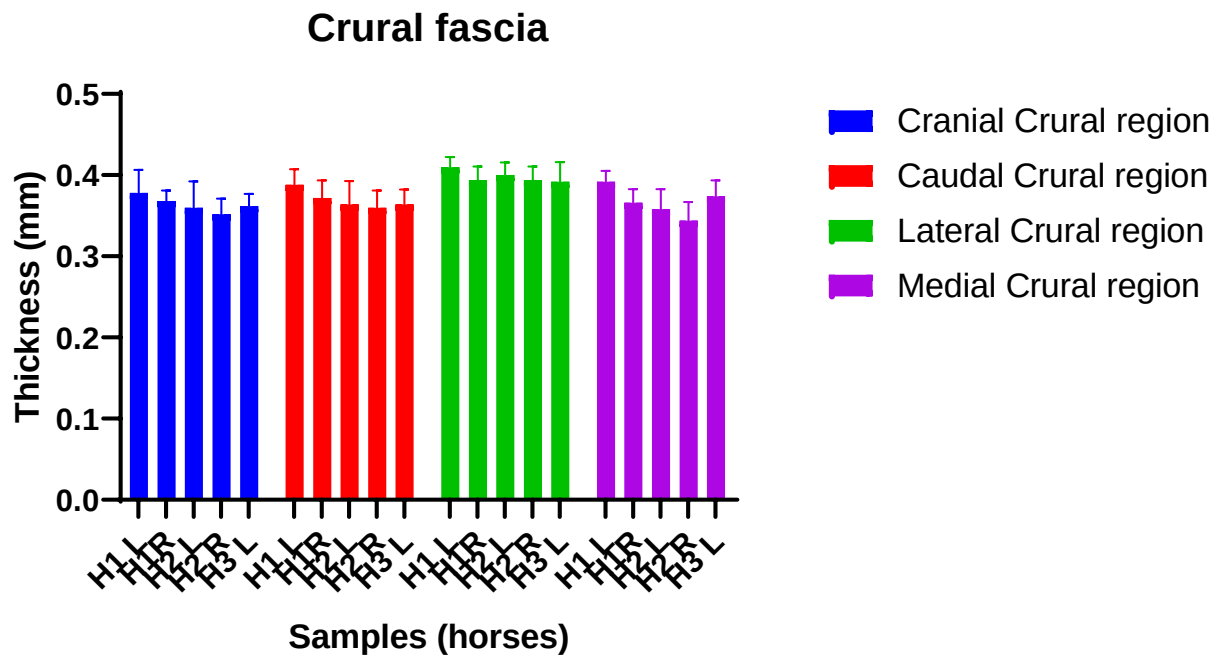


Figure 10.21: Graphical distribution of crural fascia thickness (mm) in different aspects (cranial, caudal, lateral and medial) in given horse samples, H. represents horse, L. left and right (R) sides.

Table 10. 3: Deep fascia thickness data for tarsal fascia (CF) in cranial, caudal, lateral and medial aspects with analysis of statistical significance.

Deep fascia	Mean Thickness ± SD (MM)	SEM of differences (MM)	Confidence Interval (95%) Of Mean Thickness (Mm)
Cranial	0.312 ± 0.01	0.003578	0.3021 to 0.322
Caudal	0.3116 ± 0.01	0.004956	0.2978 to 0.325
Lateral	0.3216 ± 0.01	0.004534	0.309 to 0.334
Medial	0.3124 ± 0.01	0.001939	0.307 to 0.318

Table 10. 4: Mean $\pm$ standard deviation (SD) and standard error of means (SEM) of the difference in the thickness of crural deep fascia in horse hindlimbs (n = 5) for comparison of the cranial, caudal, lateral and medial aspects. The changes in the fascial thickness are shown as an increase (+) or decrease (-) in measurement.

Group comparison	Mean Difference $\pm$ SD (mm)	Confidence Interval (95%)	P value
Cranial tarsal region vs. Caudal tarsal region	0.0004 $\pm$ 0.01	-0.01549 to 0.01629	0.9999
Cranial tarsal region vs. Lateral tarsal region	-0.0096 $\pm$ 0.01	-0.02549 to 0.006288	0.3419
Cranial tarsal region vs. Medial tarsal region	-0.0004 $\pm$ 0.01	-0.01629 to 0.01549	0.9999
Caudal tarsal region vs. Lateral tarsal region	-0.01 $\pm$ 0.01	-0.02589 to 0.005888	0.3088
Caudal tarsal region vs. Medial tarsal region	-0.0008 $\pm$ 0.01	-0.01669 to 0.01509	0.9989
Lateral tarsal region vs. Medial tarsal region	0.0092 $\pm$ 0.01	-0.006688 to 0.02509	0.3771

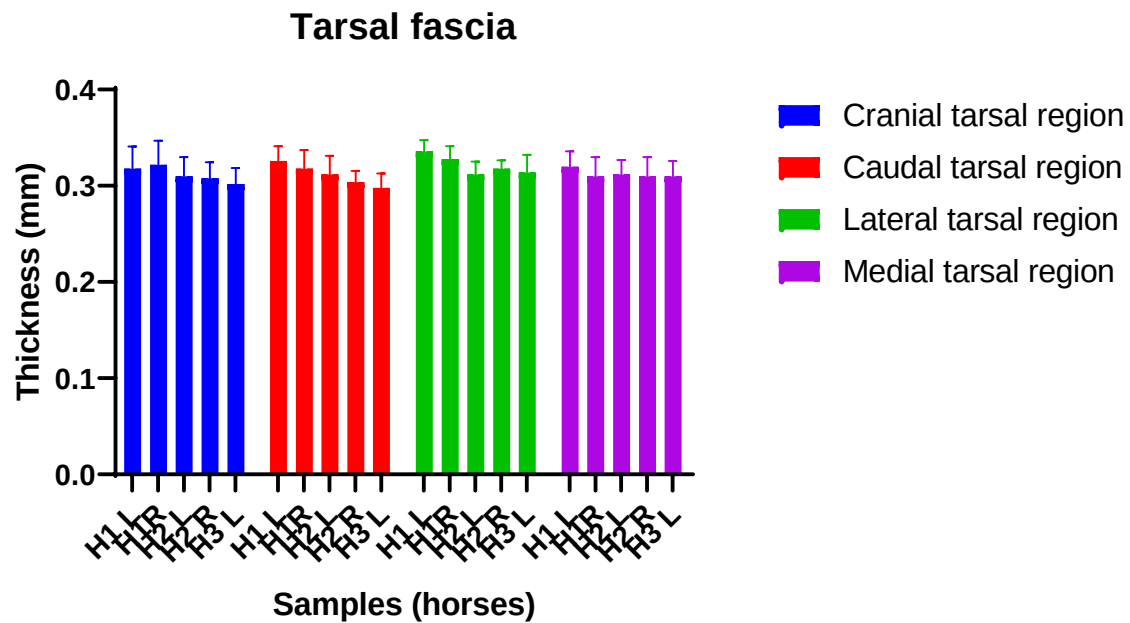


Figure 10.22: Graphical distribution of tarsal fascia in different aspects (cranial, caudal, lateral and medial) in given horse samples, H. represents horse, L. left and right (R) sides.

Table 10. 5: Deep fascia thickness data for metatarsal fascia (CF) in cranial, caudal, lateral and medial aspects with analysis of statistical significance.

Deep fascia	Mean thickness ± SD (mm)	SEM of differences (mm)	Confidence interval (95%) of mean thickness (mm)
Cranial	0.3664 ± 0.02	0.008795	0.342 to 0.391
Caudal	0.3744 ± 0.01	0.002926	0.3663 to 0.383
Lateral	0.3676 ± 0.01	0.005036	0.3536 to 0.382
Medial	0.3236 ± 0.01	0.003763	0.3132 to 0.334

Table 10. 6: Mean $\pm$ standard deviation (SD) and standard error of means (SEM) of the difference in the thickness of metatarsal deep fascia in horse hindlimbs (n = 5) for comparison of the cranial, caudal, lateral and medial aspects. The changes in the fascial thickness are shown as an increase (+) or decrease (-) signs in measurement.

Group comparison	Mean Difference $\pm$ SD (mm)	Confidence Interval (95%)	P value
Cranial metatarsal region vs. Caudal metatarsal region	-0.008 $\pm$ 0.01	-0.03066 to 0.01466	0.7459
Cranial metatarsal region vs. Lateral metatarsal region	-0.0012 $\pm$ 0.01	-0.02386 to 0.02146	0.9987
Cranial metatarsal region vs. Medial metatarsal region	0.0428 $\pm$ 0.01	0.02014 to 0.06546	0.0003
Caudal metatarsal region vs. Lateral metatarsal region	0.0068 $\pm$ 0.01	-0.01586 to 0.02946	0.8257
Caudal metatarsal region vs. Medial metatarsal region	0.0508 $\pm$ 0.01	0.02814 to 0.07346	<0.0001
Lateral metatarsal region vs. Medial metatarsal region	0.044 $\pm$ 0.01	0.02134 to 0.06666	0.0002

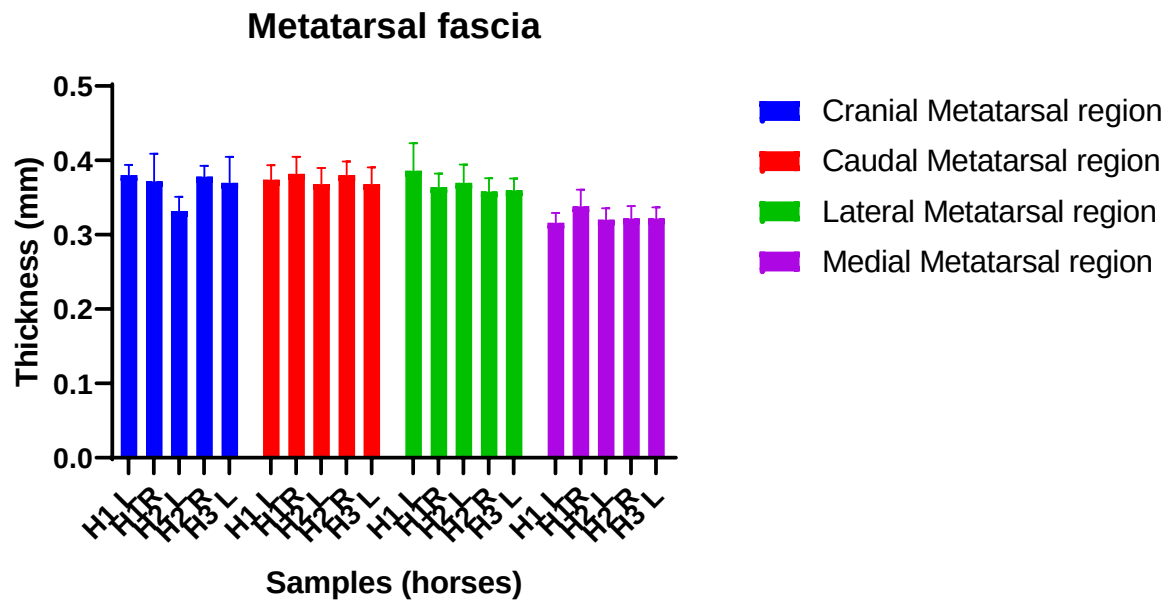


Figure 10.23: Graphical distribution of metatarsal fascia in different aspects (cranial, caudal, lateral and medial) in given horse samples, H. represents horse, L. left and right (R) sides.

Table 10. 7: Deep fascia thickness data for cranial digital fascia (CF).

Deep fascia	Mean thickness ± SD (mm)	SEM of differences (mm)	Confidence interval (95%) of mean thickness (mm)
Dorsal Aspect	0.341 ± 0.01	0.0033	0.332 to 0.351

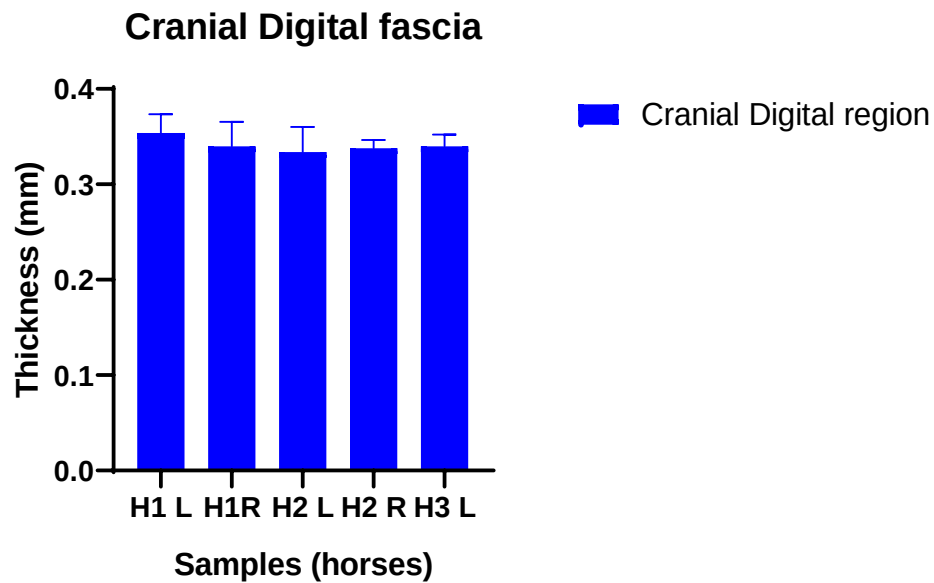


Figure 10.24: Graphical distribution of digital fascia thickness (mm) in the dorsal aspect in given horse samples, H. represents horse, L. left and right (R) sides.

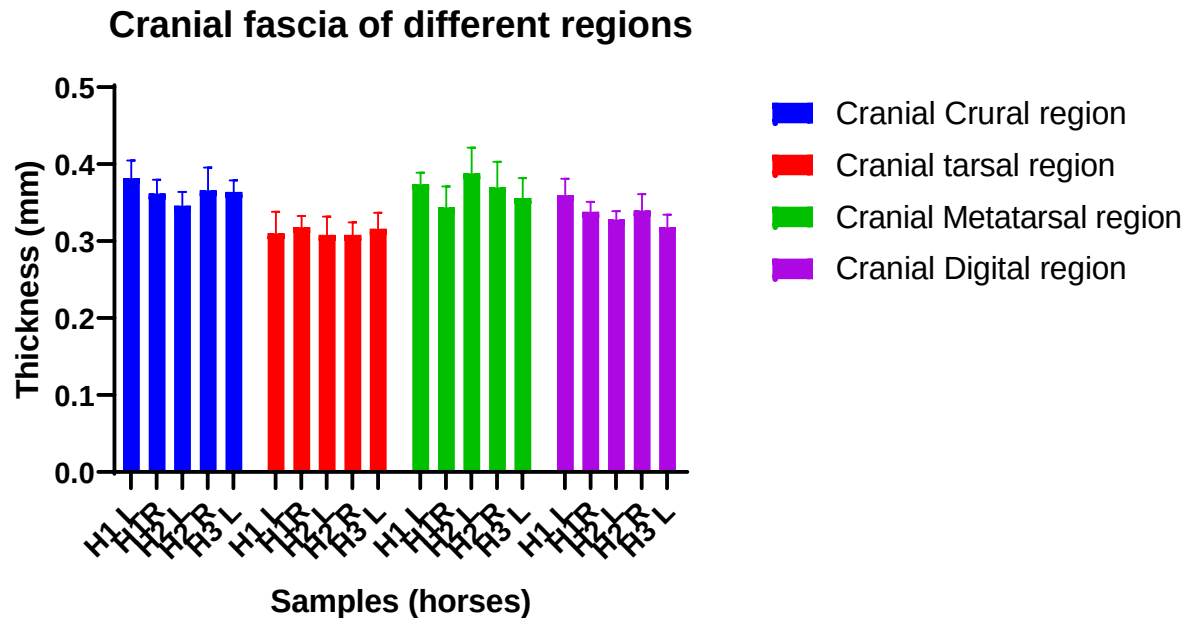


Figure 10.25: Graphical distribution of fascial thickness (mm) in the cranial aspect of hindlimb including different regions (crural, tarsal, metatarsal and digital) in given horse samples, H. represents horse, L. left and right (R) sides.

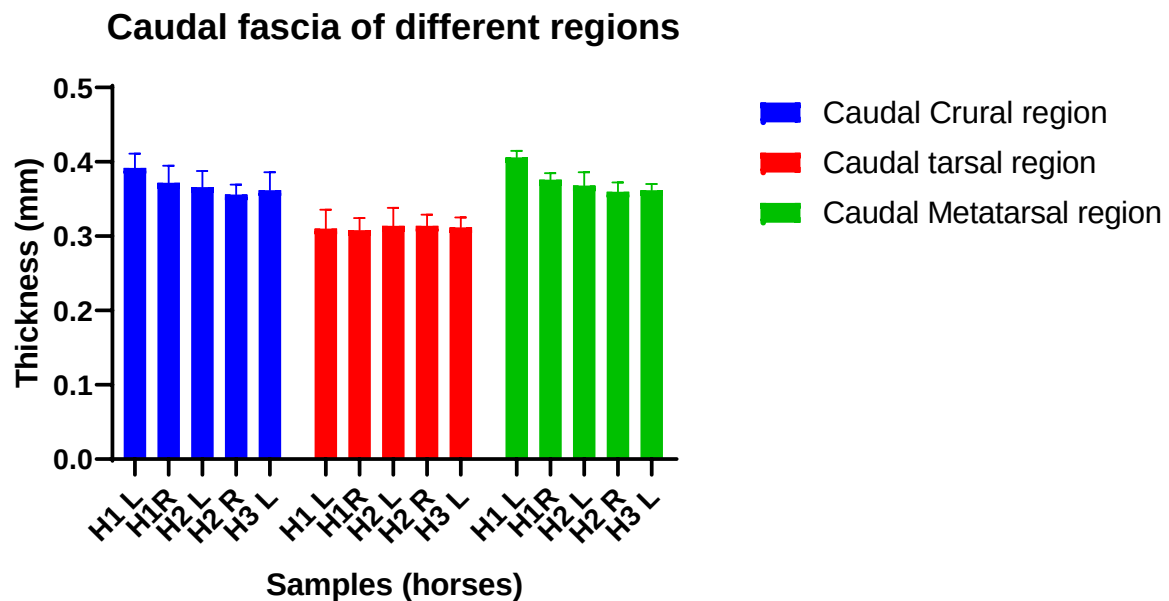


Figure 10.26: Graphical distribution of fascial thickness (mm) in the caudal aspect of the hindlimb including different regions (crural, tarsal and metatarsal) in given horse samples, H. represents horse, L. left and right (R) sides.

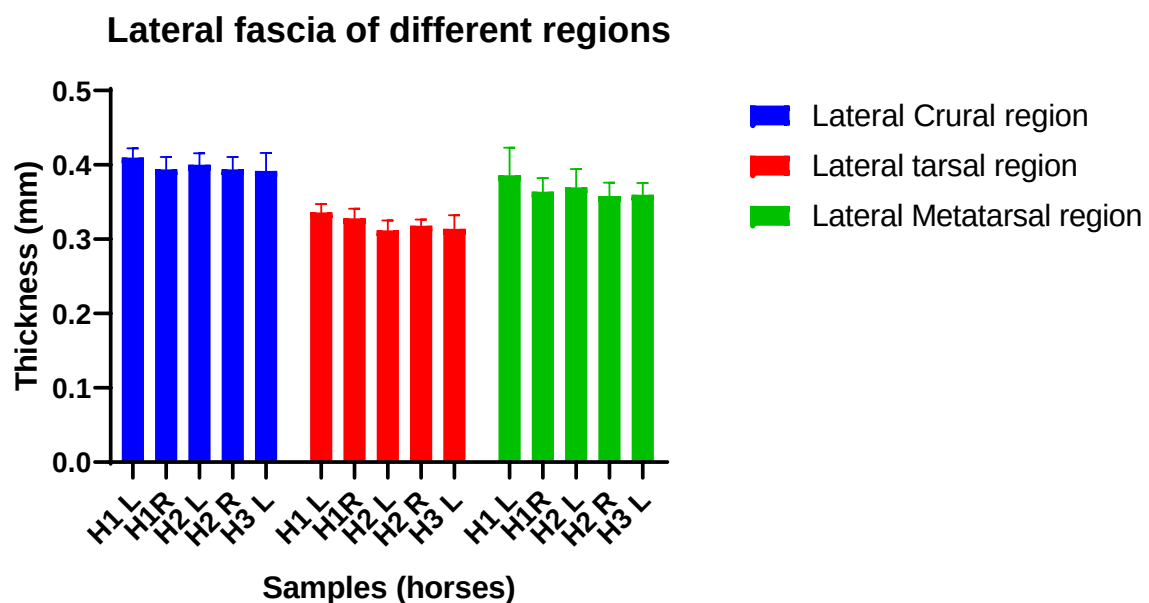


Figure 10.27: Graphical distribution of fascial thickness (mm) in the lateral aspect of the hindlimb including different regions (crural, tarsal and metatarsal) in given horse samples, H. represents horse, L. left and right (R) sides.

