DR. HELEN MS DAVIES (Orcid ID : 0000-0002-5110-2002)

Article type : Original Paper

Investigating canine elbow joint stabilisation through mechanical constraints of the deep fascia and other soft tissues

Timothy E. Chong and Helen M.S. Davies

Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville 3010, Vic, Australia

Email: h.davies@unimelb.edu.au

Abstract

The objective of this research was to investigate how the range of flexion and extension of the canine elbow joint was constrained by the mechanical connections and attachments of soft tissue structures. The skin, a section of deep fascia and several muscles from both forelimbs from 6 adult greyhounds and 7 other breeds were sequentially transected or removed, over 13 steps. During each step, repeated measurements of elbow flexion and extension were recorded using a goniometer. Only marginally significant changes to the range of flexion occurred in any of the 13 steps or overall for the greyhounds. Clearly significant changes to extension occurred in several dissection steps. Removing the skin resulted in a significant increase in elbow extension of 1.7° ± 0.3 ($P<0.001$) in the greyhounds and 1.6° ± 0.3 ($P<0.001$) in the other breeds.

Severing the deep fascia from the humerus and its connections across the elbow joint resulted in the largest significant change in elbow extension of 9.9° ± 0.3 ($P<0.001$) in the greyhounds and 6.9° ± 0.7 ($P<0.001$) in the other breeds. Transecting the biceps brachii m. close to the elbow resulted in an increase of 2.8° ± 0.3 ($P<0.001$) in the greyhounds but a non-significant change in the other breeds; and transecting the extensor carpi radialis m. from its origin resulted in an increase of 5.5° ± 0.4 ($P<0.001$) in the greyhounds and 3.9° ± 0.7 ($P<0.001$) in the other breeds. These results suggest that the collagenous framework and attachments of the skin, deep fascia, and extensor carpi radialis m., play a significant role in the function of the canine elbow by restricting it from overextension and hence stabilising it during periods of loading, in a variety of different canine breeds, and that these structures are functionally integrated into the way the forelimb supports the body weight separately from any involvement of muscle tone or muscle movements. Observations on the anatomical connections of the deep fascia between the cranial distal humerus and the antebrachial fascia highlighted its probable importance in relating movements between the shoulder and the carpus.

Keywords: canine elbow joint; fascia; extension; flexion; goniometry

Introduction

Effective and efficient joint function is an important part of locomotion for quadrupedal animals like the domestic dog. In the canine elbow joint the more proximal bone (the humerus) is maintained at an angle to the vertical during stance. Hence stabilising structures like muscles and ligaments are required to support it in this position against gravity. Understanding the passive and dynamic roles of anatomical connections which contribute to the normal function of the canine elbow joint will help determine how it may be supported and stabilised during loading. This could provide insight into the effects of surgical procedures on the function of the joint and the forelimb as a whole. Furthermore, investigating the anatomic constraints of elbow joint movements, could assist in understanding the presence and significance of mechanical stabilisers during joint function, which may contribute to the current knowledge concerning pathology or injury to the elbow.

The canine elbow is a composite joint that is currently understood to be restricted to flexion and extension by the bone shape and the thick collateral ligaments, while showing limited rotational movements (Evans & de Lahunta, 2013). Muscles that attach across the joint act as mechanical actuators of movement at the elbow. According to the literature the role of muscle has been well-studied in the canine, with papers ranging from understanding its general function and anatomy, to its moment arms, and its electromyographic activity (Tokuriki, 1973a; Tokuriki 1973b; Tokuriki 1974; Williams et al, 2008; Evans & de Lahunta, 2013). Williams et al (2008) have shown how muscular connections can have a passive role during locomotion in the absence of muscle action. However, the role of the deep fascia as a complex structure which can passively relate movements, does not seem to be well described. Evans and de Lahunta (2013) have loosely described what seems to be representative of this region of deep fascia in the canine but its orientation in relation to muscle, its connections, and its possible functional roles remain unclear. Similarly, it is not clear whether these soft tissue structures also provide passive stabilisation and restriction to the joint during movement.

The current literature contains several studies which have explored canine elbow joint range of movement mainly in terms of an holistic analysis of canine locomotion using kinematics (Pfau et al, 2011; Carr et al, 2013; Birch et al, 2015; Goldner et al, 2015). This general approach to canine movement often ignores or fails to provide an in depth understanding of what is actually occurring at the joint of interest. Some studies however have appreciated the importance of understanding joint range of movement, in order to understand its function and the surrounding structures involved (Angle et al, 2012).

In the studies mentioned above, a number of tools and methods were used to measure joint angles such as radiography, kinematic related tools and goniometry. Goniometry has been a useful tool in a number of veterinary studies for a large range of animals including horses, cats, sheep and dogs for a range of different purposes (Thomas et al, 2006; Jaeger et al., 2007 ; Liljebrink & Bergh, 2010; Lascelles et al, 2012; Govoni et al, 2012; Greene et al, 2013; Freund et al, 2016). In one study, Jaeger et al, (2002) compared and validated goniometric joint measurements with radiographic measurements in non-sedated and sedated Labrador retrievers. Their study found that goniometry was a repeatable and valid method.

In the current study, a plastic goniometer was used to investigate whether flexion or extension of the canine elbow joint was mechanically constrained by certain soft tissue structures in the forelimb. Using goniometry to detect the effect of mechanical constraints on elbow joint movement without neurological input could provide some insight into how locomotor movement is constrained at the elbow and perhaps how it is coordinated with the rest of the forelimb. Furthermore, this study may also indicate how certain structures are involved in elbow stabilisation. Understanding how soft tissue structures contribute mechanically to the elbow joint of the canine as passive stabilisers independent of muscular action, may be important in determining whether abnormality or injury to the soft tissues might affect the stability and mechanical function of the joint. The null hypothesis of this study is that a sequential dissection of soft tissue structures supporting the elbow will not result in any changes to the range of flexion or extension.