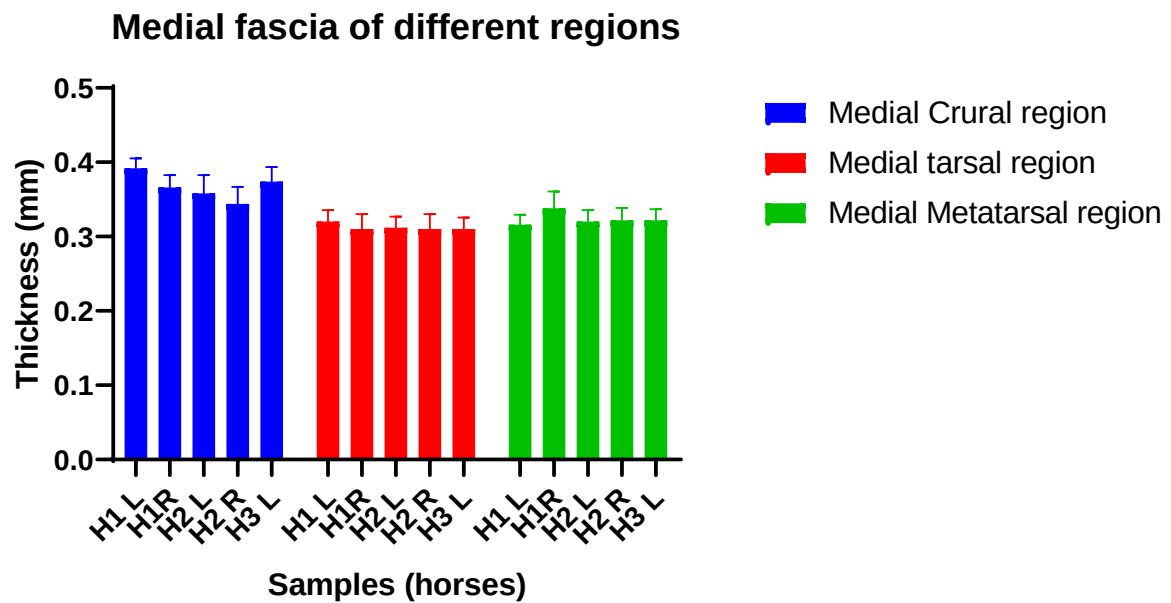


Figure 10.28: Graphical distribution of fascial thickness (mm) in the medial aspect of the hindlimb including different regions (crural, tarsal and metatarsal) in given horse samples, H. represents horse, L. left and right (R) sides.

10.4 Discussion

Previous findings in Chapter 9 clearly showed that the fascia (superficial and deep) is a connective tissue layer that provides mechanical connectivity with the skin and underlying tissue via fibrous connective tissue not only within a particular region but also between different regions of the hind limb. Similar findings were suggested in a previous study (K. L. Moore & Dalley, 1999). Further investigations were aimed to relate these findings with the composition and thickness variations that might influence the flexibility and ultimately can affect the normal range of joint motion (ROM). This will help in understanding the loading patterns along with the functional integration of structures within the hindlimb to provide support and encourage an even distribution of forces.

This study demonstrated the histological composition of the superficial and deep fascia along with thickness pattern in the equine hindlimb (from the crural region to the distal hindlimb). The results revolved around ultrasound and histology of the superficial and deep fascia to understand its architectural design and thickness variation throughout the hindlimb that may influence the joint range of motion and functions of the hind limb.

The superficial fascia was composed of a thin layer, but it split into two sublayers to envelope the cutaneous muscle (panniculus carnosus) and large blood vessels. The cutaneous muscle is well developed in many animals and described in several studies, but its relationship with the superficial fascia is still not clear. In the current study it was found to be embedded inside sublayers of superficial fascia and not attached directly to the skin.

A large number of elastic fibres were present with the collagen fibres in both sub-layers around the cutaneous muscle. These findings suggested that the superficial fascia has a role in protection of the muscle and conveying the action to the skin. Such cutaneous muscle is

present beneath the skin in most animals including horses (Higgins & Martin, 2012; Hillel et al., 2012). In addition, in a previous study Essing et al. (2013) reported that the cutaneous muscle responds to skin stimulation in the dorso-lateral aspect of the thoracic region of horses, and the contraction of this muscle localizes to the stimulation sites (Essig & Hilary, 2013). Therefore, it has been considered a functional part of the skin because its action is closely associated with the skin (Naldaiz-Gastesi, Bahri, Lopez de Munain, McCullagh, & Izeta, 2018).

The findings of the current study confirmed that the saphenous vein passes within the superficial fascia in the crus. The superficial fascia enveloped the saphenous vein in the crus as a consequence of the division of the superficial fascia into two laminae. Such a relationship of fascia and blood vessels has been studied by using dissection, histology, ultrasonography and stereomicroscopy in humans (Caggiati, 1999).

An interesting finding from this study was that the superficial fascia fuses with the deep fascia and the skin in both proximal and distal areas of the hindlimb where there is a requirement for movement, creating numerous bursae in the distal hind limb. Determination of these fusions presented a challenge through the different stages of dissection for the subcutaneous connective tissue.

Within the superficial fascia, the collagen fibres predominated and were in irregular directions, giving the tissue a woven mesh shape, and many elastic fibres were scattered between the collagen fibres. The arrangement of the collagen fibres suggested that the superficial fascia may be able to respond to multi-directional loading, thus, participating in the loading regime of the limb.

The presence of elastic fibres indicated that the superficial fascia is an elastic structure, providing mechanical resistance against stress on the limb during movement, and store elastic energy. In addition, the presence of elastic fibres in the superficial fascia more than deep fascia and skin suggests a role in sliding the skin over the deep fascia. In addition, elastic fibers in the connective tissue allows long-range deformability and negative recoil without the input of energy (Kielty, Sherratt, & Shuttleworth, 2002).

The histology showed that the nerves were surrounded by collagen fibres and the blood vessels enveloped by elastic fibres, which indicates that the superficial fascia provides protection to the nerves and blood vessels from excessive stretching. The general structures of these findings correspond to the characteristics of the superficial fascia as described in the human (Abu-Hijleh, Roshier, Al-Shboul, Dharap, & Harris, 2006; Lancerotto et al., 2011; Helene M. Langevin & Huijing, 2009).

Functionally, superficial fascia has a significant role in the skin integrity and supports subcutaneous veins, nerves and fat (Lockwood, 1991) as described in a previous study (Stecco, C., Macchi, Porzionato, Duparc, & De Caro, 2011b) the subcutaneous tissue is divided by superficial fascia into the superficial adipose tissue (SAT) and the deep adipose tissue (DAT) in human. The SAT is in a honeycomb-like structure composed of single or multiple layers of adipose lobules among fibrous septa. These septa are mostly oriented in a direction perpendicular to the skin surface, anchoring the skin to the underlying structures. While, the DAT is formed of oblique fibrous septa and contains limited elastic fibres, thus this arrangement facilitates sliding of the superficial fascia over the deep fascia (Stecco et al., 2011b) .

The current study showed deep fasciae as a sheath of connective tissue that could be divided into an external aponeurotic fascia and internal epimysial fascia. Both layers were formed by collagen fibers with a few elastic fibers embedded in the matrix of the tissue. The alignment of collagen fibers was multi-directional which indicated that the deep fascia was likely to be responding to forces from different directions, and therefore may have a role in the loading and probably stabilizing the limb. Loose connective tissue separated deep fascia from the superficial fascia and underlying muscles, potentially allowing sliding and providing cushioning between these structures. The presence of sparse amounts of elastic fibers in the deep fascia would help provide flexibility and permit free movement of the superficial fascia and skin.

This study confirmed that the deep fascia has the same structure but differed in the arrangement according to the region of the limb. This variation may be associated with its relationship with several muscles that covered and inserted into the deep fascia. The general structure of the deep fascia in the greyhound hind limbs corresponds with the deep human fascia, which has been studied previously (Stecco et al., 2008). The deep fascia in the thigh surrounds muscles, and in the lateral aspect (fascia lata) was found to be composed of three layers that provided insertion for some muscles such as the biceps femoris and tensor fascia lata muscles. This linking suggests that the deep fascia plays an essential role in the connections between muscles and transmission of force.

The alignments of collagen fibers within the fascia lata suggest several functions of this fascia; thus, it could respond to loading from more than one direction (Stecco et al., 2009; Wainwright, Biggs, Gosline, & Currey, 1982). Therefore, it allows transferring of force from the muscles with multiple insertions. Continuity between the fascia and muscles can permit

reciprocal feedback between the muscles and fascia, the fascia perceives the tension from the contraction of muscles and transmits it to a distance. This concept was highlighted by (Stecco et al., 2009), who found that the muscles in the flexor region of the human upper arm had a constant myofascial expansion between all muscles, which they existed in the same direction of the movements. Consequently, the brachial fascia and antebrachial fascia stretch when the pectoralis major and biceps brachii muscles contract during movement of the shoulder and flexion of the elbow. Similarly, contraction of the palmaris longus muscles produces stretching of the hand fascia during wrist flexion. Thus, these fascial-muscle attachments have been considered as a mirror of the muscle forces (Marshall, 2001).

Therefore, this continuity and arrangements of collagen fibers indicate that the deep fascia may transfer the muscle action from the proximal to the distal parts of the limb. In comparison to this finding, the term myofascial expansion has been mentioned in the human literature to indicate the connection of the skeletal muscle into the aponeurotic fascia.

Previous studies in humans and cats (Boyd & Roberts, 1953; Jerosch & Prymka, 1996; Michelson & Hutchins, 1995; C. Stecco & Day, 2010) have demonstrated proprioceptive nerve endings within fascia and ligaments surrounding highly mobile joints. The presence of such nerve endings in the soft connective tissue structures surrounding joints suggests that joints have an intrinsic ability to maintain their movement within a range.

In the current study, free nerve endings and mechanoreceptors were observed in both the superficial and deep fascia, which suggests that the superficial fascia is also a proprioceptive organ and supports and contributes to the movement in the limb. Ruffini's corpuscles help in perceiving the stretch of the superficial fascia, whereas Pacini's corpuscles sense pressure, and their position may contribute to distinguishing between high and low pressures;

therefore, the superficial fascia could be involved in exteroception. Pacini's corpuscles respond to sudden changes accompanying mechanical stress (Cavalcante, Rodrigues, & Mattar Jr, 2004). These findings are important when attempting to consider the functions of mechanoreceptors.

These results are interesting in relation to the functions of the hind-limb, involving its participation in the body movements. The superficial fascia is capable of perceiving the tension variations in multi-directions and contributing to proprioception, thus the presence of mechanoreceptors as activators in the collagen fibres of the superficial fascia would enhance this function due to their ability to detect stretches and integrating signals in the transverse, longitudinal and vertical directions (Stecco et al., 2018).

The fascial innervation is important when considering the functions of mechanoreceptors. Ruffini corpuscles are slowly adapting and respond to changes in stress; they transfer the action from the inactive status, internal pressure, and the direction of movement. Pacini's corpuscles adapt rapidly to mechanical stress (Cavalcante et al., 2004). Exciting these receptors within the collagen fibers of the fascia suggests that the deep fascia could also contribute to proprioception.

The deep fascia receives force from the muscle by the myofascial expansions, which maintain the basal tension of the fascia and allow stretching along different lines according to the movement performed (Stecco et al., 2007). This stretching may activate the mechanoreceptors that are embedded within the fascia, as demonstrated in the current study.

This assumption was supported by an embryonic study (Saxod, 1996), which confirmed that the capsules of the mechanoreceptors are derived from the connective tissue. This finding

corresponds to the previous study (C. Stecco et al., 2007) that confirmed the role of the deep muscular fascia of the upper limb in both static proprioception, and dynamic proprioception, which refers to the direction of the movement.

Similarly, the modern histological study of the deep palmar fascia confirmed that the Pacinian corpuscles and the free nerve endings were present among the connective fibers (C. Stecco et al., 2018). The Pacinian corpuscles were located at the border of the hand fascia and intersections of collagen fibers, which could assist in detecting stretches in multi-directions. Thus, proprioception from deep fascia can be integrated with the proprioception from the superficial fascia and skin and the proprioception from neuromuscular organs (C. Stecco et al., 2015).

Although the free nerve endings have proprioceptive functions, there could be nociceptive stimuli that relates to transfer of pain (Hoheisel, Rosner, & Mense, 2015; Standring, 2015), therefore, some receptors in the fascia may be affected by connective tissue diseases as a disorder inflammation (Schubert et al., 2006). Thus, the innervation of superficial fascia suggests that incorrect or inaccurate surgical processes can influence proprioception and the perception of pain (Stecco et al., 2018).

The range of motion (ROM) of a joint represents a significant hallmark of health of a body's musculoskeletal system and refers to the maximal distance over which a joint can be moved (Garber et al., 2011; Nelson et al., 2007; Paterson, Jones, & Rice, 2007). A thicker fascia might be associated with restricted ROM and such a relationship was found in patients with neck problems (A. Stecco, Meneghini, Stern, Stecco, & Imamura, 2014). To the author's knowledge, the present pilot study is the first to show that the equine hindlimb exhibits differences in fascial thickness not only within the same region in different aspects (cranial, caudal, lateral

and medial) but also between different regions of the hind limb (crural, tarsal, metatarsal and digital regions). Previous studies have shown that ultrasound measurements of tissue thickness were found to be reliable (Crofts, Angin, Mickle, Hill, & Nester, 2014; Skovdal, Moelgaard, & Lykkegaard Olesen, 2011) and therefore this technique offers a good opportunity to quantify the concerned structures and better understand their contributions to the joint function.

In the crural and the distal hind limb, more connective tissue envelops the skeletal muscles and their tendons. A possible reason for this consists in the presumably higher muscle mass or tensions through muscle tendons crossing a particular region. The thick connective tissue was required for higher muscle strength and higher body weight to withstand and transmit greater forces. This would be in parallel with previous data suggesting a mechanical impact of the fascia on musculoskeletal dynamics (Schleip et al., 2005).

Another noteworthy characteristic was variation of thickness in various aspects within the same region that might indicate the presence of multiple layers of aponeurotic fascia with their thickness based on the variable amount of collagen and elastin fibres present at particular aspects. The higher thickness on the lateral aspect might be indicative of distribution of tensional forces for example. Extending this idea would be helpful for the identification of normal variation of fascial thickness in activities like training exercises and similar physical activities as compared to pathological changes and would need further investigation.

Similarly, the plantar fascia is considered as functionally the most important structure for the maintenance of the longitudinal arch of the human foot and the plantar fascia is composed of three bundles with the central bundle being the most structurally important and also the

most common bundle involved in disease processes and injury (Do Carmo, de Almeida Melão, de Lemos Weber, Trudell, & Resnick, 2008; McNally & Shetty, 2010). Although, no significant changes in the plantar fascia thickness were observed in a previous study by Wilke et al. (2019) and a slight increase in the mean thickness of the plantar fascia in the running group but this also deserves further investigation.

Finally, the current ultrasound measurements can be considered reliable as five repeat measurements on the same recorded image were used as compared to a single measurement and previous studies have shown good reliability of ultrasound measurements (Crofts et al., 2014). In addition, the reliability of ultrasound measurements was increased when using the mean of three measurements compared with one measurement as shown in a previous study by Skovdal et al. (2011) of intra-and interobserver reliability for the quantitative ultrasound measurements of the human plantar fascia (Skovdal et al., 2011).

In summary, the present study provides the first evidence indicating that thickness of fascia differs normally between different regions exhibiting regional specializations in the equine hind limb: while the crural region has the thickest connective tissue overall, the metatarsal region displays a higher fascial thickness in the distal limb region. Our results also show the normal histological composition of fascia in different regions of hind limb. Any changes in fascial flexibility (in terms of internal composition with elastic and collagen fibres and mechanical connectivity) might influence the joint range of motion. Future research should continue to investigate the complex interdependence of fascial thickness, and composition, while controlling for loading distribution in larger samples (number of animals) to increase its wider potential usefulness in a larger population of animals and breeds. As these parameters may become a valuable outcome parameter in the prevention and treatment of

musculoskeletal disorders, but this topic warrants further investigation. In future trials, other variables regarding the factors potentially affecting fascial thickness and internal composition, it would also be prudent to assess muscle volume and the degree of physical activity in order to estimate their significance.

Chapter 11: GENERAL DISCUSSION

11.1 CONCEPT AND SCOPE OF STUDY:

This thesis concerns the study of bone geometry which has been widely used not only in veterinary but also in human medicine for the diagnosis and treatment of various conditions and to identify relationships between bone shape and its functions. Human medicine has used geometry for the diagnosis, treatment and to follow-up cases after surgery as well as to identify relationships between bone shape and some pathological conditions using radiographic measurements (Mamisch, Kim, Richolt, Millis, & Kordelle, 2009; Sambataro, Baccetti, Franchi, & Antonini, 2005). In racing Thoroughbreds, conformation is believed to be a significant factor in evaluating their worth where the presence of any undesirable character would decrease their value (Love et al., 2006).

The study of tarsal conformation was selected as it was widely thought to be linked to a number of injuries in racehorses (Stashak & Hill, 2002). In addition, the available conformational data in relation to tarsal pathology including deformity was scant whether using radiography or any other diagnostic tools. Radiography was used in the current study because it was the most accurate option available to identify and locate the reason for conformational defects especially using the most commonly taken lateromedial and dorsoplantar views, which could show all the tarsal joints including the joint spaces, size, shape and alignment of the tarsal bones (Bramlage & Auer, 2006).

In general, the relationship between many anatomical structures and their physiological functions are known to be dependent to each other. In this regard, the anatomical

structure of a joint such as the tarsus may provide vital information of functional mechanisms of the tarsal joint. In addition to this, functions of the tarsus can only be best explained when the underlying anatomy is well understood. This chapter reviews the significant findings of the current studies and correlates them to the available equine tarsal literature as well as discussing possible future studies.

11.2 DEVELOPMENT OF METHODOLOGY AND VALIDATION OF TARSAL CONFORMATION

Radiology is readily available and used as it is often more cost effective than any other available imaging techniques. Radiography is also routinely used in field equine practice for quick diagnosis and other conformational assessments. The tarsal conformation (morphology and alignment) could therefore be measured on radiographs if consistent reliable landmarks for tarsal measurable parameters were identified and developed. Thus, many studies in this thesis utilized radiography as the primary non-invasive imaging method. This was because bone structural outlines (morphology) and alignment are usually adequately captured on radiographs (Garcia-Elias et al., 1989; Gupta, Batra, Jain, & Sharma, 2002; Larsen, Mathiesen, & Lindequist, 1991; Oheida et al., 2016) and because detailed morphometric data of the equine tarsal bones have not been reported in the current anatomical literature. The first study specified the characteristics of the constitutive tarsal bones as bony components and identified certain landmarks that were used for identification of the internal alignment of tarsal bones on acquired radiographic images of the talus joint in later radiographic assessment of tarsal conformation.