Materials and Methods

Cadaver Limb Collection and Storage

Animal Ethics approval was not sought as forelimbs were collected from adult cadavers of dogs deceased for reasons unrelated to the study, and owners had approved the use of the cadavers in teaching and research. After being removed from the freezer (approximately -22°C) and defrosted for approximately 4-6 days in the chiller at 5-6°C, forelimbs were separated from the trunk by cutting between the scapula and the thorax and were then stored in plastic body bags in the freezer (approximately -22°C) until required. A few forelimb specimens were collected immediately from fresh cadavers and then frozen as described. When needed, forelimbs were left to defrost in the chiller in plastic body bags at 5-6°C for 4-6 days. No samples were refrozen between measurements so the maximum number of freeze, refreeze cycles for any specimen was two (as an intact cadaver and then as an intact forelimb).

Twelve Greyhound specimens consisting of six left and six right forelimbs from three males and three females were used to measure the mechanical restrictions of the elbow joint. A further 14 limbs from 7 other breeds (a miniature fox terrier, Maltese terrier, Pomeranian, pug cross, beagle, Labrador and a kelpie cross blue heeler) were measured using the same protocol.

Goniometer measurement and method of dissection

A plastic Liberty® 360° 25cm goniometer with 1° gradations was used to measure the range of flexion and extension of the canine elbow joint in the Greyhounds, and the Labrador, kelpie cross blue heeler and beagle. Due to the small size of the forelimbs, a plastic 66fitTM 360° 6-inch goniometer was used to measure flexion and extension of the elbow for the Pomeranian, Maltese terrier, miniature fox terrier, and pug cross. Measurements of flexion and extension of the joint were taken with the forelimb placed on a flat even surface so that the lateral aspect of the forelimb was the upper surface. The centre of the goniometer was placed onto the centre of the lateral epicondyle of the humerus. One arm of the goniometer was aligned close to and along the distal half or third of the humerus, where the bone could be palpated between the biceps brachii m. and brachialis m. The other arm of the goniometer was aligned with the long axis of the antebrachium (Figure 1). The angle between the two arms of the goniometer was recorded.

Flexion and extension of the joint were both measured following a single motion at the point where a large change in resistance to the motion occurred and where further movement would have required considerable force beyond the range that would have been likely to have been experienced in life. The positioning of the goniometers and the handling of the forelimb during flexion and extension is shown in Figure 1. A single investigator dissected, measured and recorded both the left and right forelimbs from a single dog in one session, always within the same day. When measuring with the goniometer, the investigator measured and recorded the range of flexion and extension of one limb, followed by the other limb, and then alternating between the two limbs. The investigator would only read the angle on the goniometer when the limb was placed into its respective position. Previously recorded measurements were progressively covered so they were no longer visible to the investigator. For each greyhound, this was repeated another four times for both the left and right elbows between each dissection step. Due to differences in forelimb shape and size in the other breeds, and the use of two different goniometers; 6 repeated goniometer measurements for each position and dissection step were taken for the non-Greyhound specimens. During dissection and measurement of each canine forelimb, any obvious signs of pathology were noted.

Prior to this study, 10 pilot studies were conducted to develop a series of ordered dissections and to determine what structures may affect elbow movement. Structures chosen for dissection were selected based on their perceived attachments and their identification as active movers of the elbow joint. The order of dissection was determined based on the potential for dissecting structures in a certain order, as well as ensuring the method of dissection could be performed consistently. The following measurements and dissections steps were identified from these pilot studies:

1. Skin intact
2. Skin removed from the lateral surface of the brachium to approximately half way to the carpus (9-10cm distal to the lateral epicondyle of the humerus in the greyhounds) and extending across the antebrachium.
3. A longitudinal incision (Approximately 2cm in the Greyhound, and considerably less, being about 10% of the length of the brachium in smaller breeds) was carefully made on the lateral aspect of the brachium through the deep fascial layers and close to the edge of the lateral head of the triceps brachii m. and superficial to the brachialis m., which exposed the brachialis m. underneath the deep fascial layers. (Figure 2).
4. The incision made in step 3 was widened (to approximately 4-5cm in the Greyhound and considerably less and to about 25% of the length of the brachium in the smaller breeds). The loose connective tissue between the brachialis m. and the fascia was then separated. This was so the fascia could be severed easily from the humerus and other regions in step 5. The attachment of the deep fascial layers to the cranial aspect of the humerus and its connections across the elbow were maintained. (Figure 3).

5. All the layers of deep fascia which joined onto the cranial aspect and around the distal half of the humerus were detached or cut. The insertion of the cleidobrachialis m. of the brachiocephalicus m. onto the humerus was difficult to separate from the insertion of deep fascia, so it was often transected as a result of severing the attachment of the deep fascia. Furthermore, the rest of its attachments to the antebrachial deep fascia were severed close to the region of the extensor carpi radialis m. origin. (Figure 4)
6. The brachialis m. was then transected and the bulk of the muscle removed.
7. Most of the cleidobrachialis m. (of the brachiocephalicus m.) and superficial pectoralis m. superficial to the biceps brachii m., were removed including the fascial layers surrounding the biceps brachii m., so that the biceps brachii m. was clearly visible.
8. The biceps brachii m. was then transected distally and the bulk of the muscle removed.
9. The extensor carpi radialis m. was detached from its origin on the humerus.
10. The tensor fasciae antebrachii m. and the fascial layer covering it were transected distally and then removed.
11. The long head of the triceps brachii m., was transected distally close to its insertion and separated from the other heads of triceps brachii m., followed by removing the bulk of this section of muscle.
12. The accessory, medial and lateral heads of the triceps brachii m. were transected and then most of them removed.
13. The anconeus m. was then detached completely from the forelimb. Part of the joint capsule intimately adhered to the anconeus m. was also removed.

Following each step, the range of flexion and extension of the elbow joint was measured and recorded with the goniometer as described. Prior to the dissection, the investigator took time to initially flex and extend the limbs enough to reduce any residual effects of rigor mortis. The presence of rigor mortis was identified if the forelimb was still rigid and if flexion and extension of the elbow was inconsistently changing when measured with the goniometer. If this occurred, the investigator would again flex and extend the elbow until consistent measurements could be achieved. Images were taken using a Canon IXUS 170 camera and Iphone SE camera.

Anatomical investigation of the deep fascia

Greyhound limb collection and storage

10 Greyhound forelimbs were collected from dogs that were euthanased for reasons not associated with this study and stored as described above.

Dissection and fascial description

Each greyhound forelimb was dissected to observe and describe the anatomy of the deep fascia on the craniolateral aspect of the distal brachium. Thickness of the fascia, direction of collagen fibres, attachments to bone and its distribution were noted. Using a Panasonic Lumix DMC-LXS camera, images of the deep fascia were taken and Photoshop used to add labels to the images. Images and video footage were taken with the elbow being flexed and extended, and the carpus being loaded and unloaded manually using pressure on the palmar surface of the paw.

Effects of refrigerated storage on flexion and extension of the greyhound elbow joint

The purpose of this part of the study was to explore whether time in refrigeration affected the range of flexion and extension of the greyhound elbow joint.

Reflecting the protocol for storage as used in this study, a large plastic body bag containing four forelimbs was removed from the freezer to defrost in the chiller on day 0. Two right and two left forelimbs from four individual greyhounds were used. On day 4, all limbs were removed from the chiller and using the goniometer, their range of flexion and extension was measured using the same method as described previously. Each measurement for flexion and extension was recorded once for each forelimb before repeating this sequence of measurements another four times. When all limbs had five recorded goniometer measurements for flexion and extension each, the limbs were then placed back into the chiller. The measurements were repeated on day 5 and day 6.

Data Analysis

Minitab® 17 Statistical 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 greyhound forelimb, or the six measurements taken from the other breeds. 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 2013 was used to plot multiple line graphs depicting separately the flexion and extension of each pair of canine forelimbs (mean of 5 or 6 measurements of left and right) for each step of the dissection.

Repeatability.

Using the WinPepi program (version 11.65), the coefficients of repeatability were calculated for the respective goniometry measurements during flexion and extension of the elbow joint to indicate the difference between each goniometer measurement. The coefficient of repeatability was calculated using the data collected for step 1.

To compare the changes in elbow flexion and extension over day 4-6 in response to the effects of time in the chiller, the data was statistically analysed using a one-way analysis of variance (ANOVA).

Results

The results are presented in Tables 1, and in Figures 5 and 6, which depict changes to flexion and extension following each step of the dissection in the greyhounds and Figure 7 which shows the data for restrictions to elbow extension in the other breeds. There were no clearly significant changes found during elbow flexion in any of the 13 steps, nor overall (Figure 5). The only consistently significant effects were found during extension.

In the greyhounds there was an increase in elbow extension (Figure 6) with the following steps: when the skin was removed (step 2, $+1.7° \pm 0.3$, $P<0.001$), with transection of the deep fascia (step 5, $+9.9° \pm 0.3$, $P<0.001$), biceps brachii m. (step 8, $+2.8 \pm 0.3$, $P<0.001$) and extensor carpi radialis m. (step 9, $+5.5° \pm 0.4$, $P<0.001$). No clear trends or differences between the left and right forelimbs were identified and there were no significant differences between male and female in the elbow measurements.

In the non-greyhound breeds, the data demonstrated similar trends in changes to extension at step 5 (+6.91° ± 0.65; $P<0.001$) for all breeds where the deep fascial connections were severed from their attachment on the humerus and across the elbow joint. Removal of the skin (step 2; +1.61° ± 0.34; $P<0.001$), and removal of the extensor carpi radialis m. from its origin (step 9; +3.94° ± 0.65; $P<0.001$); showed individually variable, but overall significant, changes to elbow extension for each breed (Figure 7). In contrast to the greyhounds, transection of the biceps brachii m. (step 8; +0.49° ± 0.26; $P=0.086$) was found to be non-significant in most individuals. The only clear change to extension at this step was found in the elbow joints from the kelpie cross blue heeler.

The repeatability coefficient for the Liberty® 360° 25cm goniometer when measuring the elbow joint during extension was 4.7°. This had a 95% confidence interval of 3.9° to 5.9°. The repeatability coefficient for this goniometer measuring the elbow joint during flexion was 2.8° with a 95% confidence interval of 2.3° to 3.5°.

The repeatability coefficient of the 66fitTM 360° 6-inch goniometer when measuring elbow extension was found to be 4.3°. This had a 95% confidence interval of 3.6° to 5.5°. The repeatability coefficient for the same goniometer measuring the elbow joint during flexion was 3.1°. This had a 95% confidence interval of 2.6° to 4.0°.

When comparing the overall changes in the study (Table 1), significant changes were found for both flexion ($P<0.05$) and extension ($P<0.001$) of the greyhound elbow joint and just for extension in the other breeds ($P<0.001$).