11.2.1 Radiographic anatomy of the equine tarsal joint

Before the study reported by (author) Akbar et al. 2019, all available previous studies of equine tarsal radiographs (Caron, 1988; Clegg, 2003; Dahn & Ueltschi, 1989; Shelley & Dyson, 1984; Shively & Smallwood, 1980; Tyson et al., 2004; Vanderperren, Raes, Van Bree, et al., 2009) studied the tarsal conformation and very few tarsal parameters were reported. None of these previous authors investigated the effects of having a reference line, point or any form of standardization that would ensure some uniformity of the

radiographs being measured or identify accurately any variations due to changes in alignments between objects and x-ray source on the measured radiographs unlike the paper by the current author and colleagues (Akbar et al., 2019).

11.2.2 Lateromedial view at zero angle (ZLM):

The hindlimbs (cadavers) used to develop the radiographic parameters were from different breeds including Thoroughbreds, Arabians, Warmblood horses and Quarter horses. The current study defined the ZLM view in lateromedial tarsal radiographs for morphometrical purposes. This view was based mainly on a vertical landmark for evaluating the vertical rotation and a horizontal landmark for the horizontal rotation. The rotation around the sagittal axis of the tarsus was neglected as it only changed the position of an object on the radiograph and not the measurements (Y. J. Yoon et al., 2002). The selection of the specific profiles of the ZLM for both horizontal and vertical rotations was as close as possible to the common lateromedial views which were described in the literature and those available from clinical archives. This meant that the ZLM would be easily achievable for clinicians as long as they followed the common techniques of tarsus radiography.

Despite the final methodology for radiographing the ZLM which was described in Section 3.2.1 of Chapter 3, a number of different attempts were performed to achieve the view. These trials were aimed to identify easily palpated bony landmarks on the tarsal region such as the medial and lateral malleolus of the distal tibia. The strict use of such landmarks as guides to make the best alignment between cassette, tarsus and radiography machine was thought to be sufficient to get the ZLM. However, after a number of attempts, it was realized that the morphological variations between tarsi of different horses were also

important to allow the identification of specific gross features to be a sufficient guide to achieve identical radiographs. Such a result helped to clarify the difficulties in dealing with tarsal morphology. Hence, it is suggested that identifying a zero degree for any radiographic view that is planned to be used for morphometrical purposes should rely mainly on small landmarks on the radiographs, while the gross landmarks would assist in approaching the aimed zero view.

11.2.3 Dorsoplantar view at zero angle (ZDP):

The current study defined the ZDP view in dorsoplantar tarsal radiographs for morphometrical purposes. This view was also based mainly on a vertical landmark for evaluating the vertical rotation and a horizontal landmark for the horizontal rotation. The rotation around the sagittal axis of the tarsus was neglected for the reasons given above. The selection of the specific profiles of the ZDP for both horizontal and vertical rotations was as close as possible to the common dorsoplantar views which were described in the literature and those available from clinical archives. This meant that the ZDP would be achievable for clinicians as long as they followed the common techniques of tarsus radiography.

Despite the final methodology for radiographing the ZDP which was described in Section 4.2.1, a number of different attempts were performed to achieve the view. These trials were aimed to identify easily palpated bony landmarks on the tarsal region such as the medial and lateral malleolus of the distal tibia. The strict use of such landmarks as guides to make the best alignment between cassette, tarsus and radiography machine was

thought to be sufficient to get the ZDP. Hence, it is suggested that identifying a zero degree DP for any radiographs that is planned to be used for morphometrical purposes should rely mainly on small landmarks on the radiographs, while the gross landmarks would assist in approaching the correct alignment for the zero view.

11.2.4 Reference line:

The reference line (RL) was developed to provide a base to measure the angulation of features of interest in the tarsal region. The angles of these tarsal features were measured in relation to the RL and each angle was given a separate name. The medial and lateral landmarks of the RL (which were assessed using Thoroughbred specimens only) were chosen as being easy to detect on radiographs, and less susceptible to pathology. These types of features were considered in some previous conformational studies (Barr, 1994; Caron, 1988; Major et al., 1996; Oheida et al., 2016). This reference line would have morphometrical and biomechanical advantages in understanding the possible effects of the angulation of tarsal features in relation to the different postures of the limb.

11.3 STRENGTHS OF THE STUDY: TARSAL MEASURABLE PARAMETERS

11.3.1 Parameters on ZLM and ZDP views:

For the routine radiographic evaluation of the tarsus, different projections can be considered to be evaluated but the lateromedial (LM), and dorsoplantar (DP), are the most commonly taken views among others including the dorso-lateral plantaro-medial oblique (DL-PIMO), plantaro-lateral dorso-medial oblique (PIL-DMO) as well as two additional views (J. A. Butler et al., 2008b; Per Eksell et al., 1999; Laverty et al., 1991; Shively &

Smallwood, 1980) .

The current study established 37 and 49 measurable tarsal morphometric parameters on ZLM and ZDP views of the tarsus respectively. They were aimed to represent as much as possible of the tarsal morphology especially, the features which were frequently involved in subsequent pathology reported in the literature.

In order, to remove potential effects of the variable size of horses, the measurements were designed in the form of angles and ratios. The ZLM parameters included 13 individual angles, 11 correlated angles and 13 ratios. Similarly, the next study established 49 measurable tarsal morphometric parameters on the ZDP view of the tarsus. The parameters included 11 individual angles, 19 correlated angles and 19 ratios. All the landmarks of the developed parameters were identified and specified by the author.

All the anatomical features in the current study were located between the distal tibial malleolus and articular surface with the proximal articular surfaces of the metatarsal bones. This region was intentionally selected to focus the study on the tarsal internal morphology, excluding any influence of surrounding bones and enable the use of archival radiographs within acceptable range of rotations that lacked most of the tibia and third metatarsal shafts in future studies. Hence the parameters of such landmarks would allow the accurate evaluation of the tarsal morphology in more detail than previously described morphometry such as the tarsal angle (Biewener, Thomason, & Lanyon, 1988; Caron, 1988; Gnagey et al., 2006) which might represent the relationships of the longitudinal axes of the tibia and third metatarsal rather than the morphology of the tarsus itself.

Measurements of the length and width of calcaneus and talus, and the dorsal heights of the central and third tarsal bones on the LM view have been reported by (Dahn & Ueltschi, 1989) but that study lacked information about the bony land marks that were used to

ensure consistency of similar radiographs without any rotations, or the potential effects of other rotations during radiography nor did they address the reliability of the developed parameters.

11.3.2 Intra-rater repeatability:

The intra-rater repeatability of the parameters in the current study was tested. Based on ICC and Lin's concordance correlation coefficient (CCC), the angular parameters generally showed a higher accuracy than the ratio parameters. The individual parameters had the most consistent measurements among all the parameters. However, as the values of the ratios were much smaller than the angular parameters, this means that the range of values in each ratio was also smaller compared to the angular parameters. The CCC could be influenced by the range of values, hence it could be assumed that the small range of values in the ratios affected their correlation coefficient (Lin & Chinchilli, 1997). The same reason might explain the results when testing the reliability of measurements of the parameters using digital radiographs where the angular parameters generally showed higher agreement than the ratio parameters.

Despite the general findings of the angular parameters, Bland Altman plots were best to visualize the level of agreements between repeat measurements, as some angles showed poor accuracy and/or wide limits of agreement. Based on the results of these parameters, the parameters with easily identifiable or clear landmarks had a higher repeatability. In respect to the ratio parameters, the lowest repeatability and/or reliability was found in WCalc/aHCalc ratio. The appropriateness for clinical use should be based to some extent on the reliability of parameters.

11.3.3 Effect of vertical and horizontal rotations of radiography machine on the developed parameters:

The rotations of x-ray projection angles, any misalignment such as postural changes of the subject (tarsus of hind limb) or radiographic plates can commonly happen during equine field or clinical radiography. These resultant rotations during acquisition of radiographs can subsequently affect the tarsal measurements from these acquired images and may also affect the quality of radiographs.

If the X-ray machine was rotated either inward (-ve) or outward (+ve) along its vertical axis during radiography and either upward (+ve) and downward (-ve) along its horizontal axis it would result in misalignment of the x-ray beam with the tarsus (hind limb). This type of rotation of the x-ray machine that affected beam projection in this way was referred to as vertical and horizontal rotations of the projection angle of the x-ray beam. The effect of such rotations was the focus of the study in chapter 5. Most of tarsal parameters developed in preceding chapters, were affected by vertical and horizontal rotations of the projection angle of the x-ray beam.

The majority of parameters in the current study showed variable responses to vertical rotations of the x-ray beam depending upon the location of the landmarks. The lower horizontal rotation resulted in bigger changes in all the rotational angles than in the upper horizontal rotation. The quality of radiographs was not diminished as the projection angle of the x-ray beam vertically rotated away from the ZLM and ZDP view.

In a similar study by Oheida et al., (2017), it was demonstrated that vertical rotation of the limbs from the ZDP position produced the most significant changes in the measured parameters of the equine carpus. The vertical rotation was referred to as the rotation of the limbs either inward (-ve) or outward (+ve) along their vertical axes and serially

radiographed. These were attributed to the landmark sensitivities for the carpal parameters, which were observed to easily distort with vertical rotations of the limb (Yoon et al., 2002; Oheida et al., 2016).

Each pair of landmarks was connected in lateromedial orientation to represent the horizontal anatomical postures of bone surfaces or their articular surfaces for the developed tarsal parameters identified on the acquired digital radiographs in the current study (Akbar et al., 2019). The relationships between the bony landmarks were found to be changed during the rotation of the subject which consequently affected their representing parameters (Y.-J. Yoon et al., 2002). In addition to this, in cephalometric studies, rotation around the vertical axis has been reported to affect the horizontal measurements while rotation around the horizontal axis affected the vertical measurements (Malkoc et al., 2005). In the current study however, both vertical and horizontal rotations of the radiography machine resulted in a significant effect on the majority of the angles. The results did not indicate a clear relation between the value of tarsal angles and the amount of change. Therefore, it seems necessary to consider other possible factors which might cause this disagreement.

Finally, one of the important aims of this thesis was to identify an acceptable range of radiography machine rotation at which the developed tarsal parameters experienced minimum distortion or change. According to current analysis, the rotational angle of 5° resulted in less than 1° of change in the majority of tarsal parameters in each rotation of radiography machine in the vertical and horizontal direction. These findings were in agreement with a previous study on the equine carpus Oheida et al. 2017 and a cephalometric study by Ahlqvist et al. (1988) who indicated that an increase in the rotation resulted in an increased distortion of the angles (Ahlqvist et al., 1988; Oheida et al., 2017b).

Therefore, it can be suggested that the rotations of 5° range simultaneously from ZLM and ZDP in either of the rotations in both the vertical and horizontal axes or 10° in one axis while 0° in the other axis would be acceptable but require more caution when measuring the least reliable of the tarsal parameters that were most affected and changed by these vertical and horizontal rotations.

11.3.4 Inter-rater repeatability:

After developing and addressing the effects of rotations, a further aim was to check reliability and repeatability of some selected parameters (based on their landmark's repeatability in previous studies) through different observers (author and two independent observers). No significant differences between 3 raters' measurements were observed in selected parameters. Although narrow limits of agreement were found between rater 1 and 3 (Lin's CCC 0.9) in HT/aHCalc and HT/PHCalc ratio measurements (−0.02 to 0.02 limits of agreement for Bland Altman plots respectively) suggested that landmarks were easier to identify. However, some parameters showed wider limits of agreement such as in the Proximo-Caudal Angle of Calcaneus between rater 2 and 3 with a Lin's CCC of 0.26 and a range of −2.47 to 5.57.

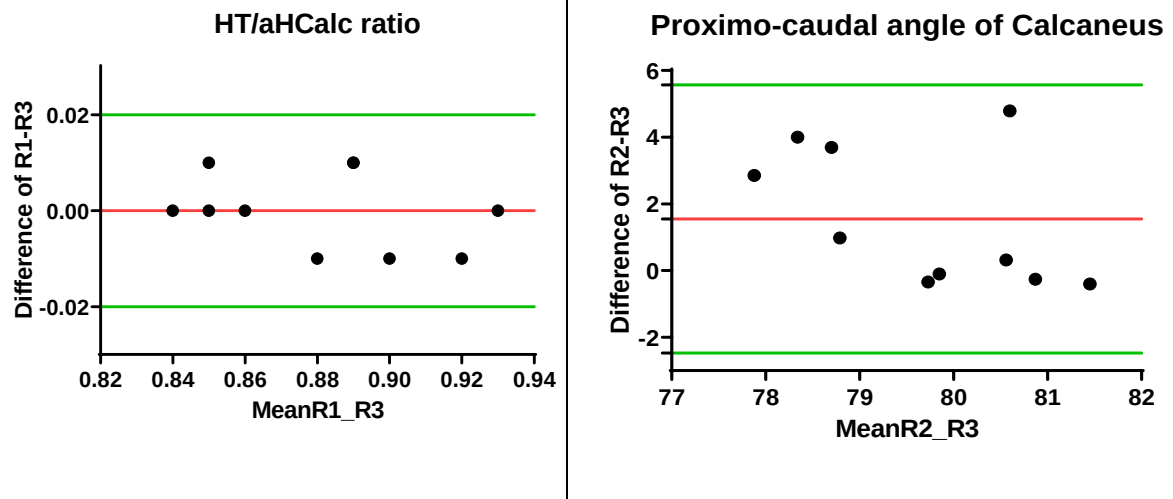


Figure 11.1: Mean Bland Altman plots of HT/aHCalc and Proximo-Caudal Angle of Calcaneus parameters. Green lines in the graph indicate limits of agreement. Abbreviations; R1. 1st rater. R2, 2nd rater. R3, 3rd rater.

All selected parameters and methodology were reproducible and repeatable by different observers. The experience and practice or training of independent observers has also great significance. Taking repeated measurements from the same radiographs after an interval either by a single observer after a defined period (intra-rater repeatability) or by 2 or more observers (inter-rater repeatability) is a proven test of the validity of a measuring instrument and reliability of the values of measurements obtained or calculated (Bland & Altman, 2007; Oheida et al., 2016; Petrie & Watson, 2013). Thus, establishing validated and repeatable landmarks for the Zero Lateromedial (ZLM) and Zero Dorsopalmar (ZDP) parameters ensured that all measurements were taken from a standardized position or reference point for subsequent tarsal evaluation.

11.3.5 RELATIONSHIPS BETWEEN TARSAL BONE MORPHOLOGY, LIGAMENT MORPHOMETRY, JOINT ALIGNMENT, LOADING BIOMECHANICS AND ROLE OF THE LIGAMENTS AND FASCIA IN LIMB COORDINATION AND JOINT STABILITY AND MAINTENANCE OF NORMAL RANGE OF MOTION.

Tarsal bones attach to one another by tarsal ligaments and are the fundamental parts of the tarsal joint (Getty 1975). The shapes of the equine tarsal bones and the biomechanical properties of the interconnecting major and small intertarsal ligaments between tarsal bones largely determine the alignment of the tarsus at rest or during locomotion or loading of the joint.

Wedge angles were considered important in previous studies on the equine carpal bones because of previous reports arising from clinical observations and anecdotal beliefs that these were conceived to measure the topographical anatomy of the proximal and distal articular surfaces (Rooney 1969; Bramlage et al., 1988; Deane and Davies 1995a). The angles of the articular surfaces of each carpal bone also facilitate sliding (“wedgeness”) of the carpal bones into a “locked up position” with adjacent bones during loading conditions. The congruity of carpal bones is also believed to prevent carpal damage as it results in transfer of sudden stress from bone into the elastic intercarpal ligaments (Deane & Davies, 1995a; Olusa, Murray, & Davies, 2019). Such findings highlight the importance of bone shapes and their interconnecting ligaments, although tarsus has different anatomy and less motion of bones during locomotion as compared to the carpus, but no such findings are available for the equine tarsus.

As any condition cannot be well managed until it can be measured quantitatively, thus the protocols developed here for accurate measurements would enable an objective assessment of conformation of the equine tarsus. In addition to this, 14 tarsal parameters measured on both DP and LM radiographic views were selected based on their locations

around the tarsus and introduced for measuring loading alignment and stability in chapter 7.

Increased load on the tarsal joint produced measurable radiographic changes in the alignment and direction of movement of the tarsal bones. Change of internal alignment of the tarsal bones in response to loading could be observed through tarsal conformational parameters. For instance, a progressive decrease in Calcaneoquartal- Distal Talocalcaneal Joint Angle (CalcQ-DTaloCalc.) indicated increasing tarsal loading.

The joints are believed to attain maximum surface contact of the articular surfaces only in the close packed position (MacConaill, 1946). This pilot study was clearly in agreement with the idea that the geometrical properties of the articular surfaces of bones play a vital role in determination of the stability of their joints (Sledge, 1993) as changes in parameter's values were observed when measuring the articular surfaces of tarsal bones like Calcaneoquartal- Distal Talocalcaneal joint angle and TMT-Tt Joint angle. Similarly, six parameters were found to be very helpful in assessment of changes in alignment of the tarsus under loading. It is reasonable to suggest that any changes in these angles from their established “normal” values or within their normal range, may impact on the loading intensity and integrity of the proximal metatarsal bones and could be related to soreness or fracture of these splint bones.

The parameters (angles) such as Tarsometatarsal- proximal intertarsal joint, Tarsometatarsal-proximal central joint, Distal intertarsal-tarsometatarsal joint and Tarsometatarsal- tibial metaphyseal/malleolar joint angles might be useful to indicate the likelihood of certain pathologies as the locations of their landmarks are commonly affected areas of the tarsus. These parameters may assist in the choice of good performance horses, but this needs further detailed investigations.

The muscles of the body are responsible for the movements of different parts due to their ability to contract and relax. These muscular tissues are controlled by the central nervous system. Skeletal muscles can be divided or classified in different categories according to the functions they perform or based on the orientation of the line of pull to the joint structures such as extensors, flexors, abductors, rotators and adductors (Clair 1975; Nickel et al., 1986). The tendons are the connective tissue structures that connects muscles to bones, while ligaments are the connective tissues which join bones to each other (Clair 1975; Sisson, 1975).

Although some brief anatomical description of the major collateral tarsal ligaments have been reported in equines (Barker et al., 2013; Ross, Genovese, & Reef, 2002; Updike, 1984), individual tarsal ligaments have not been considered to be of significant importance in the veterinary literature, other than to provide general elasticity and stability (Sisson 1975; Dyce et al., 2002). The use of radiographic bone morphology along with the morphometry of tarsal ligaments to investigate the functional stability during the loading of the equine tarsal joint has not been available in the current literature.

In this study, severing all major tarsal ligaments caused significant changes in tarsal flexion ($P < 0.001$). Thus, they play a part in normal joint range of motion. A tough connective tissue structure with three distinct fiber bundles in a convex shape links the talus and calcaneus in a transverse orientation and was identified and named the talus and calcaneus tarsal bone intertarsal ligament (Talus-CalC ITL). Another Calcaneus Quartal intertarsal ligament (CalC-Q ITL) in a longitudinal direction provided linkage in between the calcaneus (proximal row) and fourth tarsal of the distal row of tarsals, suggesting a similar orientation and providing additional support to the long plantar ligaments at the plantar aspect of tarsus to provide extra stability and prevent displacement and transfer of loads

as exerted by heavy proximal musculature through the calcaneal tendon.

Through the fibers arrangement of this intertarsal ligament, the joint between the talus and calcaneus is able to undergo a slight proximo-distal or dorsoplantar sliding movement between these two tarsal bones during flexion and extension which keeps calcaneus in alignment with talus and the distal row from the plantar aspect during loading through the distal end of tibia to the trochlea of talus. These findings suggest that these ligaments can play a vital role in slight gliding movements of tarsal joint and these movements agree with previous observations such as Rooney (1969) who suggested that the tarsal bones rotate to the lateral side during the first one-half of stride and in a medial direction during the second half of the stride. In addition, lack of caudal soft tissue structures during the loading of the limb (in the loading apparatus) should also be considered as these caudal soft tissue structures might compress the calcaneus against the talus and can contribute to stability of the tarsus while loading.