Following dissection of the Pomeranian forelimbs, clear signs of arthritic changes to the elbow joints were found, especially in the right elbow. All other elbow joints appeared free of significant obvious pathology.

Before transection of the extensor carpi radialis m. from its origin, it was also noted that manual extension of the elbow allowed the carpus to ‘snap’ or ‘flick’ into an extended position. Severing this muscle from its origin onto the humerus, not only resulted in an elbow which could extend further, but it also interfered with the carpus’s ability to sustain an extended position. This occurred despite other extensors of the carpus remaining intact.

The average flexion and extension of the greyhound elbow joint in response to storage at 5-6°C over days 4, 5 and 6, were calculated and are shown in Table 2. Using ANOVA, the results suggest that the changes in extension of the elbow over the three days that were measured were non-significant (P=0.471). Furthermore, the changes in flexion of the elbow over the three days were also found to be non-significant (P=0.993).

Observation and description of the deep fascia restricting elbow extension in the greyhound

When the greyhound forelimbs were dissected a layer of superficial fascia could be identified covering the deep fascia of interest. Incising through this layer of fascia on the lateral aspect of the forelimb in the region of the brachialis m. revealed the layer of deep fascia that restricted elbow extension.

This section of deep fascia, which appeared to be thicker than other areas of brachial fascia, extended distally along the extensor carpi radialis m., where it merged and became part of the antebrachial fascia on the forearm. On the brachium, this section of deep fascia divided into two deep fascial layers, where each layer was distributed either underneath or above the lateral head of the triceps brachii m.

This section of deep fascia was attached continuously along the cranial aspect of the humerus from the deltoid tuberosity distally to the level of, and just lateral to, the insertion of the cleidobrachialis m. (distal part of the brachiocephalicus m.). At the region where the deep fascia attaches along the cranial surface of the humerus, the attachment of connective tissue was quite thick and solid. (Figure 8 and Figure 9)

Where the insertion of the fascia ended lateral to, and at the level of, the cleidobrachialis m. insertion, the deep fascia continued medially onto the brachium as well as distally distributing and merging with the antebrachial fascia.

To understand where and to what extent the fascia was restricting the movement or extension of the elbow joint, the elbow was flexed and extended while the fascia was being palpated. Flexion of the elbow joint resulted in a lack of tension and stress throughout the fascia in this area of the forelimb. However, during extension of the elbow joint, the fascia became quite taut and under tension around the region of the brachialis m. and extensor carpi radialis m. Furthermore, the tension seemed to distribute from where the fascia was attached onto the cranial surface of the humerus, relatively along the length of the brachialis m. and through the antebrachial fascia along the whole length of the extensor carpi radialis muscle. Tugging on the deep fascia from the region of the humerus, seemed to influence movement at the carpus. Increasing tension caused extension of the carpus, and even slight extension of the digits.

In the region superficial to the brachialis m., collagen fibres were clearly seen as a component forming the layer of fascia (Figure 10). Other fascial areas of the brachium did not show such clearly visible collagen fibres. These collagen fibres were white in colour, and were cross-hatched at the most proximal section of the fascia. Fibres running transversely, were also superficial to the lateral head of the triceps brachii muscle. However, the cross-hatching of collagen fibres became less apparent for the majority of the fascia covering the brachialis and extensor carpi radialis muscles. Collagen fibres running in a proximal to distal direction along the length of the brachialis m. could be seen. These fibres coursed towards the elbow joint and distally towards the antebrachium.

Discussion

This study focused on the passive mechanical connections and attachments of certain soft tissue structures and their effect on the canine elbow joint. The results demonstrated that the removal or transection of soft tissue connections of canine cadaver forelimbs led to changes to the range of elbow joint extension when measured with a goniometer. This indicated that the restrictions to elbow joint movement as a result of the collagenous framework and attachments of muscles and deep fascia, may have an important role in forelimb function.

The canine elbow joint is supported and stabilised by a complex interaction of passive and dynamic connective tissues during locomotion. Manipulation of cadaver specimens demonstrated that these connections tend to relate the movements of the elbow with the rest of the forelimb, so it seems likely that they help to constrain and direct the mechanical movements of the whole forelimb.

The results showed that the deep fascial connections restricting the elbow joint during extension and loading were likely to play a role in the stability of the joint in a range of breeds. This confirmed that the structure was not specific to athletic breeds such as the greyhound, and that it appears to serve as an integrating structure for general functional movements of the forelimb in a range of very different canines. When assessing the effects of transecting the connections of certain musculature, there was an increased variation in changes to extension between the breeds. This could be explained by differences in how bulky the musculature was between breeds and between individuals. Amount of exercise and breed purpose could impact the importance of the fascial architecture and connections of muscles such as the biceps brachii m. and extensor carpi radialis m.

Furthermore, it was observed that the arthritic changes to the elbow joints of the Pomeranian were associated with differences in the range of flexion and extension. In the right elbow joint during extension, there was a general lack of response to the dissection except when the deep fascia was cut in step 5, which suggests that pathological changes to the bones and other tissues of that elbow joint had some direct mechanical effect on joint movement. Only a few studies have assessed elbow joint range of movement in dogs with pathology (Burton et al. 2008; Caron et al. 2014; Galindo-Zamora et al. 2014).

The removal of the skin led to a clearly consistent slight increase in elbow extension. These changes suggest that the elastic properties of the skin could contribute to the mechanical restriction of the elbow joint during extension. To our knowledge, previous studies have not included consideration of the possible role of skin in the mechanics of limb movement in the canine. Its potential role in the mechanics of elbow joint movement highlights the requirement for further research into understanding its contribution to joint function.