The joints are said to be in complete congruity only in the close-packed position (when maximum surface contact of the two articular surfaces occurs) (MacConaill, 1946). In determination of the mechanism of fracture production, it must be recognized that the joint rotates during both flexion and extension. Any asynchronous movements of the tarsal bones that may be initiated by damage to the intertarsal ligaments must also be considered.

Finally, these findings, constitute a significant contribution to the current understanding of the biomechanics of the equine tarsal joint. These findings can also be helpful in understanding the pathogenesis of injuries related to certain tarsal ligaments or chip fractures within the tarsal joint.

The recent progression of research of anatomy and function of fascia has emphasized the

significance of considering the individual elements of the musculoskeletal system in terms of their body-wide fascial connectivity (Helene M Langevin, 2006; T. Myers, 2009; Schleip, Mechsner, Zorn, & Klingler, 2014; C. Stecco, Macchi, Porzionato, Duparc, & De Caro, 2011a). This conceptual thinking is in line with the conclusions drawn from chapters 9 and 10 prompted by the fascial investigations of the equine hindlimb. Overall, it was observed that all hind limb structures were interconnected to each other in some way through the surrounding network of fascia.

The most recent research by Wilke et al. 2019, demonstrated that multiple connections of fascia and stiffness (thickness) of the fascia can directly limit joint range of motion (ROM) as described in the diagram below regarding its architectural features (Figure 11.2).

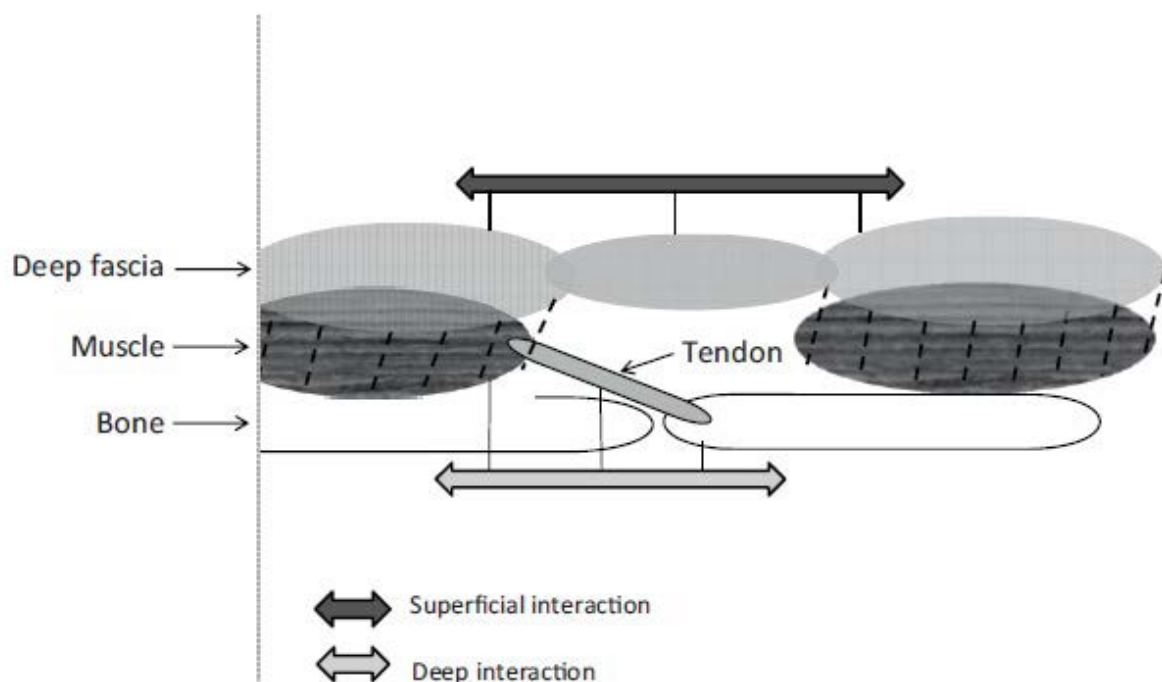


Figure 11.2: Schematic drawing adopted from Wilke et al. 2019 illustrating the mechanical interactions that might restrict range of motion (ROM) of a joint. The superficial interactions are based on fiber continuity of two myofasciae. The deep interactions are

based to the myotendinous connections between the two articulating bones, while additional interactions are expected to occur between fascia and muscles.

In general, it was found that the fascia of different regions of the hind limb was anatomically complex because of the different integrated layers of fascia and also their relationship with the bones and musculotendinous units (Wilke et al., 2019). The different layers of fascia not only provide mechanical connectivity between described structures of a particular region but also between the structures of a particular region. These regions comprise of the crus (proximally), metatarsal (distally) and the tarsus.

The stress-strain properties are dictated by the fibre orientation of any material. Strain applied to fascia was shown to stimulate the proliferation of fibroblasts and caused them to be orientated along the same stress lines as the direction of applied force (Gehlsen, Ganion, & Helfst, 1999). Hence, the macroscopic observations of the collagen fibres constituting the fascia of the hind limb and their orientation and arrangements can indicate the tension paths for force distribution and stretch when under loading.

Generally, the superficial fascia layer over the entire limb was observed to have an irregular arrangement of fibres. This could suggest a balance of strength and flexibility in multiple directions rather than a unidirectional strain and tension distribution as would be expected with the more regular arrangement of fibres comprising the deep fascia. In contrast, the more organized arrangement of fibres of deep fascia highlights a more defined directional tension distribution and allows the determination of direction that provides the most resistance to strain forces, and which allows the most elasticity.

The superficial and deep fascia provide mechanical connectivity with the skin and underlying tissue through fibrous connective tissue attachments not only within a region but also in different regions of the hind limb. These findings are in line with previous findings (Elbrønd & Schultz, 2015; K. L. Moore & Dalley, 1999; Skovdal et al., 2011).

In last, the present findings also provide the first evidence of thickness variation of fascia across the different regions of the equine hind limb highlighting the regional specializations: the crural region has the thickest fascia and the metatarsal region had more thickness of fascia in the distal limb region. Our results also demonstrate the normal histological composition of fascia in different regions of hind limb including the normal pattern of elastin and collagen fibre orientations for load transfer and their relative quantity or ratio for particular flexibility and level of strength around different regions suggesting that any changes in fascial flexibility in terms of internal composition can influence the joint range of motion.

Functionally, this might be significant in managing unstable loading forces that may occur as a result of uneven ground surfaces as it highlights the potential to adapt and redirect the forces along multi pathways. In this way, the superficial fascia layer with an irregular pattern has the potential to deal with any uneven forces which the deep fascia cannot optimally manage.

11.4 LIMITATIONS OF THE STUDY:

It is believed to be important in understanding the functional working of the tarsus to know how the joint loads and works in different stress conditions along with help of surrounding structures. Such understanding can be a great help in possibly modulating the training of equine athletes to be within a safe limit of loading and thus reduce the risks of tarsal damage. However, there were some limitations to this project which might need considerations for complete understanding of tarsal function.

The observation of a complex 3-dimensional structure like the tarsal joint and its motion and rendering such movements into 2-dimensional (horizontal and vertical) planes such as on the radiographic image tends to over-simplify the tarsal structures and limit useful information concerning the third transverse (z-axis) plane that could have been possible. Moreover, transferring the 3D tarsal configuration into 2D images would be another source of error. Such transference might induce some errors in measurements (Weller, Pfau, Babbage, et al., 2006).

The development of these tarsal parameters aimed to include all the features as well as places of origin and insertion of the intertarsal ligaments on LM and DP radiographs of tarsus. However, a number of features were difficult to measure consistently, such as the proximal and distal articular surfaces of the central and the medial and the lateral tarsal intertarsal ligaments. In addition, the morphometrical details of the first and second tarsal bones which are usually fused in the horse, forming a bone of a very irregular shape (Getty, 1975a) were beyond the scope of the current research.

Another limitation of this study was the small sample size. In larger populations more differences might exist due to differences in breed, or through individual variation, age, sex and type of performance. The loading of tarsal joint without the caudal soft tissues in

the loading apparatus was also difficult because of angle of hock and lack of loading of calcaneus because of tendons removal. Establishing the reference line was a useful tool in measuring the parameters in the study. However, this character might vary in other equine breeds or individuals. Hence, the use of this reference and loading strategies should be further investigated.

11.5 CONCLUSION AND FUTURE DIRECTIONS:

This research developed reliable measurable tarsal parameters using LM and DP radiographs, allowing adaptability of the measurement protocol to live horses in field radiography or archival radiographs (acquired images of tarsus within acceptable vertical or horizontal based on the landmarks and have adequate areas captured in LM and DP radiographs). Thus, providing a way to develop a baseline database for future reference. Such measurement protocols allowed the identification of any particular tarsal conformation for early diagnosis or other purposes for investigations into the equine tarsus using a radiographic technique.

In the routine examination of yearling Thoroughbreds such conformational studies would allow early diagnosis of any conformational deviations of the tarsus and potentially save money and misplaced effort in the racing industry as well as protecting the horses from injury. Prospective studies are required to confirm that the measurement of these parameters in radiographs of yearling Thoroughbreds are commercially viable. Predictions about the future racing career of the horses would then be made based on the radiographic measurements and the horses' career followed to examine the reliability of these predictions.

Although explaining the mechanism of alteration in the landmarks during vertical and horizontal rotations was not possible for some parameters, information described in this study and in combination with other studies (Auer, 1991; Bramlage & Auer, 2006; Caron, 1988; Dahn & Ueltschi, 1989; Ghafari et al., 1995; Major et al., 1996; Malkoc et al., 2005; Marks, 2000; Vanderperren, Raes, Van Bree, et al., 2009; Witte & Hunt, 2009; Yoon et al., 2001; Y. J. Yoon et al., 2002) should be considered in future studies for the development of any radiographic measurements.

Significant findings were found in mechanical connections of fascia and its role in proprioception around the tarsus. Fascial exploration such as pattern of fascial layers and myofascial connections provided significant understanding of the areas around the tarsus requiring higher mechanical strengths and regions where fibers connected with tendon fibers as an extra support during movement of the joint. In addition to this, this investigation of the connectivity fascia demonstrated a complete integration of structures of hind limb which urges caution in functionally isolating muscles and joints of the limb. It further suggests that investigation of the fascial connectivity beyond the crural region to other body parts would be worthwhile for better understanding of the postural patterns and the development of locomotory disabilities in the horses.

In parallel line, the mapping of fascial trains in human body has proven quite useful for visualizing the locomotory patterns (Myers, 2009) and some equine fascial lines have been described in terms of spinal flexion or extension, lateral flexion and rotations which all can be influenced by fascial contraction and can be very helpful in describing both static and dynamic postures in the equine (Elbrønd & Schultz, 2015). Thus, consideration of the various locomotory disabilities observed in horses, the mapping of the equine fascial trains may also prove to be equally beneficial. In addition to this, investigation into the

proprioceptive mechanisms contributing to joint control in the hind limb may further contribute to the current understanding of the hind limb biomechanics. This is an area that was only briefly investigated throughout this thesis but has great potential for future research and the understanding of joint mechanics.

Finally, the stability and efficiency of the equine tarsal joint with other joints in hind limb largely depends on the mechanical connectivity present between different structures and also the passive force distribution, but this needs further research to see the change in pattern of force distribution with injury or other types of movement restrictions. Such findings may provide further help in the understanding of development of particular pathologies and may facilitate in its control or help in direct treatment. The pilot study on the 3-dimensional model of the tarsus (Appendix A) was a first step to address this limitation in future studies. The tarsal conformational data generated from these studies will be useful to create better and validated 3D models of the tarsal joint. This will improve our understanding of tarsal biomechanics and pathogenesis of injury.

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differences in extra-muscular myofascial force transmission. *Medical Engineering & Physics*, 28(3), 214-226.

APPENDICES:

13.1 Development and analysis of three-dimensional representations with computer model of complex equine tarsus

INTRODUCTION

The tarsus is a complex joint that can be represented in two-dimensional scans but a complex 3D structure that is geometrically positioned at about the middle of the equine hind limb, whose mechanical behavior is difficult to analyze. To obtain such information, experimentation can be a direct mean. However, it is often unwieldy and can be costly. On the other hand, using a model, permits one to simulate the tarsus behavior in different situations like sound, pathological, and instrumented. The use of model can also be helpful to obtain some information that is not directly accessible through experimentation such as the stress distribution inside the tarsal segment.

Tarsal bones are group of seven small bones and 3 long bones which locate in the middle of hind limb of horse. Bone can be defined in biological terms as connective tissue. It is classified as a tissue, because, a tissue is nothing but an aggregation of similarly specialized cells, which are united to perform a particular function. It is known as a connective tissue, since it binds together and is the support to various parts of the body. Bone material properties are the tissue level mechanical properties that describe the constituent material and are independent of the size and shape of the bone. Material properties include the tissue

ultimate stress and modulus of elasticity. These tissue properties are determined by machining precise samples from the bone of interest and testing them in a particular loading mode.

The material properties are influenced by compositional measures such as mineral density, collagen content, and ash fraction. In addition to composition, factors such as collagen fibre orientation, mineral crystal size, and the microstructural organization (e.g., lamellae, osteons) also influence material behaviour. From a mechanical perspective, the composition and organization of the material clearly influence the tissue's ability to bear loads, but most measures, except for mineral density, have not yet been related directly to the tissue properties derived from mechanical tests (Van der Meulen, Jepsen, & Mikić, 2001).

In the horse, the third metatarsal or 'cannon' bone bears all of the load transmitted from the phalanges to the tarsus. The second and fourth metatarsals are substantially reduced, like their corresponding numbers in the metacarpus, coming to lie along the proximo-plantar aspect of either side of the third metatarsal. The tibia is the sole weight-bearing bone between the hock and stifle joints as the fibula being greatly reduced.

The orientations of the tibia and metatarsus differ with respect to the ground reaction force. This results in a difference in the distribution of bending versus axial loads/forces borne by these two bones. In contrast, the carpus is almost straight during load bearing. The corresponding fore and hind limb bones have similar curvatures as the tibia is curved slightly along its diaphysis (concave caudally) analogous to the radius, whereas the metatarsus is straight (analogous to the metacarpus), while differing in conformation (Biewener et al., 1988).

Different methods and procedures have been employed by different researchers in modelling and analysis of such bones. In one of the studies, a new procedure for the automatic generation of finite element meshes of bone segments from computed tomography (CT) data sets was described. This new method allows a direct automatic generation from the CT data and produces a very accurate unstructured hexahedral mesh. The accuracy of the method was established using the CT images of an artificial femur showing range of attenuation values comparable to those of a human femur. While some authors still prefer to create a CAD model of the bone segment from the CT data and then to build a mesh from it, direct generation methods such as the so-called 'voxel meshes' have become quite popular in the last few years. This method directly converts the regular voxel lattice of the CT data set into a sub-sampled regular lattice of eight-nodes to iso-parametric elements (brick elements). The resulting mesh is commonly referred to in non-medical literature by the term 'Cartesian mesh' (Kumbhalkar, Nawghare, Ghode, Deshmukh, & Armarkar, 2013). In this experiment, a computer based three-dimensional (3D) representation/computer modelling of tarsus was developed through the use of laser scanning and CT, using Finite Element Analyses.

MATERIALS AND METHODS

Dissection of the equine tarsal joint

The cadaveric right and left hind limb were collected from one adult thoroughbred horse collected from the Department of Pathology, FVAS, The University of Melbourne, Werribee campus were used in this study. Information regarding age, sex, breed was recorded. The horse was euthanized or died for other reasons not related to this study. The horse selected for this study was not associated with the tarsus injuries or/and locomotor disorders of hind limb. The hind limb was disarticulated at the distal one-third of the tibial bone. A careful dissection of bones was performed for understanding the attachments sites of different ligaments and general anatomic morphology. After the dissection the bones were obtained by boiling and cleaning without damaging the bone geometry for their scanning.

3D Laser Scanning

3D Laser Scanning is a non-contact, non-destructive technology that digitally captures the shape of physical objects using a line of laser light. 3D laser scanners create “point clouds” of data from the surface of an object. In other words, 3D laser scanning is a way to capture a physical object’s exact size and shape into the computer world as a digital 3-dimensional representation. In this project, Artec space spider 3D scanner was employed for completing the scanning tasks.

Artec Space Spider is a high-resolution 3D scanner based on blue light technology. It is perfect for capturing small objects or intricate details of large industrial objects in high resolution, with steadfast accuracy and brilliant colour. The software supporting the Artec spider is Artec studio 10. It is the latest software for advanced 3D scanning and data

processing. Some of the features of the Artec studio 10 are

- Powerful algorithms to maintain total control of tracking.
- Automatic scan alignment correction during scanning.
- Texture colour mapping.
- Boosted auto-alignment and non-rigid global alignment capabilities
- More texture editing tools

Scanning procedure

1: Scanning with geometry and texture tracker.

The Artec 3D scanner operation is based on the structured light 3D reconstruction method. This optical method allows digitizing a fairly large area of the object surface in a single frame. The scanner uses an incoherent light source to this end. It allows registration algorithm to track and align scans using both texture and geometrical features of the object that is being scanned and imported into Artec Studio software.

(version 0.7.3.39; Artec Group, Luxembourg, Luxembourg)

2: Selecting and preparing the objects for scanning.

The Artec 3D scanner operation is based on the structured light 3D reconstruction method. This optical method allows digitizing a fairly large area of the object surface in a single frame. The scanner uses an incoherent light source to this end. Since the method of capturing 3D frames is optical, there are certain difficulties in getting accurate results. Some objects are difficult to scan. Some of the examples of the objects are objects made of transparent

materials very dark objects and objects with a dark surface, shiny or reflective objects, objects with very fine details.



Figure 13.1: Scanning of right calcaneal bone of thoroughbred horse using ARTEC 3D scanner.

3: Revising and editing scans

Sequence of frames obtained at each scanning session is saved into a separate scan. The list of all the scans in a given project is displayed in Workspace of the application.

4: Scan alignment

Each scan is captured with a different orientation in 3D space. The align tool allows you to pick common points from each scan to put the scanned pieces together. This is usually done

before performing global registration. Immediately after scanning, the ARTEC Studio application may not contain enough information about the relative position of multiple scans. To combine all scans into a single model, the data must be converted into a single coordinate system, i.e. registration must be performed. The first stage of performing this task is the semi-automatic alignment of scans using the Align tool.

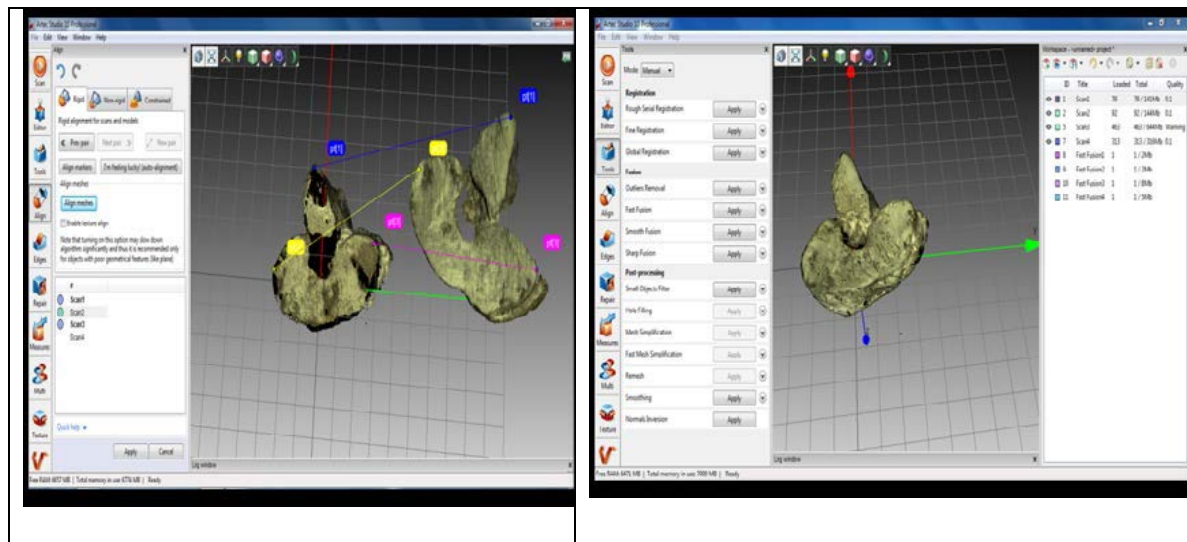


Figure 13.2: Alignment of scans of third tarsal bone in ARTEC 3D software

5: Global Registration

The next process after the alignment is global registration. The global registration algorithm converts all one-frame surfaces into a single coordinate system using information on the mutual position of each pair of surfaces. To do it, a set of special geometry points is selected on each of the frames followed by a search for pair matches between the points on different frames. For the algorithm to perform correctly, initial approximation is required which is performed in the process of using the Align tool.