Some dissection steps exhibited statistically significant changes in flexion or extension of less than 1°. An average difference in flexion or extension of less than 1° is less than the gradations of the goniometer. It seems more likely that these “significant” differences in flexion and extension of the elbow were the result of statistical error and therefore may not indicate a restriction of the joint. This is evident in the representation of changes in angle in the graphs where no consistent pattern is evident in the relevant steps. As a result, steps which indicated statistical significance but showed changes of less than 1°, were not considered in this study as mechanical restrictors of the elbow. As a result, none of the dissected structures were considered to mechanically constrain elbow joint flexion. This could suggest that the flexion of the elbow joint may be mechanically constrained primarily by structures forming the joint such as the bones, ligaments or joint capsule. However, several of the antebrachial muscles which were not dissected in this study could have some passive mechanical effect on the flexion of the elbow joint. Ultimately, further investigation into these structures would be required.

The dissection of the biceps brachii m. led to a statistically significant increase in extension of the greyhound elbow joint. This suggests that the fascial architecture and attachments of the muscle contributes to the mechanical restriction of the elbow joint during extension. This would be in addition to the underlying muscle tone and neuromuscular response in a live greyhound. The biceps brachii m. has mainly been described as a muscle which flexes the elbow, and extends and stabilizes the shoulder joint during the stance phase of locomotion (Goslow et al. 1981). Furthermore, it has also been reported to create its greatest moment arm across the elbow and shoulder joint during the stance phase, providing stability to the forelimb and preventing it from collapsing (Williams et al. 2008). However, no other study to our knowledge has specifically related its muscle attachment and fascial architecture to its potential contribution to elbow stability by restricting it during elbow extension in the greyhound. In the other breeds, overall there was no significant change in elbow extension when biceps brachii m. was transected, and only the kelpie cross blue heeler showed any difference at all (Figure 7). This could be due to these breeds not being specifically bred for athletic performance, or just that the individuals used in this study were generally lacking in athletic fitness.

The extensor carpi radialis m. mechanically constrained the extension of the elbow joint in the greyhound as well as all the other breeds. It showed a greater contribution to the restriction of elbow extension than the biceps brachii m., which suggests that it may have a larger involvement in stabilizing the elbow during extension. To our knowledge, studies have only identified muscular activity in the extensor carpi radialis m. during flexion of the elbow and extension of the carpus, based on the use of electromyography (Tokuriki, 1973a; Tokuriki 1973b; Tokuriki 1974). However, the current study shows that the muscle has a passive role in restricting elbow extension. Further observation also demonstrated that the attachments and connections of the extensor carpi radialis m. have a passive influence on other parts of the limb by increasing the extension of the carpus when the elbow is extended. This was supported by further evidence when severance of this connection led to a clear and consistent reduction in carpal extension in all specimens when the elbow was manually extended. This additional mechanical role could potentially highlight the coordination between joints and overall forelimb movement during locomotion independent of muscle tone or contraction. The relating of joint movements would also allow specific loading of the forelimb, and may help to prevent the forelimb from collapsing, by ensuring that both the elbow and carpal joints are stabilised when extended, in addition to the support provided by active muscles and other related structures in a living dog. These descriptions could reflect a passive stay apparatus type function for the extensor carpi radialis m. and associated fascia, similar to the equine forelimb. Nevertheless, the results from this study highlights the importance of the extensor carpi radialis m. during the weight bearing phase of locomotion.

The greatest change in extension however, based on the structures which were dissected in the current study was the transection of the deep fascia from the humerus and its connections across the joint. During the dissection of the deep fascia, the insertion of the cleidobrachialis m. (of the brachiocephalicus m.) was severed whenever it’s insertion could not be separated from that of the deep fascial attachment to the humerus. However, measurement of any effect of the brachiocephalicus m. or other extrinsic muscles on elbow movement would require the forelimb to be retained in situ on the cadaver. In the current study all the extrinsic muscles were transected during removal of the limbs. Hence any potential effect of these muscles on the elbow could not be included and the lack of restriction or tension during elbow extension from the cleidobrachialis muscle or the superficial pectoral m. before the transection of any remnant insertions, highlights that it was the deep fascia restricting the elbow joint in this stage of the current study. The results suggest that this section of deep fascia is the greatest contributor to the passive mechanical stability of the elbow by preventing elbow overextension. Elbow extension typically occurs when the forelimb of the canine makes contact with the ground during movements such as walking, trotting, galloping or jumping. During ground contact, the forelimb is likely to be subject to a variety and variability of forces and loads depending on the type of activity being performed (Gillette & Angle, 2014). This would mean that the skin, biceps brachii m. (in the greyhound only), extensor carpi radialis m. and deep fascia contribute to the stabilisation and restriction of movement of the elbow during extension independent of any muscle activity. The large change in elbow extension as a result of dissecting a section of deep fascia (+9.9° ± 0.3 in the greyhounds and +6.91° ± 0.65 in the other breeds $P<0.001$) highlights its greater contribution to the stability of the elbow compared to the other structures studied in this experiment. Because the elbow loads when extended, the ability of the deep fascia to stabilize or restrict the extension of the elbow, would also require it to repetitively endure high loads and stress during locomotion. Its ability to tolerate these stresses could relate to its composition allowing strength and stability, which may also assist in the loading and transmission of forces as suggested for fascial structures in other sites and species (Huijing et al, 2003; Fratzl, 2008; Benjamin, 2009; Stecco et al, 2011).