6: Outliers removal

Outliers are small surfaces not connected to the main surfaces. These small surfaces are subject to removal, because they may spoil the model or produce unwanted fragments. There are two ways to do it erase them before fusion (preventive approach) and after fusion (so called furthering approach).

7: Fusion

Fusion is a process of creation a polygonal 3D model. It can be described as melting and solidifying the frames obtained and processed. This is the most interesting part of the processing because polygonal 3D model is what exactly people wait from 3D scanning

Scanning process procedure

8: Model Editing

The resulting fusion model may contain surface defects due to scanning or registration errors. A number of tools exist in the Artec Studio application for correcting such errors

The Repair tool is used for correcting the model's triangulation errors.

- The Small Objects Filter is used for filtering and deleting small objects near the surface of the model.
- The Edges tool is used for filling holes and smoothing out the model's edges semi automatically.
- The Smooth brush tool is used for smoothing surface areas with the most noise manually.
- The Mesh Simplification algorithm is used to reduce the number of polygons within a model while minimizing the loss of accuracy.
- The Remesh algorithm is used to create isotropic mesh while keeping the processed

mesh as close to the original as possible.

- Scanning process procedure summary

Conversion of 3D scans to solid body and assembly in solid works

Reduce the mesh size in Mesh Mixer

Due to complex geometry and large number of mesh size, it is hard to convert these scans to solid models in solid works. So before importing the files in solid works, the files are imported in Mesh mixer software. Mesh mixer is a prototype design tool based on high resolution dynamic triangle meshes. Mesh mixer was used basically to reduce the number of mesh without compromising the geometry of 3D scans. The size of mesh was reduced by manipulating the three reduce features given in the software. They are as follows

- Triangle budget- This tool reduces the number of triangles on the scan surface.
- Reduce percentage- This tool reduces the overall count of mesh.
- Max deviation - Max deviation option allows us to neglect the error on the surface of scanned body.

Given below are the procedure/steps applied to reduce the mesh size of the scan in the mesh mixer tool.

Scan files to solid body

The main objective of this process is to convert the 3D scans to solid body in solid works. The exported scan mainly contains integration of mesh data. Scan mesh data is converted to

series of small patches and then extruded to form a solid model. The whole process is represented in flow chart below followed by schematic diagrams.

In solid works mesh prep wizard and surface wizard tools are used for converting 3D scans to solid models, mesh prep wizard mainly reduces the noise and also helps in reduction in number of target mesh. Surface wizard creates the boundaries to patches and from the patches on the surface of mesh models and then extrude the formed patched to solid body. Because of the complexity of the bone there are many intersections within the surface which need to be reduced to zero. These intersecting surfaces are not recognised by solid works and ignoring these surface errors will eventually lead to disappear that particular solid part which is associated with the intersecting lines. The boundary feature lines can be modified by relaxing, deleting, moving or creating new lines. Some of the examples of feature lines are shown in the figure below.

Solid works modelling of cartilages

Cartilages are smooth and elastic tissue, which is a rubber like padding and also works as a protection on bone ends. It also helps in smooth movement between two bones which are in contact with each other. Modelling of cartilages was done in solid works using deform option. The cartilage's thickness in horse tarsal bone is around 1.5mm- 2mm [12]. A thickness of 2mm was applied for modelling the cartilages. On all the major joints, cartilages were modelled.

Flow chart represents the process of cartilage modelling and the images depict the modelling of cartilages.

Assembly of tarsal bones

After the modelling of cartilages, the assembly of the bones and cartilages is done in solid works, all the solid parts were inserted individually in assembly module. Before the application of the mate relationship, it was assured that all the different parts of tarsal bones are at correct alignment and placed as close to each other. After aligning all the bones close to each other, mate relation was applied but due to complex geometry and uneven surfaces, a common reference plane was not generated. As a result, the standard mate options were not available to create the relationship between the surfaces of bones. The aligned bones were placed over each other ensuring that the surface boundaries did not intersect within each other. The images depict the assembly of tarsal bones in Solid works. After the completion of the assembly of bones, the file was converted into solid part document.

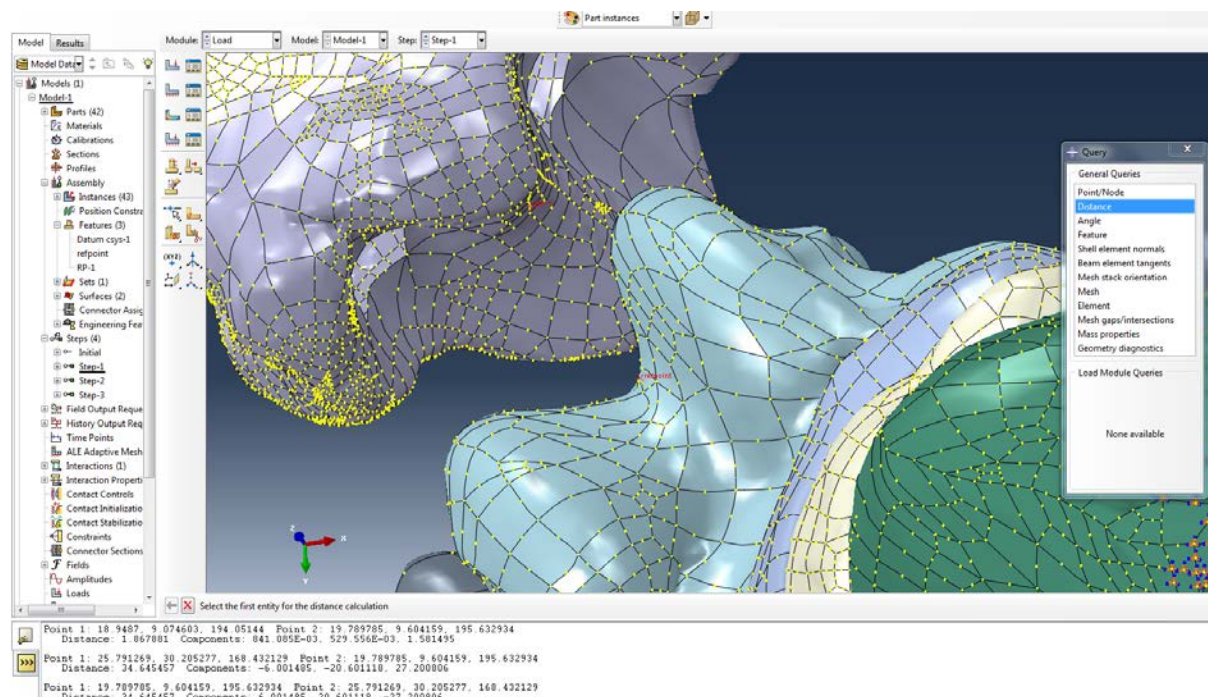


Figure 13.3: Assembly of distal articular surface of tibia and dorsal articular surfaces of talus ridges.

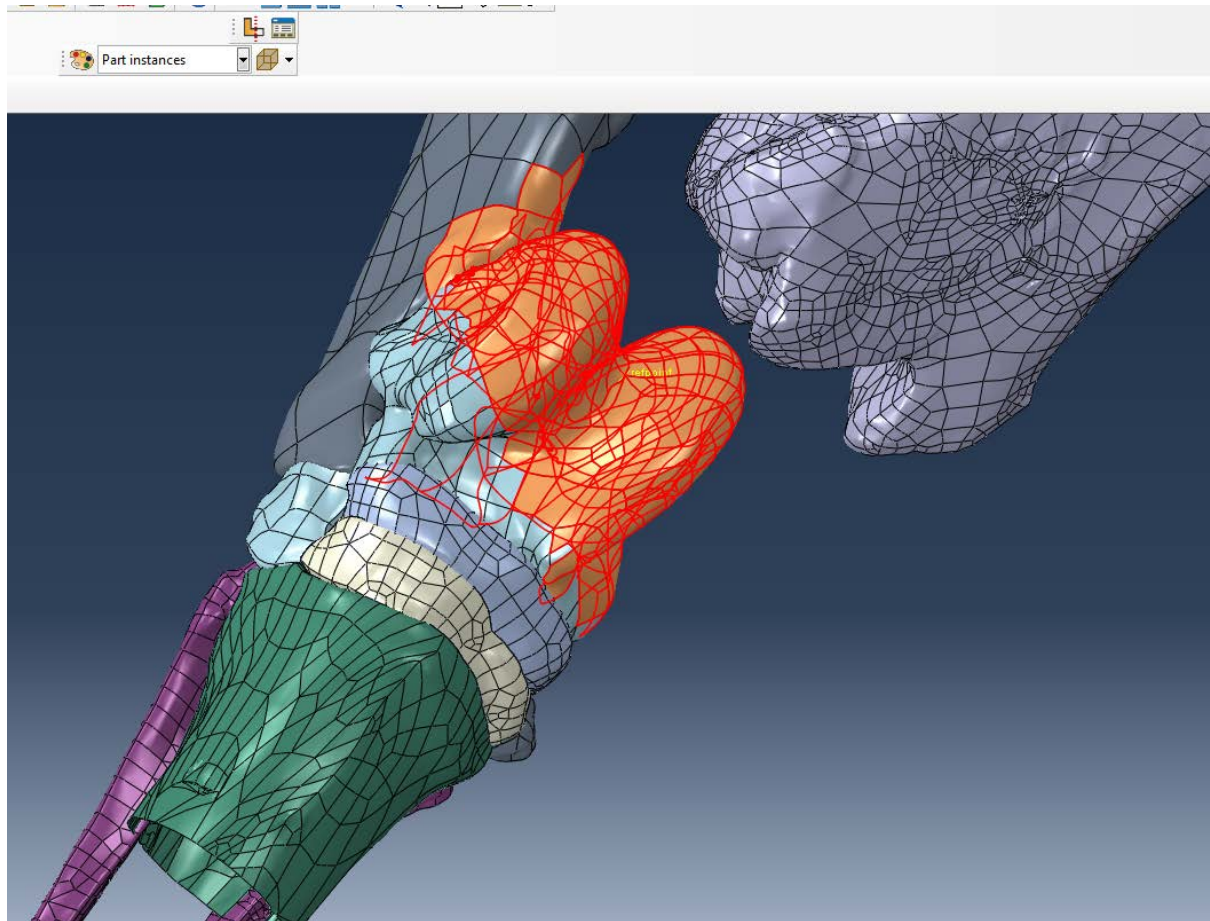


Figure 13.4: Assembly of distal articular surface of tibia with rest of assembled tarsal bones highlighted area indicate the contact areas on dorsal articular surfaces of talus ridges.

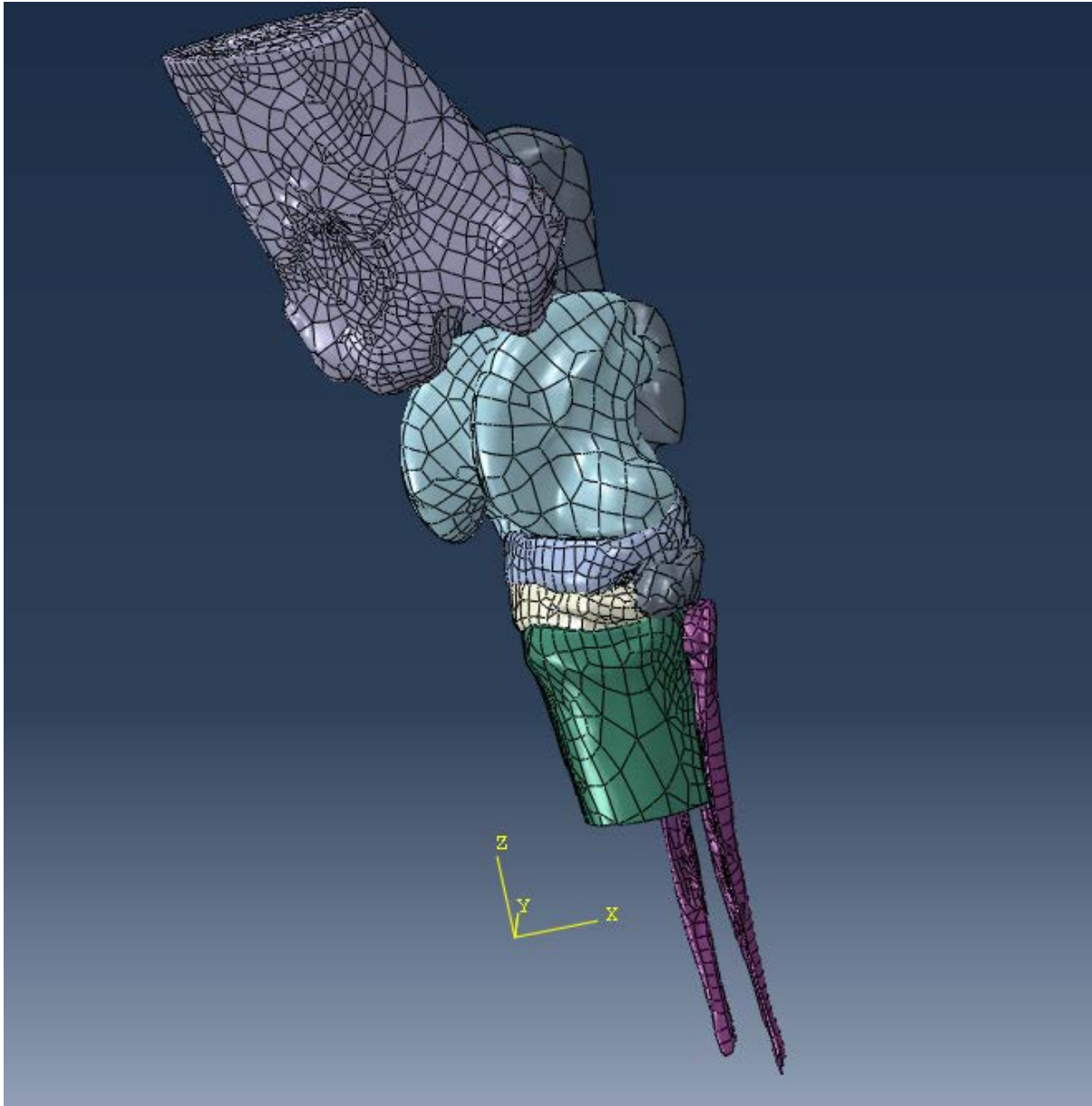


Figure 13.5: Assembly of distal articular surface of tibia with rest of assembled tarsal bones at dorsomedial angle.

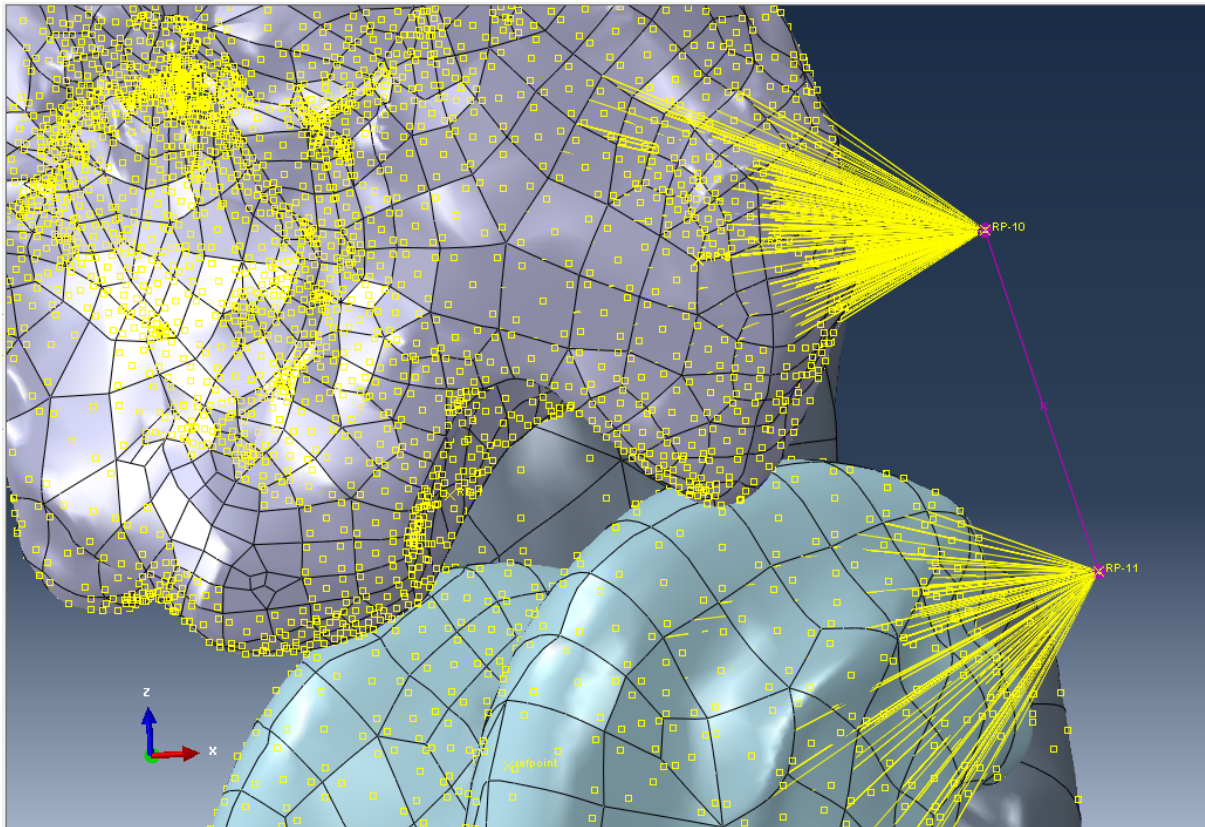


Figure 13.6: Assembly/replacement of medial collateral ligament attachment sites between distal end of tibia with talus bone.

RESULTS

Some of the key observations and findings on the methodology of this capstone project were:

- 1) The 3D scans gave around one million polygons for every single bone which had to be reduced for modelling in Solid works. Even after using mesh mixer the mesh size was still large which resulted in more time for modelling in Solid works.
- 2) The geometry of the tarsal bone was too complex for modelling in Solid works. The conversion of the 3D scans to solid models took around two months of time. The number of self-intersections was too many to reduce it to zero. The mesh size was reduced in Solid works and mesh mixer for getting a reliable model closer to bone structure. However, this compromised the mesh quality leading to poor geometry.
- 3) The assembly in Solidworks was also not effective in the modelling of the hock joint. The mate relations option could not be employed as Solidworks could not find any common reference planes.
- 4) The reduction in the number of meshes to almost half of the original mesh size resulted in the generation of holes leading to improper geometry.
- 5) The abaqus software is basically a solver-based software which is not compatible for meshing complex geometry.
- 6) The student version of abaqus is not suitable for the analysis of the complex bone geometry since complex geometry cannot converge results with less number of nodes.