Observations made from this study have described a clear site of attachment to the humerus and an expansive distribution of fascial connections in the region of the greyhound forelimb. These observations also seem to relate to what is described by Künzel et al (1993) where there are descriptions of the craniolateral brachial and antebrachial fascia for the horse, cow and pig. That description suggests that the anatomy of the deep fascia in this region of the greyhound forelimb is comparatively similar. However, based on descriptions by Künzel et al (1993), the attachment of the deep fascia on the cranial surface of the humerus is described as ending at the radial fossa in the species studied. In the current study, the attachment of the deep fascia was observed to end at the level of insertion of the cleidobrachialis m., which is proximal to the radial fossa. Künzel et al (1993) also did not explore the functional significance of the deep fascia in these animals. Based on the observations in the current study, this section of deep fascia seems to have a role in relating the movements of the shoulder, elbow and carpus, hence assisting the coordination of the canine forelimb in a way similar to how the deep fascia potentially connects different segments of the body allowing peripheral motor coordination in humans as suggested by Stecco et al. (2008). Its extensive distribution throughout the canine forelimb and the evidence provided in the current study, support this finding. The possibility that there is a passive mechanical coordination of the joint of the canine forelimb, may ensure that the forelimb can be stabilised during extension. Like the horse, which has a well-recognised passive stay apparatus, where the connectivity and supportive mechanisms of the tendons and ligaments minimizes muscular effort in the limb, these connections in the canine may have a similar yet more subtle function in the canine forelimb. In addition to relating movements through these fascial connections, the distribution and merging of the brachial deep fascia from the humerus to the antebrachial fascia, could indicate the transmission and distribution of stress or load through this region of the forelimb. The arrangement of collagen fibre directions is an indication of the resistance it has to traction running in the same direction of the fibres (Stecco et al. 2011). Collagen fibre's morphological characteristics are also related to stability, toughness and strength (Fratzl, 2008). So the cross-hatching of the collagen fibres at the most proximal section of the deep fascia close to the deltoid tuberosity, suggests the presence of a multi-directional pull or strain on the fascia, which could be providing stability in reaction to loads from numerous directions. Distally, according to the direction of collagen fibres, which run towards the elbow joint, and the distribution of deep fascia onto the antebrachium, it would suggest a transmission and distribution of load in a direction which has a relationship to how movements between the brachium and antebrachium are integrated.

In addition to the passive mechanical forces created as a result of the fascia restricting the elbow during extension, and the coordination of forelimb movement, the transmission of tension is generated by muscular contraction from muscles such as the brachialis m. and extensor carpi radialis m. This would also suggest that the deep fascia would play a role in the transmission of stress during elbow flexion because the brachialis m. is a known flexor of the elbow joint, and clearly contraction of the muscle would increase tension in the covering fascia.

The attachment of the deep fascia to the cranial aspect of the humerus could indicate some role in distributing load and stress between the humerus and the distal connections of the forelimb. This could be the case when the elbow is extended and the deep fascia is constraining the joint by tension acting on its attachment to the bone. The direction of the attachment and the effect of tension on this fascial connection that might be expected to occur when the limb is extended, suggests that it would cause a compressive force on this region of the humerus. This may highlight a role in the loading of the humerus. This attachment and relationship with the periosteum of the bone highlights the continuity and integration of connective tissues behaving like a system rather than as a separate entity. Gerlach and Lierse (1990) refer to this sort of relationship as a bone-fascia-tendon system, noting the crural fascia, the iliotibial tract, and femoral and crural intermuscular septa as examples. The human iliotibial tract is thought to stabilize the human knee joint (Gerlach and Lierse, 1990; Flato et al, 2017). It connects the ilium to the tibia and more distally (Gerlach and Lierse, 1990), and has been described as a coalescence of the aponeurotic covering of the tensor fascia lata m. and gluteus maximus m. as well as the fascia lata (Flato, 2017). In a similar way the deep fascia of the canine brachium appears to be a coalescence of the fascia associated with the muscle sheaths of the brachialis m., the triceps brachii long and lateral heads, and the extensor carpi radialis m. extending into the antebrachial fascia. As such, any contraction of the large triceps muscle would increase tension on the deep fascia and assist in resisting elbow extension, hence passively stabilising the elbow whenever triceps was active. The fibre directions visible in Figure 10 support this possibility. The potential effects of contraction of brachialis and extensor carpi radialis on the deep fascia is less clear, although the bulk of extensor carpi radialis m. alone passively restricted elbow extension while brachialis did not in the current study. The passive stay apparatus in the horse is another example of bone, tendon and ligaments acting on each other as a system to passively stabilise and support the limbs. The tension band effect created by the iliotibial tract in humans could relate to a role of this region of deep fascia acting on the humerus based on the angle it sits at during stance (Gerlach and Lierse, 1990). Fascia or membranes of similar composition have been shown to have a possible role in the mechanical loading of bone structures based on modelling and finite element analysis (Curtis et al, 2011; Fechner et al, 2013). The ischiopubic membrane, which has similar fascial characteristics in the domestic fowl, was shown to have a mechanical significance in influencing the mechanical loads on the scapus pubis by distributing loads to other adjacent structures (Fechner et al, 2013). This mechanism ultimately contributes to the development of more delicate bone structures despite the high mechanical loading (Fechner et al, 2013). The temporal fasciae in Macaque monkey skulls also has a similar role in providing stabilisation and reducing loads around the zygomatic arches (Curtis et al, 2011). These studies highlight the possible role of the deep fascia in the loading and distribution of load onto the canine humerus and may warrant further investigation.

Based on the significant effect that the deep fascia has on an extended elbow, small tears or damage to this area could realistically be expected to affect the stability and loading of the joint. It is understood that the distribution of load and contact pressure within the canine elbow joint may be affected by joint angle (Preston et al, 2000; Mason et al, 2005; Cuddy et al, 2012). So if damage to the fascia is enough to cause a slight increase in extension as well as induce instability to the joint, then perhaps abnormal changes to contact pressure and the distribution of load through the joint could be expected. Indeed, such a change in load and stability to the joint is likely to lead to functional implications. Alternatively, damage to the deep fascia could also lead to pain and pain-avoidance responses that could cause increased stiffness, and restrict elbow movement, hence leading to other complications. However, further investigation is required to understand the potential problems resulting from damage to the deep fascia in this region of the forelimb. Ultimately, it is evident that the deep fascia and potentially other soft tissues contribute to providing a passive mechanical restriction of the elbow when extended, hence providing a potential means of increasing stability and preventing an unnatural overextension of the joint. With elbow loading also being determined by joint angle, these structures ensure that the elbow is specifically loaded through regions within the joint that are capable of withstanding such loads during movement, especially when muscles are fatigued and the joint must rely on these passive connections for stability.

Limitations and implications to the study

In this study, canine cadaver forelimbs were collected based on availability, timing and storage space. The collection of history and information on each canine such as age and medical history, would have been a beneficial addition to the study. However, time constraints and the accessibility of such information made this difficult. Nevertheless, the selection of canines in this study provides a representation of the racing greyhound population in Victoria. The other breeds of dogs were a random sample of available specimens but included a range of older ages and very variable levels of fitness as well as some breeds that have been selected for a small size rather than any athletic attributes unlike the greyhounds. Hence the similar results to the greyhounds suggest that these findings might be applicable to all canine breeds and types.

The detachment of the forelimb from the body of each canine meant that the study was unable to assess whether structures proximally associated with the trunk or shoulder region had an effect on the elbow joint. Dissecting the forelimb alone, allowed the investigator to access or dissect the medial side of the forelimb by simply turning it over with the lateral aspect face down, rather than having to develop a different and less convenient method to dissect those medial structures.

It must also be acknowledged that a canine cadaver limb does not represent the complete function of the forelimb. The study is dealing with a complex biological system where other components of the forelimb will also affect the elbow joint in a live canine. For example, the presence of muscle tone and muscle reflex responses would be expected to play a large role in the stability of the elbow joint or forelimb, affecting the range of flexion and extension of the elbow joint. However, the lack of neurological input also benefits the study in demonstrating that the changes in extension of the elbow joint were the result of mechanical connections being severed and therefore independent of any neurological effects on muscle contraction.

Dissections on each forelimb were not exactly identical. However, it may also be deduced that minor variations in each dissection were not enough to affect similar trends in changes to the extension of the elbow joint. Also, not every structure of the forelimb was dissected. Investigation may be needed to understand whether antebrachial structures have an effect on the flexion or extension of the elbow joint. How structures may mechanically affect the rotation of the joint should also be explored.

It is also worth considering the type of incision used to incise the deep fascia in step 3 and step 4. In this study, an incision sagittal to the long axis of the forelimb and along the deep fascia was used in these steps. A transverse and a sagittal or longitudinal incision could potentially lead to different results and change the interpretation of the constraints created by certain structures on the range of movement of the elbow joint. In comparison to a transverse incision of the deep fascia, a sagittal incision could lead to a greater change of tension in underlying structures such as the brachialis m. in this study, allowing it to expand and move more. Understanding this, it could be suggested that a loss of tension from the brachialis m. as a result of a sagittal incision of the deep fascia superficial to it, may affect its perceived passive constraint on the elbow. However, should a change in restriction to the elbow occur, then this would have been made obvious in steps 3 or 4. This would therefore suggest that the sagittal incision of the deep fascia either did not have a perceived effect on the elbow joint or was undetectable in this study.

The length of incision made is also worth comment in this study. Length of incision was relatively consistent in the greyhound specimens, but obvious size difference in the other breeds meant that changes to the length of incision needed to be adapted to the size of each specimen. Length of incision for each breed was not recorded for the other breeds. However, no obvious effect was observed based on different lengths of incision into the deep fascia in step 3 and step 4.

Another source of error worth noting is the effect of freezing and defrosting on tissue mechanics and constraints on the range of elbow movement. Changes to meat quality as a result of freezing and thawing is a well-studied area in meat science (Leygonie et al, 2012). Physical quality parameters of meat affected by freezing and thawing such as moisture loss, protein denaturation, and shear force, include several factors that could potentially have some effect on the specimens used in this study (Leygonie et al, 2012). The same changes to muscle may also apply to other soft tissue structures such as the deep fascia. This would therefore suggest that there are possible effects of freezing and defrosting on what constrains the elbow joint. However, difficulties in acquiring suitable specimens and ethical constraints on specimen collection and use mean that constant access to fresh material left the study with no choice but to use freezing and refrigeration as a means of storage and preservation. Furthermore, we were also limited by the type of freezing and refrigeration used. Several studies have explored the effects of freeze-thaw cycles in several different animal meats from sheep, pigs and chicken, all demonstrating that multiple freeze-thaw cycles can affect meat quality and should be limited (Qi et al, 2012; Ali et al, 2015; Zhang et al, 2017). It should therefore be acknowledged that there could be similar effects to the structure of the soft tissue structures dissected. However, this would need to be explored in the conditions the current study was conducted in to determine if there are any significant effects on soft tissue. To help limit or avoid excessive freeze-thaw cycles, the specimens were only frozen and thawed a maximum of twice and only frozen at all when unavoidable.