DISCUSSION

The model presented in this study contained sufficient elements to create an adequate external geometry of the bones of the equine tarsus. Although the internal architecture of the tarsal bones such as the subchondral bone, trabecular and cortices were not considered in this pilot study, this model provides a useful first step in a many-step process towards development of a more detailed tarsal model that can be used for further studies such as investigation of axial and transitional force distribution patterns during various loading conditions of the tarsus. While the underlying motivation for this project which was to develop a computational tool that could ultimately be used to identify variables that could predispose to tarsal damage have not yet been realized, the specific goal of this study, which was to develop a geometrically accurate 3-D solid surface representation of the tarsal joint, was achieved. A major interest of any 3D model study is its finite element analysis (FEA) as it provides baseline discretization for the model (Henninger, Reese, Anderson, & Weiss, 2010). The use of quadratic tetrahedral elements in this study enhanced the accuracy of the model as formations for tetrahedral elements have been reported to be accurate for finite element deformation (Babuska & Oden, 2004; Weiss, Schauer, & Gardiner, 1996).

The gaps between the articular surfaces of the tarsal bones which are naturally filled with cartilage and synovium were replaced with a solid model and analysed linearly as a solid structure. Whether the replacement of cartilage and synovium with a solid model might have significantly influenced the result of the FEA will require further investigation. Furthermore, because of technical difficulties during compressional loading of the model.

The full version of Abaqus is required for the meshing and convergence of the results. The use of CT scans or MRI scans and using a registration and segmentation software for

registering the images and importing into a finite element analysis software would have been more accurate results.

Validation is a critical part of any computer model because it greatly increases the confidence in any prediction based on the model. However, to reliably validate a model, any data generated from the model should be compared to experimental data from other sources. There is currently sufficient data in terms of morphological parameters of tarsal bones that could be used to validate this model for assessment of tarsus geometry or alignment in computer model. Then loading of tarsus could generate more useful information regarding the high load points within the tarsus geometry and their pattern of transfer in future studies for further better understanding of loading biomechanics of tarsus.

In conclusion, this study provides a geometrically three-dimensional representation of surface boundaries of the equine tarsus. Successful modelling of the most bony components of the equine tarsus is a step forward in understanding the joint's complex biomechanical functions. This model will be useful in further studies on conformation, contact and stress distribution patterns within the tarsal joint.

ADVANTAGES OF COMPUTER 3D MODELLING OF TARSUS

The significant findings of the morphometrical measurements have promoted the testing of the pattern of load distribution. The tarsus is a complex joint that can be represented in two-dimensional scans but is a complex 3D structure.

The analysis of bones through computer modelling has gained an increasing importance in biomechanical studies. The degree of reliability of the analysis is proportional to the concordance between the model and the simulation. The end purpose of this study was to model a three-dimensional computer model of tarsus by 3D scanning methods (Laser scanning) and Computed Tomography (CT) and to utilize the 3D model in stress analysis.

This pilot study was to model a hock joint which can be used for analysis and investigation of the material properties and geometry of tarsal bones. A 3D digital model was constructed and FEA analysis was done on the model in Abaqus software. All materials in the model were assumed to be linearly elastic and isotropic.

Mechanical properties for the tarsal bones and cartilages have been taken from the previous literature. The scanning of bones was done by NEXTENGINE and ARTEC 3D scanners and later by a CT machine. The scanned data which was obtained from 3D scanning contained the information about just the bone surface which is not sufficient for the analysis. The scanned mesh was converted to a solid body in Solid works. Modelling of cartilages and the assembly of all the bones was also done in Solid works. The finite element analysis explains static and dynamic behavior of the hock joint. The study is still incomplete and promote further future analysis.

The validation of model can be performed by applying the conformation parameters on

tarsal geometry of model and their monitoring with the normal range along with effects of rotations obtained from cadaveric limbs from previous studies can give the likelihood of model geometry with the real tarsal geometry for further analysis.

On the other hand, using a model, permits one to simulate the tarsus behavior in different situations like sound, pathological, and instrumented. The use of model can also be helpful to obtain some information that is not directly accessible through experimentation such as the stress distribution inside the tarsal segment. In addition, the angulation of these significant features seemed to have a direct contribution to the maintenance of stability in the tarsal joint during different movement phases.

Appendix B:

Some common Pathologies of tarsus:

Degenerative joint disease

Degenerative joint disease of tarsal joints is the most common cause of tarsal lameness, it also is known as a common cause of hind limb lameness in horses (Rachel C Murray, Dyson, Weekes, Short, & Branch, 2005). This disorder is often bilateral and mostly involves the centrodistal, tarsometatarsal joints while occasionally the talocalcaneal–centroquartal and, rarely the talocalcaneal joints (Butler et al., 2000; Martin Gough & Munroe, 1998; Vanderperren, Raes, Hoegaerts, et al., 2009).

Radiography is the most common imaging technique used for the evaluation of this disease in horses. The lateromedial and the dorsolateral–plantaromedial oblique projections are the most significant as usually the lesions begin at the dorsomedial aspect of joints. However, the disease may affect the dorsolateral aspect of the centrodistal and tarsometatarsal joints in Icelandic horses, so in such cases the plantarolateral-dorsomedial oblique projection is the most appropriate view. The dorsoplantar projection is the least useful because of the normally uneven border of the third tarsal bone (Per Eksell et al., 1999; May, Wyn-Jones, & Peremans, 1986; Verschooten & Schramme, 1994; Wyn-Jones, 1988).

The radiographic findings such as irregularity, ankylosis (narrowing or loss of the joint space), subchondral bone lysis, sclerosis, enthesiophytosis of the interosseous foramen, periarticular osteophytes, enthesiophytes, periarticular bone proliferation and periosteal bone formation are associated with degenerative joint disease (Fig. 1.16) (J. A. Butler et al., 2000; Martin Gough & Munroe, 1998; Verschooten & Schramme, 1994).

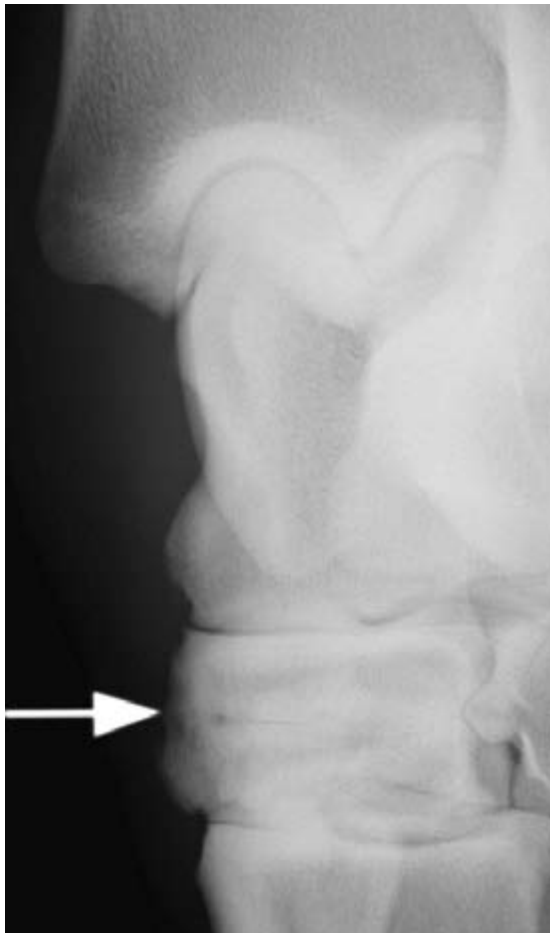


Figure B.1: Dorsolateral-plantaromedial oblique (DL-PIMO) view adapted from Vanderperren et al., 2009 of a Shetland foal with degenerative joint disease of the centrodistal joint of tarsus (arrow). The space of the centrodistal joint is narrowed and not visible at its dorsal aspect.

Cartilage degeneration, thinning of the articular surfaces in loaded areas, osteophytes, formation of enthesophytes and thickening of the joint capsule can be best visualized by ultrasonography (Redding, 2001). Scintigraphy can also be used in cases of limited response to perineural analgesia or inconclusive radiographs with a less common presentation of degenerative joint disease and can detect some subtle changes not visible in radiographic examination (Ehrlich, Seeherman, O'Callaghan, Dohoo, & Brimacombe, 1998; Martin Gough

& Munroe, 1998). Degenerative joint disorders can also be evaluated by using magnetic resonance imaging (R. Murray & Mair, 2005).

FRACTURES

Aetiology of tarsal bones fractures

Traumatic injuries and luxations occur because of substantial forces on the equine tarsus while precise diagnosis and appropriate management of such lesions can be a challenge for practitioners, a thorough understanding of anatomy and radiographic appearance is therefore, quite significant (Bolt, Williams, & Burba, 2002). External factors such as a fall or kick by another horse, limbs trapped (e.g., in a hole, cattle guard, bridge) or entangled in a fence may result in fractures particularly in exposed areas like the lateral aspect of the hock, (Gross, 1964; AJ Nixon, 1996; Wheat & Rhode, 1964). The immediate trauma during various types of athletic performances and forces causing them may not be often observed (S. Jakovljevic, C. GIBBS, & J. Yeats, 1982; Tulamo, Bramlage, & Gabel, 1983). Pathological tarsal fractures are not reported frequently. The immaturity of bones in neonatal foals has been reported to cause fractures of the third and central tarsal bones and might occur bilaterally (Dewes, 1982). Some pathological fractures along the medial border of calcaneus including sustentaculum tali may arise as a result of septic tenosynovitis and cellulitis of overlaying deep digital flexors and soft tissues (Tulleners & Reid, 1981a). Tarsal joint luxation occurs by similar traumatic forces resulting in fractures and associated with tarsal bones fractures (Gross, 1964; AJ Nixon, 1996). Sometimes tarsal joint luxation might be self-inflicted because of violently kicking of a horse against a wall (Moll et al., 1987).

Clinical diagnosis

Tarsal bone fractures result in relative non-specific clinical symptoms and most of fractures remain closed with exception of fractures of calcaneus. Various symptoms like degree of swelling, lameness, crepitus and heat in surrounding structures varies considerably according to the extent of the trauma and location of injuries. However, most of the horses with tarsal bone fracture show resistance to palpation on the site of fracture or flex their hock by force (Gross, 1964; S. Jakovljevic, C. Gibbs, & J. J. Yeats, 1982). There is a communication and fragments dislocation between tarsal joints may be worse with full weight bearing, so intra-articular anaesthesia should be attempted continuously for diagnosis of tarsal bone fractures (Winberg & Pettersson, 1999). The intra-articular anaesthesia also has been used in small tarsal bones fractures to localize such fractures (Tulamo et al., 1983).

Radiographic examination of affected sites is the most common evaluation technique used for determination and confirmation of specific suspected tarsal bone fracture and its location and extent (Bolt et al., 2002). The equine tarsus can be evaluated by a thorough radiographic examination which includes at least four radiographic views including lateromedial, dorsopalmar, dorsomedial-plantrolateral oblique and dorsolateral-plantaromedial oblique views. For some additional information multiple oblique views at different angles or the lateromedial flexed view might be helpful as tuber calcaneus, sustentaculum tali, tarsus groove and plantar aspects of medial trochlear ridge of talus tarsal bone can be best evaluated by a flexed plantroproximal-plantrodistal or skyline view (Farrow, McNeel, Morgan, & Resch, 1976; Foerner, 1992). A thorough understanding of normal radiographic appearance is required because of the complex anatomy of the tarsus to avoid any misdiagnosis and enable

localization of some specific lesions (Kneller & Losonsky, 1989; Shelley & Dyson, 1984; Shively & Smallwood, 1980).

Early identification and localization of lesions and injuries may be aided by advanced diagnostic imaging technologies where no radiographic changes are detectable such as computed tomography, nuclear scintigraphy (Goodrich & Trostle, 1997; Stover, Hornof, Richardson, & Meagher, 1986; Tulamo et al., 1983). Ultrasonography provides important diagnostic information about concurrent soft tissues, for imaging superficial subchondral bone and articular cartilages of the trochlear ridges of talus tarsal bone along with distal intermediate ridges of distal tibia in adult horses and distal tarsal bone in foals (Virginia B Reef, 1998a; Ruohoniemi et al., 1995; Julia E Tomlinson, Redding, & Sage, 2000).

Fractures of the tibial malleoli

The distal articular surface of the tibia is flanked by lateral and medial malleoli, make up the most prominences of lateral and medial sides. These both malleoli are very susceptible to external trauma as very thin soft tissue covers these areas (Dyce et al., 1996). Fractures of malleoli are most common traumatic injuries of the hock, affecting both malleoli but mostly lateral malleolus is affected more commonly as compared to medial malleolus of distal tibia (Foerner, 1992; S Jakovljevic et al., 1982; I. Wright, 1992). This might be due to its great exposure to any external impact trauma, such as kicking by others.

Malleolar fractures commonly occur because of the short collateral ligaments avulsion as they tensed during the hock flexion. It is hypothesized that avulsion can occur due to sudden increased torque or impact on a partial flexed hock (AJ Nixon, 1996; Updike, 1984; I. Wright,

1992). An acute onset of lameness which might be moderate to severe along with pronounced swelling of soft tissues are major clinical signs of malleolar fractures. The flexion of hock and direct pressure by palpation over affected area may induce a painful response (Bolt et al., 2002). The radiographic evaluation and confirmation of clinical diagnosis can be achieved by dorsoplantar view which can provide most of information, oblique views can provide precise location of fragments and any communication which are quite helpful for defining the surgical approach (Foerner, 1992). The dorsoplantar view in all cases indicates the fractures while dorsomedial-plantarolateral oblique view 75% of cases fracture was observed (Wyn-Jones, 1988). The fracture fragments might displace plantarodistally because of their attachment to the short collateral ligaments (AJ Nixon, 1996).

Fractures of lateral malleolus tend to be smaller size and multiple in number, such fractures are difficult for internal fixation as compared to medial malleolar avulsion. The fragments smaller than 3 x 1 cm are best to remove surgically, fragments smaller than 1cm or less can be removed by arthroscope. The fragments resected from attached synovial and collateral ligament tissues from the dorsolateral pouch of tarsocrural joint (McIlwraith, Nixon, & Wright, 1990). The presence of new periosteal bone on the dorsal aspect of the tibial bone might be related to the chronic injuries (Clegg, 2003; I. Wright, 1992).

Fractures of calcaneal tarsal bone

The fractures of calcaneal tarsal are relatively rare (KE Sullins, 2002). These fractures occur always due to trauma, externally impact arise from different factors such as kicking by other horses or against a solid barrier, fall (Foerner, 1992; S Jakovljevic et al., 1982). Falling upon a slippery surface may also result in calcaneal fractures because of extensive tension on

calcaneal tendon (Scott, 1983). Most of the calcaneus fractures are open either because of lacerations of skin accompany with external trauma or penetration of fracture fragments into skin (AJ Nixon, 1996). Some of chronic fractures may have developed osteomyelitis and other secondary complications including septic calcaneal bursitis, superficial digital flexor tendinitis and tenosynovitis of the tendon of deep digital flexor (AJ Nixon, 1996; Scott, 1983).

Fractures of calcaneus might be smaller and extensive, the small fractures can occur at various prominences which are widely exposed to any external trauma and fractures of calcaneal tuberosity or complete fracture of calcaneal tuber or communicated fractures of body of calcaneus regarded as extensive fractures of calcaneus (Ferguson & Presnell, 1976; Foerner, 1992; Jones, 1976). The fractures of tuber calcaneus may also be related with luxation and medial displacement of the tendon of superficial digital flexor (Scott, 1983).

Fractures of sustentaculum tali of calcaneus may also cause substantial damage of to the tendon of deep digital flexor. The pathological fractures of sustentaculum tali of calcaneus secondary to septic tenosynovitis and tendinitis of the tendon of deep digital flexor has been reported (Tulleners & Reid, 1981a). The swelling of at the proximo-plantar aspect of the hock and crepitus and the lameness is usually severe in horses with fractures of calcaneus. Skin lacerations over the calcaneal region in association with lameness and history of trauma are big indication of the fractures and should be confirmed through radiographic examination. The complete radiographic evaluation including lateromedial, dorsoplantar and oblique views, another flexed plantaroproximal- plantarodistal or sky line projection might be of great value to evaluate the entire tuber calcaneus and the surface of flexor and sustentaculum tali of calcaneus (Farrow et al., 1976; Foerner, 1992).

Fractures of talus

The talus is one of the proximal row tarsal bones and is composed of two oblique trochlear ridges, which articulate with the distal tibial cochlea dorsally. The body of talus articulates with calcaneus caudally and with central tarsal distally. The trochlear articulation with distal tibial cochlea constitutes the major component of tarsocrural joint and accounts for major mobile joint of hock, while the other tarsal joints have almost no mobility. The talus trochlear ridges are covered and protected by minimum soft tissues and are more susceptible to external impact trauma. The most common sites of fractures are distal aspects of lateral trochlear ridges and proximal aspect of medial trochlear ridge of talus (Foerner, 1992; S Jakovljevic et al., 1982; KE Sullins & Stashak, 1983; Tulleners & Reid, 1981a).

The fractures of medial trochlear ridge in distal portion may also occur, the spurs and or small fragments at this region are incidental findings (Bohanon, 1999; Watrous, Hultgren, & Wagner, 1991; I. Wright, 1992). Both talus trochlear ridges might be affected by osteochondritis dissecans, however, distal trochlear ridge fractures must be differentiated from osteochondritis dissecans fragments particularly in case of lateral trochlear ridge is affected (Bohanon, 1999). The osteochondritis dissecans fragments mostly occur in yearlings and weanlings while there might be a seldom severe lameness as compared with true fractures (AJ Nixon, 1996).

The sagittal fractures of the body of talus tarsal bone tend to minimum displacement and occur usually after a twisting fall or even in a horse after successful completion of race in rare cases (Meagher & Mackey, 1990; AJ Nixon, 1996). Severe trauma such as falls, kicks by other horses etc. can lead to comminuted or sagittal fractures of talus tarsal. The trochlear ridge fractures required surgical treatment to alleviate a persistent effusion of tarsocrural joint and

lameness. Small fragments can be often removed by surgical approaches, arthroscopically or limited arthrotomy (Bohanon, 1999; Foerner, 1992; C. W. McIlwraith, 1983; AJ Nixon, 1996; KE Sullins & Stashak, 1983; Tulleners & Reid, 1981b).

For better localization and visualisation of fractures through radiographic examination, the dorsoplantar view or a slightly oblique projections can be used (A Nixon, 1996). Advanced imaging technologies such as scintigraphy, computed tomography and magnetic resonance imaging should be used when available as in a study, radiographic examination failed to identify the fractures in 9/11 racing horses (Davidson, Ross, & Parente, 2005).

Fractures of other tarsal bones

Other tarsal bones include the remaining tarsal bones including central, first and second fused tarsal or as separated, third and fourth tarsal bones. The fractures of tarsal bones are most common slab fractures induced by trauma, the third tarsal bone is most commonly affected as compared to the central tarsal while the second and fourth tarsal bones are affected in rare cases (Bohanon, 1999; Tulamo et al., 1983).

The slab fractures of the central tarsal bone occur at the dorsomedial aspect most commonly, whereas third tarsal bone fractures mostly observed at the lateral or dorsolateral aspects (Bolt et al., 2003). The radiographic localization and identification might be difficult in early diagnosis, especially in case of minimal swelling and the fracture is not displaced, as the line of fragment may not be visible clearly on standard radiographic projections (Bolt et al., 2003; Stover et al., 1986; Tulamo et al., 1983).

The fractures of tarsal bones can be best visualised on different radiographic views such as lateromedial projection for central tarsal bone and dorsomedial-plantarolateral oblique projection for third tarsal bone (Lindsay et al., 1982; Tulamo et al., 1983). The fractures of the fourth tarsal bone and fractures of the proximal aspects of the fourth metatarsal bone are frequently combined and can be diagnosed by radiography (Bolt et al., 2003).

Along with standard projections, oblique views of tarsus may be necessary for confirmation of suspected fractures and might reveal many fracture planes (Bolt et al., 2003; Tulamo et al., 1983). The horses with suspected clinical tarsal fractures but negative radiographic assessment should be re-examined after ten days to examine periosteal proliferation and bone resorption (Bolt et al., 2003).

Along with radiographic examination other techniques can also provide important information such as the fractures of the central and third tarsal bone can be easily identified with ultrasonography. The hairline fractures or non-displaced fractures, comminuted fractures or reattached bony fragments in marginal fractures can be localized and diagnosed by ultrasonography (Bolt et al., 2003; Denoix, 1996; Virginia B Reef, 1998b).

The fracture line can be identified as a hypoechoic to anechoic line through the cortical bone, tarsocrural joint effusion with central tarsal bone fractures can be visualized due to the communication between the tarsocrural joint and the talocalcaneal-centroquartal joint by ultrasonography. In contrast, TTB fractures do not have visible joint effusion (Tulamo et al., 1983). There was increased radioactive uptake on the dorsal aspects of the small tarsals in proximal row of the tarsus joint is present with a tarsal bone fracture on scintigraphy. The fracture planes can be defined in both simple and comminuted fractures by computed tomography (Bolt et al., 2003; Stover et al., 1986).

Appendix C:

Chapter 5: EFFECTS OF ROTATIONS

Cassette holder:

The cassette holder was comprised of two parts, which comprises cassette holding bars and a base of cassette holder. These two parts were combined by a screw in such a way that cassette holding bars can be rotated vertically on the same center of rotation on the base of cassette holder. The digital radiographic cassette can be placed in space between two cassette holding bars and was placed in such a way that the dorsal surface of digital cassette which faced the radiography machine was exactly perpendicular to the head of the radiography machine during each vertical deviation of radiography machine (Figure 4.2).

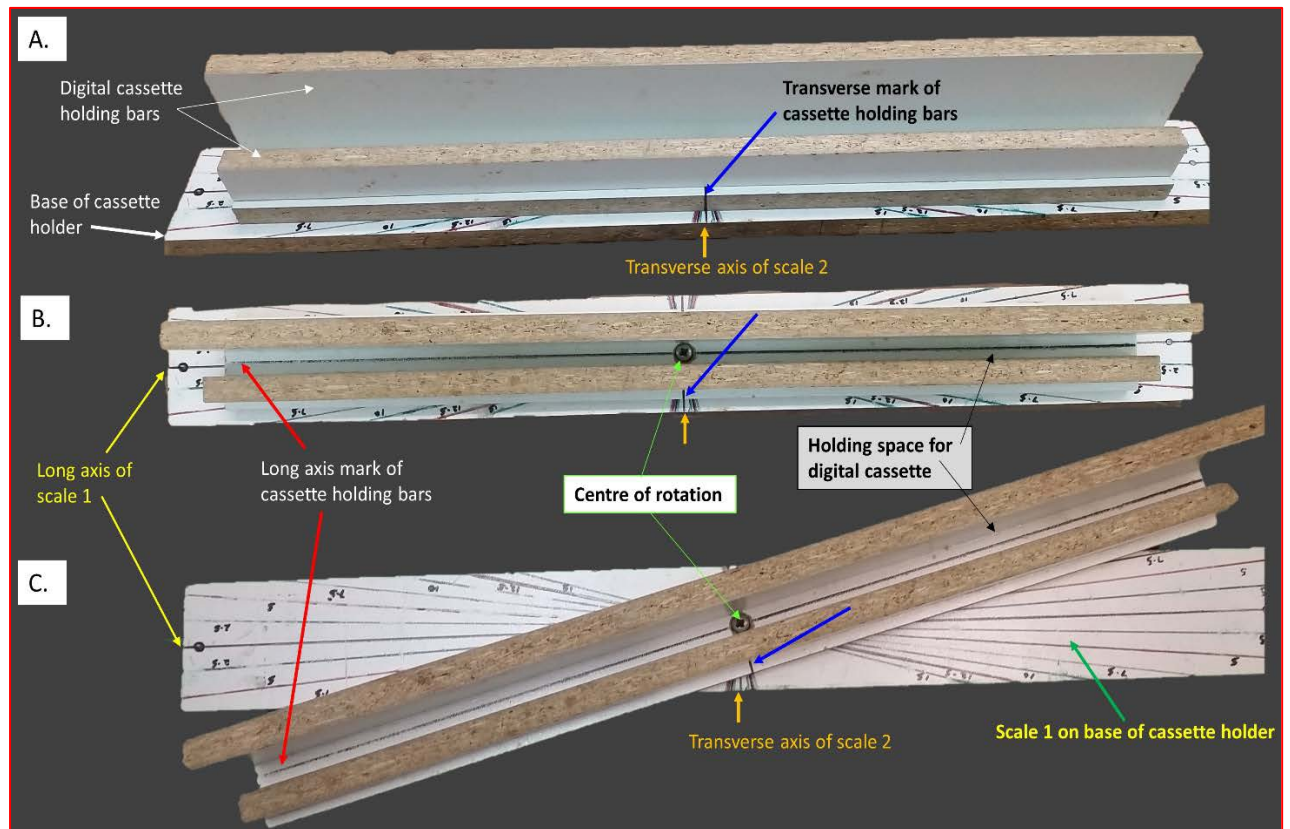


Figure C.1: Cassette holding device used in this study for holding the digital cassette and its adjustment according to the head of radiography machine. A. Frontal, B. Dorsal (0° adjustment), C. Rotation of cassette holding bars ($\pm 15^\circ$) over scale of radiographic base showing combined at center of rotation. Scale 1 was drawn along long axis and scale 2 along the transverse axis on base of cassette holder at an interval of 2.5 degrees (unit). The long axis mark (red arrows) and transverse (blue arrows) mark of cassette holding bars were used to align exactly for zero rotation (B) and monitor rotation of cassette holding bars on the base of cassette holder according to scale 1 and 2.

Digital cassette and radiographic processor:

Digital cassettes were used. The radiographs were produced automatically using a digital radiographic scanner machine. Radiograph plates were scanned using scanner (FireCR+ Veterinary 80 CR reader) and imported into DicomPACS® DX-R X-Ray acquisition software. All the radiographs were saved in laptop directly to a special program (as mentioned above). The radiographs were then collected after being filed and labelled according to which specimen they came from.

Positioning wedges:

The positioning wedges were made of wood (Figure 4.1). It was designed to fit between the two transparent perspex radiographic sheets used for horizontal rotation of radiographic machine (Figures 4.8 and 4.9). There are four wedges used to make rotations of 2.5°, 5°, 7.5° and 10° around the horizontal axis of the radiographic machine. These wedges were also used at same time to rotate the digital radiographic cassette holder according to displacement of radiography machine head as shown in figures 4.6 and 4.10.

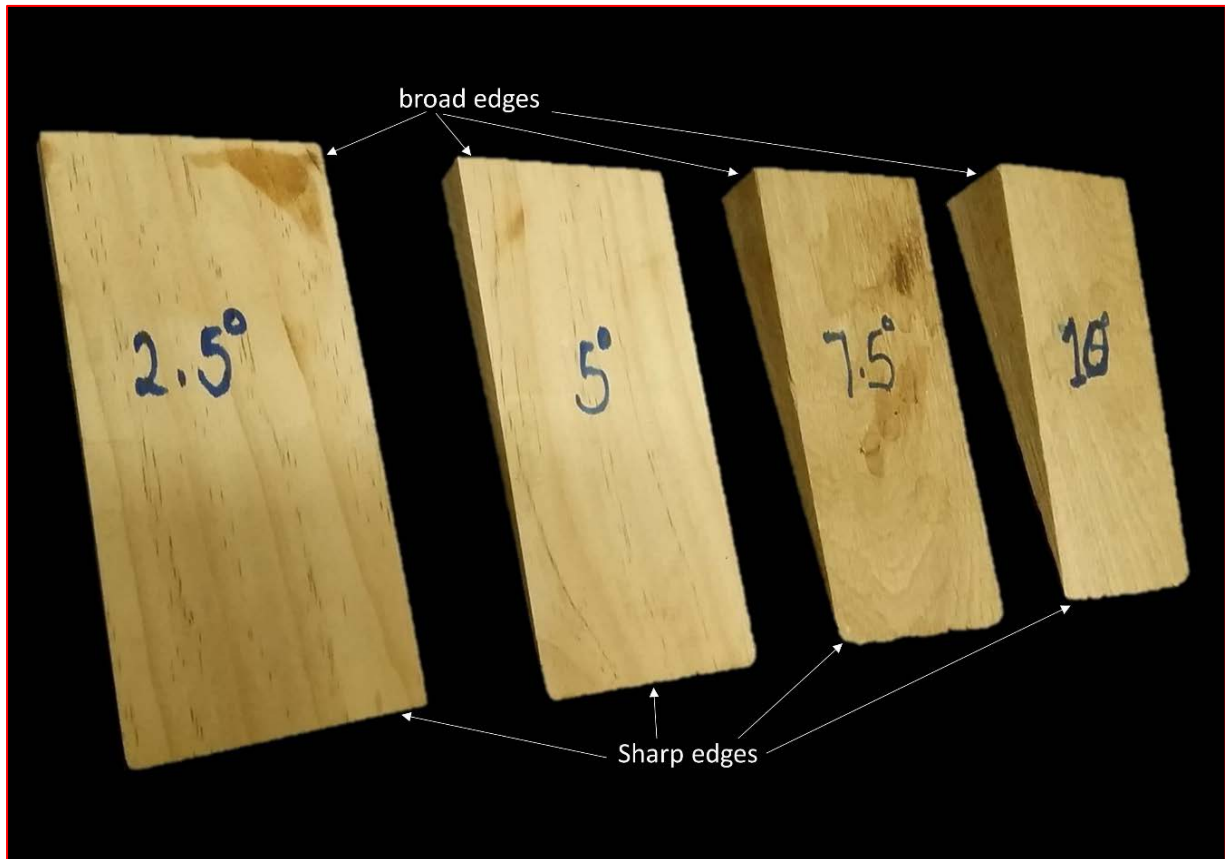


Figure C.2: The positioning block labelled with 2.5°, 5°, 7.5 and 10° with broader and sharp edges used to cause a horizontal rotation of the radiography machine and radiographic cassette holder to keep them align during each rotation.

Table C.1: Descriptive statistics of individual angular parameters in different rotational positions

PARAMETERS	ROTATIONAL POSITIONS	DESCRIPTIVE STATISTICS					
		Means $\pm$ SD	Std. Error Mean (SEM)	Median	95 % CI of Mean		Test of Normality (Shapiro-Wilk) Sig.
IAP-1, LM. DISTAL INTERTARSAL-METATARSUS JOINT ANGLE	V+15° from ZLM	97.22 $\pm$ 2.58	0.67	96.86	95.97	98.65	0.877
	V+10° from ZLM	97.80 $\pm$ 2.37	0.61	97.15	96.48	99.12	0.230
	V+5° from ZLM	97.66 $\pm$ 2.68	0.69	97.23	96.18	99.15	0.344
	ZLM-V0°	96.79 $\pm$ 1.91	0.49	97.00	95.73	97.84	0.514
	V-5° from ZLM	96.66 $\pm$ 3.05	0.79	97.10	94.97	98.34	0.793
	V-10° from ZLM	95.77 $\pm$ 3.10	0.80	95.70	94.06	97.49	0.781
	V-15° from ZLM	94.55 $\pm$ 2.93	0.76	94.88	92.93	96.17	0.930
IAP-2, LM. MIDDLE INTERTARSAL-METATARSUS JOINT ANGLE	V+15° from ZLM	98.06 $\pm$ 3.41	0.88	96.90	96.17	99.95	0.062
	V+10° from ZLM	97.77 $\pm$ 3.70	0.95	96.96	95.72	99.81	0.324
	V+5° from ZLM	96.54 $\pm$ 3.62	0.93	95.27	94.53	98.54	0.425
	ZLM-V0°	95.07 $\pm$ 3.09	0.80	94	93.36	96.79	0.127
	V-5° from ZLM	94.22 $\pm$ 3.41	0.88	93.75	92.33	96.11	0.669
	V-10° from ZLM	92.83 $\pm$ 4.01	1.04	92	90.61	95.05	0.361
	V-15° from ZLM	90.32 $\pm$ 4.54	1.17	89.36	87.80	92.83	0.265
IAP-3, LM. PROXIMAL INTERTARSAL-METATARSUS JOINT ANGLE	V+15° from ZLM	93.58 $\pm$ 3.57	0.92	93.54	91.60	95.55	0.271
	V+10° from ZLM	93.05 $\pm$ 3.30	0.85	92.20	91.22	94.87	0.116
	V+5° from ZLM	91.38 $\pm$ 4.13	1.07	90.78	89.09	93.67	0.107
	ZLM-V0°	90.71 $\pm$ 3.55	0.92	89.63	88.74	92.67	0.005*
	V-5° from ZLM	88.70 $\pm$ 3.39	0.88	88.00	86.82	90.58	0.336

	V-10° from ZLM	87.06 ± 3.07	0.79	87.00	85.36	88.76	0.270
	V-15° from ZLM	85.67 ± 3.31	0.85	85.36	83.84	87.50	0.632
IAP-4, LM. CAUDO-DISTAL FACET OF CALCANEUS-4TH TARSAL- METATARSUS JOINT ANGLE JOINT ANGLE.	V+15° from ZLM	83.43 ± 5.74	1.48	83.34	80.25	86.61	0.836
	V+10° from ZLM	84.55 ± 5.47	1.41	85.60	81.51	87.58	0.641
	V+5° from ZLM	84.37 ± 5.16	1.33	84.06	81.52	87.23	0.559
	ZLM-V0°	84.07 ± 4.92	1.27	84.75	81.35	86.80	0.587
	V-5° from ZLM	84.38 ± 4.06	1.05	85.90	82.13	86.63	0.210
	V-10° from ZLM	84.50 ± 4.30	1.11	85.13	82.12	86.88	0.492
	V-15° from ZLM	83.56 ± 4.54	1.17	82.80	81.04	86.08	0.886
IAP-5, LM. TALOCALCANE JUNCTION- METATARSUS JOINT ANGLE	V+15° from ZLM	133.12 ± 2.15	0.55	133.12	131.93	134.30	0.470
	V+10° from ZLM	134.80 ± 2.50	0.65	134.50	133.41	136.18	0.962
	V+5° from ZLM	136.30 ± 2.50	0.65	136.06	134.91	137.68	0.999
	ZLM-V0°	137.93 ± 2.16	0.56	138.2	136.73	139.12	0.305
	V-5° from ZLM	139.70 ± 2.80	0.72	140.44	138.15	141.25	0.122
	V-10° from ZLM	141.77 ± 2.88	0.74	142.50	140.18	143.36	0.073
	V-15° from ZLM	144.17 ± 3.35	0.86	144.56	142.32	146.02	0.216
IAP-6, LM. DORSO-DISTAL CALC- TALOCALCANE JUNCTION ANGLE	V+15° from ZLM	111.72 ± 2.29	1.18	111.23	109.19	114.24	0.475
	V+10° from ZLM	114.08 ± 3.40	0.88	113.70	112.19	115.96	0.406
	V+5° from ZLM	115.97 ± 2.39	0.62	115.90	114.65	117.29	0.900
	ZLM-V0°	117.20 ± 2.29	0.59	116.37	115.93	118.47	0.108
	V-5° from ZLM	120.21 ± 3.29	0.85	119.40	118.39	122.04	0.328
	V-10° from ZLM	122.77 ± 4.08	1.05	124.43	120.04	125.04	0.020
	V-15° from ZLM	125.82 ± 3.02	0.78	126.20	124.14	127.49	0.802
IAP-7, LM. DORSO-DISTAL CALC- METATARSUS ANGLE	V+15° from ZLM	22.55 ± 4.89	1.26	23.00	19.84	25.25	0.707
	V+10° from ZLM	21.24 ± 3.81	0.98	21.60	19.13	23.36	0.737
	V+5° from ZLM	20.43 ± 3.17	0.81	21.61	18.67	22.18	0.057

	ZLM-V0°	20.75 ± 3.54	0.91	22.06	18.78	22.71	0.068
	V-5° from ZLM	19.69 ± 3.26	0.84	18.40	17.88	21.49	0.431
	V-10° from ZLM	18.87 ± 5.17	1.33	18.10	16.00	21.073	0.072
	V-15° from ZLM	18.88 ± 4.54	1.17	18.40	16.37	21.40	0.338
IAP-8, LM. TALO-METATARSUS JOINT ANGLE	V+15° from ZLM	140.77 ± 1.52	0.39	141.20	139.93	141.61	0.365
	V+10° from ZLM	140.90 ± 2.54	0.66	141.36	139.49	142.31	0.852
	V+5° from ZLM	141.66 ± 2.27	0.59	142.40	140.40	142.92	0.034
	ZLM-V0°	142.86 ± 2.62	0.68	143.50	141.41	144.31	0.488
	V-5° from ZLM	144.46 ± 2.42	0.62	145.00	143.12	145.80	0.894
	V-10° from ZLM	146.42 ± 2.59	0.67	147.20	144.98	147.86	0.014
	V-15° from ZLM	147.86 ± 2.51	0.65	148.41	146.47	149.26	0.006
IAP-9, LM. CALCANEAL-METATARSUS JOINT ANGLE	V+15° from ZLM	164.83 ± 2.54	0.65	164.50	163.42	166.24	0.928
	V+10° from ZLM	164.88 ± 2.58	0.67	164.20	163.44	166.31	0.990
	V+5° from ZLM	165.21 ± 2.54	0.66	165.05	163.80	166.61	0.980
	ZLM-V0°	165.65 ± 2.23	0.58	165.50	164.41	166.88	0.952
	V-5° from ZLM	165.91 ± 2.62	0.68	165.66	164.45	167.36	0.703
	V-10° from ZLM	166.39 ± 2.17	0.56	166.60	165.18	167.59	0.423
	V-15° from ZLM	137.20 ± 2.36	0.61	167.42	165.89	168.51	0.604
IAP-10, LM. SUSTENTACULUM CALACANI-METATARSUS JOINT ANGLE	V+15° from ZLM	24.80 ± 4.24	1.09	25.64	22.45	27.16	0.167
	V+10° from ZLM	25.78 ± 3.94	1.101	25.86	23.60	27.96	0.276
	V+5° from ZLM	25.66 ± 3.28	0.85	25.53	23.85	27.48	0.233
	ZLM-V0°	25.85 ± 2.49	0.64	25.90	24.47	27.23	0.633
	V-5° from ZLM	26.49 ± 2.48	0.64	25.98	25.11	27.86	0.164
	V-10° from ZLM	27.14 ± 2.64	0.68	26.65	25.68	28.60	0.332
	V-15° from ZLM	27.18 ± 2.07	0.53	26.52	26.02	28.33	0.117
IAP-11, LM.	V+15° from ZLM	39.62 ± 5.00	1.29	41.00	36.85	42.39	0.245

CALCANEO-SUSTENTACULUM CALACANI JOINT ANGLE	V+10° from ZLM	40.78 ± 4.00	1.03	40.11	38.57	43.00	0.867
	V+5° from ZLM	40.29 ± 3.16	0.81	40.40	38.55	42.05	0.640
	ZLM-V0°	40.30 ± 2.72	0.70	39.80	38.79	41.81	0.644
	V-5° from ZLM	40.66 ± 2.59	0.67	40.50	39.23	42.10	0.660
	V-10° from ZLM	40.91 ± 2.41	0.62	40.44	39.57	42.24	0.810
	V-15° from ZLM	40.04 ± 2.95	0.76	40.96	38.40	41.68	0.765
IAP-12, LM. TALOCALCANEUS AXISAL ANGLE	V+15° from ZLM	54.85 ± 2.60	0.67	54.00	53.41	56.29	0.409
	V+10° from ZLM	54.29 ± 3.50	0.90	53.00	52.35	56.23	0.229
	V+5° from ZLM	53.18 ± 3.12	0.81	52.30	51.46	54.92	0.419
	ZLM-V0°	51.63 ± 3.21	0.83	50.79	49.85	53.41	0.230
	V-5° from ZLM	49.72 ± 3.06	0.79	50.30	48.02	51.41	0.473
	V-10° from ZLM	47.53 ± 3.33	0.86	47.55	45.69	49.38	0.599
	V-15° from ZLM	45.27 ± 2.74	0.71	45.85	43.76	46.79	0.140
IAP-13, LM. ANTERIO-POSTERIOR ANGLE OF PROXIMO-CAUDAL ANGLE OF CALC	V+15° from ZLM	76.63 ± 2.82	0.76	76.82	75.00	78.26	0.782
	V+10° from ZLM	76.63 ± 2.50	0.65	76.60	75.07	78.06	0.817
	V+5° from ZLM	77.17 ± 2.89	0.75	76.97	75.34	78.75	0.828
	ZLM-V0°	77.91 ± 3.01	0.78	78.26	75.99	79.54	0.142
	V-5° from ZLM	78.40 ± 2.92	0.75	78.17	76.51	79.84	0.297
	V-10° from ZLM	79.32 ± 2.93	0.76	78.88	77.41	80.71	0.224
	V-15° from ZLM	80.88 ± 3.12	0.80	80.80	78.86	82.16	0.364

Figure C.3: Change of individual angle 1 with respect of different vertical rotations.

Comparison of different positions on individual angle1

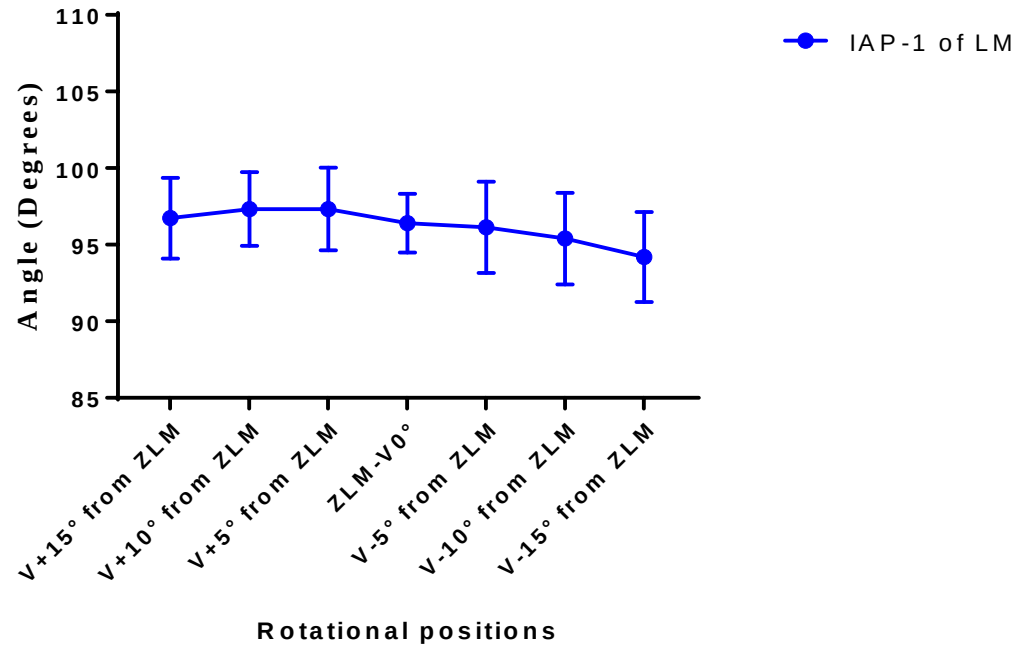


Figure C.4: Change of individual angle 1 with respect of different vertical rotations depicting the individual variation in all 15 samples.

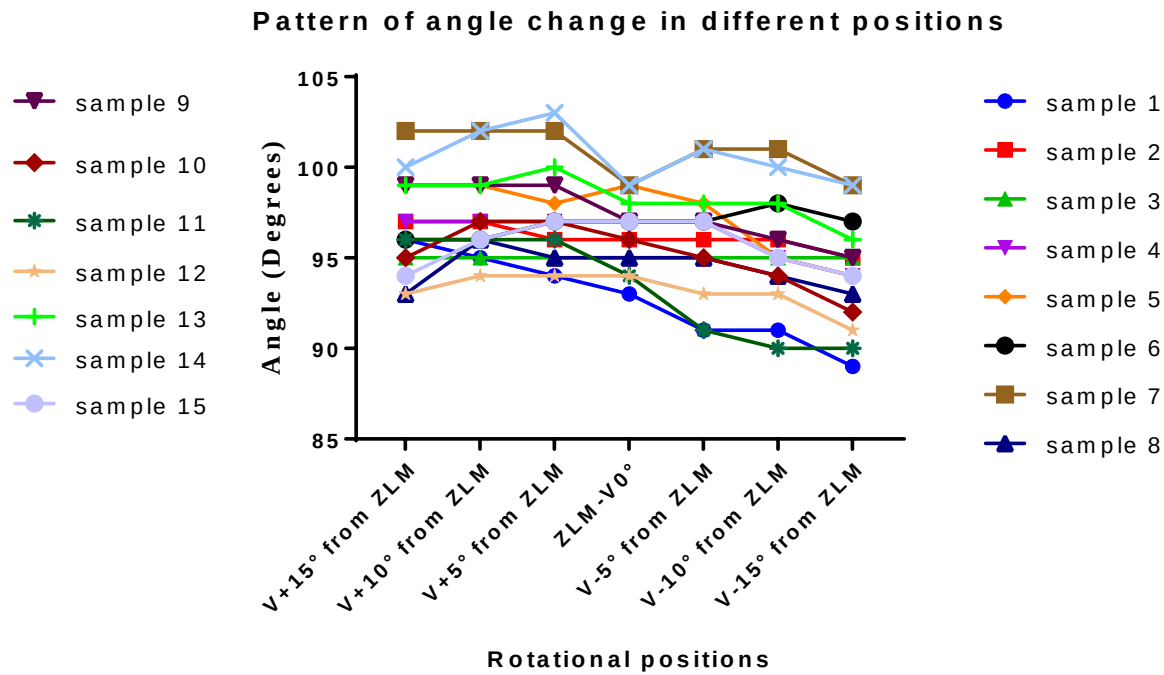


Table C.2: Comparison between parameter values in all upper (+) and lower (-) Vertical rotations in 15 tarsi. All angular measurements were in degrees.

PARAMETERS	ROTATIONAL POSITIONS	PAIRED T-TEST						
		Means $\pm$ SD	Paired Mean Difference (ZLM-V0°-others) $\pm$ SD	Std. Error of Mean Difference	95 % CI of the Difference		Paired sample correlations (ZLM-others)	Sig. (2-tailed) p<0.05
					Lower	Upper		
IAP-1, LM. DISTAL INTERTARSAL-METATARSUS JOINT ANGLE	V+15° from ZLM	97.22 $\pm$ 2.58	-0.44 $\pm$ 1.66	0.43	-1.36	0.48	0.765	0.326
	V+10° from ZLM	97.80 $\pm$ 2.37	-1.02 $\pm$ 1.12	0.29	-1.59	-0.17	0.885	0.003*
	V+5° from ZLM	97.66 $\pm$ 2.68	-0.88 $\pm$ 1.28	0.33	-1.59	-0.17	0.898	0.019*
	ZLM-V0°	96.79 $\pm$ 1.91	0	0	0	0	0	0
	V-5° from ZLM	96.66 $\pm$ 3.05	0.13 $\pm$ 1.29	0.33	-0.59	0.84	0.968	0.709
	V-10° from ZLM	95.77 $\pm$ 3.10	1.01 $\pm$ 1.77	0.46	0.03	1.99	0.856	0.044*
	V-15° from ZLM	94.55 $\pm$ 2.93	2.24 $\pm$ 1.71	0.44	1.29	3.18	0.832	0.000**
IAP-2, LM. MIDDLE INTERTARSAL-METATARSUS JOINT ANGLE	V+15° from ZLM	98.06 $\pm$ 3.41	-2.99 $\pm$ 1.85	0.48	-4.01	-1.96	0.842	0.000**
	V+10° from ZLM	97.77 $\pm$ 3.70	-2.69 $\pm$ 1.78	0.46	-3.68	-1.70	0.877	0.000**
	V+5° from ZLM	96.54 $\pm$ 3.62	-1.46 $\pm$ 1.74	0.45	-2.42	-0.49	0.877	0.006*
	ZLM-V0°	95.07 $\pm$ 3.09	0	0	0	0	0	0
	V-5° from ZLM	94.22 $\pm$ 3.41	0.86 $\pm$ 1.48	0.38	0.04	1.67	0.902	0.041*
	V-10° from ZLM	92.83 $\pm$ 4.01	2.24 $\pm$ 1.75	0.45	1.27	3.21	0.911	0.000**
	V-15° from ZLM	90.32 $\pm$ 4.54	4.75 $\pm$ 2.33	0.60	3.47	6.05	0.881	0.000**

IAP-3, LM. PROXIMAL INTERTARSAL- METATARSUS JOINT ANGLE	V+15° from ZLM	93.58 ± 3.57	-2.86 ± 1.98	0.51	-3.96	-1.77	0.845	0.000**
	V+10° from ZLM	93.05 ± 3.30	-2.34 ± 1.18	0.30	-2.99	-1.68	0.943	0.000**
	V+5° from ZLM	91.38 ± 4.13	-0.67 ± 1.79	0.46	-1.66	0.32	0.903	0.170
	ZLM-V0°	90.71 ± 3.55	0	0	0	0	0	0
	V-5° from ZLM	88.70 ± 3.39	2.01 ± 1.66	0.43	1.09	2.93	0.887	0.000**
	V-10° from ZLM	87.06 ± 3.07	3.65 ± 1.87	0.48	2.61	4.69	0.849	0.000**
	V-15° from ZLM	85.67 ± 3.31	5.04 ± 1.79	0.46	4.05	6.03	0.867	0.000**
IAP-4, LM. CAUDO-DISTAL FACET OF CALCNEO-4TH TARSAL- METATARSUS JOINT ANGLE.	V+15° from ZLM	83.43 ± 5.74	0.64 ± 1.90	0.49	-0.41	1.69	0.948	0.211
	V+10° from ZLM	84.55 ± 5.47	-0.47 ± 2.63	0.68	-1.93	0.98	0.877	0.498
	V+5° from ZLM	84.37 ± 5.16	-0.30 ± 1.86	0.48	-1.33	0.73	0.933	0.541
	ZLM-V0°	84.07 ± 4.92	0	0	0	0	0	0
	V-5° from ZLM	84.38 ± 4.06	-0.30 ± 2.34	0.60	-1.60	0.99	0.882	0.621
	V-10° from ZLM	84.50 ± 4.30	-0.43 ± 2.47	0.64	0.94	-0.67	0.865	0.513
	V-15° from ZLM	83.56 ± 4.54	0.51 ± 3.85	0.99	-1.62	2.65	0.672	0.613
IAP-5, LM. TALOCALCANE JUNCTION- METATARSUS JOINT ANGLE	V+15° from ZLM	133.12 ± 2.15	4.81 ± 1.12	0.29	4.19	5.43	0.865	0.000**
	V+10° from ZLM	134.80 ± 2.50	3.13 ± 1.06	0.27	2.54	3.72	0.908	0.000**
	V+5° from ZLM	136.30 ± 2.50	1.63 ± 0.86	0.22	1.15	2.11	0.942	0.000**
	ZLM-V0°	137.93 ± 2.16	0	0	0	0	0	0
	V-5° from ZLM	139.70 ± 2.80	-1.77 ± 1.10	0.28	-2.38	-1.16	0.934	0.000**
	V-10° from ZLM	141.77 ± 2.88	-3.84 ± 0.98	0.25	-4.39	-3.30	0.963	0.000**
	V-15° from ZLM	144.17 ± 3.35	-6.24 ± 1.53	0.39	-7.09	-5.40	0.936	0.000**
IAP-6, LM.	V+15° from ZLM	111.72 ± 2.29	5.48 ± 3.15	0.81	3.74	7.23	0.774	0.000**

DORSO-DISTAL CALC-TALOCALCANE JUNCTION ANGLE	V+10° from ZLM	114.08 ± 3.40	3.12 ± 2.62	0.68	1.67	4.57	0.640	0.000**
	V+5° from ZLM	115.97 ± 2.39	1.23 ± 2.21	0.57	0.1	2.45	0.555	0.049*
	ZLM-V0°	117.20 ± 2.29	0	0	0	0	0	0
	V-5° from ZLM	120.21 ± 3.29	-3.01 ± 2.99	0.77	-4.67	-1.36	0.472	0.002*
	V-10° from ZLM	122.77 ± 4.08	-5.57 ± 4.19	1.08	-7.89	-3.25	0.235	0.000**
	V-15° from ZLM	125.82 ± 3.02	-8.61 ± 3.43	0.89	-10.52	-6.71	0.185	0.000**
IAP-7, LM. DORSO-DISTAL CALC-METATARSUS ANGLE	V+15° from ZLM	22.55 ± 4.89	-1.80 ± 3.14	0.81	-3.53	-0.06	0.768	0.043*
	V+10° from ZLM	21.24 ± 3.81	-0.49 ± 3.35	0.86	-2.35	1.36	0.587	0.577
	V+5° from ZLM	20.43 ± 3.17	0.32 ± 1.86	0.48	-0.71	1.35	0.851	0.519
	ZLM-V0°	20.75 ± 3.54	0	0	0	0	0	0
	V-5° from ZLM	19.69 ± 3.26	1.06 ± 2.26	0.58	-0.19	2.31	0.781	0.091
	V-10° from ZLM	18.87 ± 5.17	1.88 ± 3.92	1.01	-0.29	4.05	0.652	0.085
IAP-8, LM. TALO-METATARSUS JOINT ANGLE	V-15° from ZLM	18.88 ± 4.54	1.86 ± 3.38	0.87	-0.01	3.74	0.675	0.051
	V+15° from ZLM	140.77 ± 1.52	2.09 ± 1.70	0.44	1.15	3.03	0.789	0.000**
	V+10° from ZLM	140.90 ± 2.54	1.95 ± 1.84	0.47	0.94	2.97	0.747	0.001*
	V+5° from ZLM	141.66 ± 2.27	1.19 ± 0.97	0.25	0.66	1.74	0.930	0.000**
	ZLM-V0°	142.86 ± 2.62	0	0	0	0	0	0
	V-5° from ZLM	144.46 ± 2.42	-1.60 ± 1.47	0.38	-2.42	-0.79	0.831	0.001*
IAP-9, LM.	V-10° from ZLM	146.42 ± 2.59	-3.56 ± 1.83	0.47	-4.57	-2.54	0.753	0.000**
	V-15° from ZLM	147.86 ± 2.51	-5.01 ± 2.19	0.56	-6.22	-3.79	0.634	0.000**
	V+15° from ZLM	164.83 ± 2.54	0.82 ± 0.76	0.20	0.39	1.24	0.957	0.001*
	V+10° from ZLM	164.88 ± 2.58	0.77 ± 0.82	0.21	0.32	1.22	0.953	0.003*

CALCANEO-METATARSUS JOINT ANGLE	V+5° from ZLM	165.21 ± 2.54	0.44 ± 0.83	0.21	-0.01	0.90	0.949	0.056
	ZLM-V0°	165.65 ± 2.23	0	0	0	0	0	0
	V-5° from ZLM	165.91 ± 2.62	-0.26 ± 0.88	0.23	-0.74	0.23	0.946	0.273
	V-10° from ZLM	166.39 ± 2.17	-0.74 ± 0.74	0.19	-1.15	0.33	0.943	0.002*
	V-15° from ZLM	137.20 ± 2.36	-1.55 ± 1.07	0.28	-2.14	-0.96	0.892	0.000**
IAP-10, LM. SUSTENTACULUM CALACANI-METATARSUS JOINT ANGLE	V+15° from ZLM	24.80 ± 4.24	1.04 ± 2.98	0.77	-0.61	2.70	0.726	0.196
	V+10° from ZLM	25.78 ± 3.94	0.06 ± 2.33	0.60	-1.23	1.35	0.830	0.920
	V+5° from ZLM	25.66 ± 3.28	0.18 ± 1.77	0.46	-0.80	1.17	0.845	0.694
	ZLM-V0°	25.85 ± 2.49	0	0	0	0	0	0
	V-5° from ZLM	26.49 ± 2.48	-0.64 ± 2.27	0.59	-1.90	0.62	0.583	0.294
	V-10° from ZLM	27.14 ± 2.64	-1.29 ± 1.92	0.49	-2.36	-0.23	0.719	0.021*
	V-15° from ZLM	27.18 ± 2.07	-1.33 ± 2.66	0.69	-2.81	0.14	0.334	0.073
IAP-11, LM. CALCANEO-SUSTENTACULUM CALACANI JOINT ANGLE	V+15° from ZLM	39.62 ± 5.00	0.68 ± 3.78	0.96	-1.41	2.77	0.667	0.499
	V+10° from ZLM	40.78 ± 4.00	-0.48 ± 2.37	0.61	-1.79	0.82	0.818	0.441
	V+5° from ZLM	40.29 ± 3.16	0.00 ± 1.61	0.41	-0.89	0.90	0.859	0.997
	ZLM-V0°	40.30 ± 2.72	0	0	0	0	0	0
	V-5° from ZLM	40.66 ± 2.59	-0.37 ± 2.39	0.62	-1.68	0.96	0.596	0.563
	V-10° from ZLM	40.91 ± 2.41	-0.61 ± 1.92	0.50	-1.68	0.46	0.724	0.241
	V-15° from ZLM	40.04 ± 2.95	0.26 ± 3.24	0.84	-1.54	2.05	0.349	0.764
IAP-12, LM. TALOCALCANEUS AXIAL ANGLE	V+15° from ZLM	54.85 ± 2.60	-3.21 ± 2.07	0.54	-4.36	-2.07	0.765	0.000**
	V+10° from ZLM	54.29 ± 3.50	-2.66 ± 1.79	0.46	-3.65	-1.67	0.861	0.000**
	V+5° from ZLM	53.18 ± 3.12	-1.55 ± 1.12	0.29	-2.17	-0.93	0.938	0.000**

IAP-13, LM. ANTERIO-POSTERIOR ANGLE OF PROXIMO- CAUDAL ANGLE OF CALC	ZLM-V0°	51.63 ± 3.21	0	0	0	0	0	0
	V-5° from ZLM	49.72 ± 3.06	1.91 ± 1.37	0.35	1.15	2.67	0.906	0.000**
	V-10° from ZLM	47.53 ± 3.33	4.10 ± 1.55	0.40	3.24	4.96	0.888	0.000**
	V-15° from ZLM	45.27 ± 2.74	6.36 ± 2.20	0.57	5.14	5.58	0.738	0.000**
	V+15° from ZLM	76.63 ± 2.82	1.14 ± 1.40	0.37	0.33	1.94	0.891	0.010*
	V+10° from ZLM	76.63 ± 2.50	1.28 ± 1.53	0.40	0.44	2.13	0.862	0.006*
	V+5° from ZLM	77.17 ± 2.89	0.74 ± 1.45	0.38	-0.07	1.54	0.879	0.071
	ZLM-V0°	77.91 ± 3.01	0	0	0	0	0	0
	V-5° from ZLM	78.40 ± 2.92	-0.49 ± 1.24	0.32	-1.18	0.19	0.912	0.147
	V-10° from ZLM	79.32 ± 2.93	-1.41 ± 1.30	0.34	-2.13	-0.69	0.905	0.001*
	V-15° from ZLM	80.88 ± 3.12	-2.97 ± 1.66	0.43	-3.89	-2.06	0.854	0.000**

Figure C.5: Change of individual angle 10 with respect of different vertical rotations.