This current study did however explore the effects of the chiller, and whether the amount of time spent (days) in the chiller had any effect on the range of movement of the elbow joint in the greyhound. Throughout the whole study, limbs were initially packaged in groups of four in large plastic body bags and stored in the freezer at approximately -22°C. During the defrosting process, each bag of four forelimbs was moved from the freezer to the chiller on day 0 to defrost. The first pair of forelimbs was dissected on day 4 and the second pair dissected on day 6. The temperature of the chiller was usually set between 5-6°C. If soft tissue is directly exposed to the conditions inside the chiller for too long, dehydration of the tissue may occur. The forelimbs can therefore be affected by the length of time and the conditions in the chiller, which could affect elbow joint movement. As a result, to test the effects of the chiller over time, a similar preparation involving the defrosting process was used for this study. The results demonstrated that the length of time spent in the chiller which reflected the time spent before dissection, did not significantly affect the range of movement of the joint. Performing a similar study method on fresh material to see if the same results can be obtained would have been a valuable addition. Although there are limitations to the use of refrigeration and freezing for preservation of specimens, it is however a better option compared to more traditional methods requiring the fixation of tissues, which potentially affects the integrity of the fascia and muscles explored and prevents a more natural movement of the forelimb.

Additionally, a single investigator was responsible for all dissections, measurements and recordings in this study. Hence the investigator was not completely blind and it could be argued that there is the chance that the study could be affected with unintentional bias. Several measures were taken to help reduce the chances of this occurring such as progressively covering up recorded data each time a measurement was taken, or aligning the goniometer with the flexed or extended elbow before reading the gradations of the goniometer. Ultimately, the recording and measurement of the angle of the elbow with the goniometer relied on the investigator's ability to have a consistent approach to the study.

However, it may also be questioned whether the results found with one investigator will vary significantly compared to the results obtained by another investigator performing the same study. In one study, Jaeger et al (2002) assessed inter-observer variability with goniometric measurements in the Labrador retriever, and demonstrated there were no significant differences in measurements between the 3 independent investigators that were used. Furthermore, Jaeger et al, (2007) also demonstrated that goniometric joint measurements compared to radiographic measurements in cats were repeatable and valid, based on measurements from a single investigator. In fact, even if another investigator used a different approach to align the goniometer but remained consistent in their measurement, it would be likely that the same structures would be found to mechanically restrict the elbow joint, due to size of the statistically significant changes seen in the current study.

The Liberty® 360 degree 25cm goniometer demonstrated a repeatability coefficient of 4.7° for extension and 2.8° for flexion. These values represent the value in which goniometer measurements would vary between each other. For the repeatability of the Liberty® 360 degree 25cm goniometer, repeated goniometer measurements of the greyhound forelimbs were used when the skin was intact in the dissection. The reason why the repeatability was calculated only with the skin intact was that experience suggested that it was likely to be the most difficult and variable step to measure with the goniometer compared to later steps where the alignment and measurement with the goniometer became easier.

The repeatability coefficients for this study highlights that the variation between goniometer measurements could be affected due to several factors. This would include factors such as the alignment and handling of the goniometer, the handling of the forelimb, the placement or position of the forelimb and the subsequent rotation of the forelimb. Furthermore, as mentioned previously, the alignment of the goniometer became easier, as structures were progressively removed at each step. Nevertheless, the repeatability coefficient and the possible effect of these factors support why repeated goniometer measurements were used in this study, ultimately reducing the effect of these sources of error. Furthermore, significant results when the p-value was less than 0.001 for structures mechanically constraining the elbow during extension highlight the consistency of these measurements when using the goniometer. Ultimately, the use of the goniometer in this study proved to be an effective tool for identifying and measuring the effect of these mechanical restrictors on the range of flexion and extension of the canine elbow joint.

In conclusion, this study highlights the presence of mechanical restrictors of the canine elbow joint. These constraints included the skin, deep fascia, biceps brachii m. and the extensor carpi radialis m., which all contributed to restricting the elbow joint during elbow extension in the greyhound, and similarly significantly limited elbow extension in the other breed dogs except for the biceps brachii m. This suggests that they are involved in maintaining stability in the joint during extension. Further research into these structures is warranted, especially the role of the deep fascia and the extensor carpi radialis m. in quadruped forelimb function.

Acknowledgements

The authors have no conflict of interest. They would like to thank Brendan Kehoe and Dinidu Wijesurendra for their technical assistance.

Author Contributions

T.E.C wrote the manuscript. H.M.S.D assisted with the writing, drafting and critical revision of the manuscript. Both authors were involved in the design, collection of data and interpretation of data.

References

Ali, S., Zhang, W., Rajput, N., Khan, M.A., Li, C., & Zhou, G. (2015). Effect of multiple freeze-thaw cycles on the quality of chicken breast meat. *Food Chem*, **173**, 808-814.

Angle, T., Gillette, R., & Weimar, W. (2012). Kinematic analysis of maximal movement initiation in Greyhounds. *Aust Vet J*, **90**, 60-68.

Benjamin, M. (2009). The fascia of the limbs and back - a review. *J Anat*, **214**, 1-18.

Birch, E., Boyd, J., Doyle, G., & Pullen, A. (2015). The effects of altered distances between obstacles on the jump kinematics and apparent joint angulations of large agility dogs. *Vet J*, **204**, 174-178.

Burton, N., Dobney, J., Owen, M., & Colborne, G. (2008). Joint angle, moment and power compensations in dogs with fragmented medial coronoid process. *Vet Comp Orthop Traumatol.*

Caron, A., Caley, A., Farrell, M., & Fitzpatrick, N. (2014). Kinematic gait analysis of the canine thoracic limb using a six degrees of freedom marker set. *Vet Comp Orthop Traumatol*, **27**, 461-469.

Carr, J., Millis, D., & Weng, H. (2013). Exercises in canine physical rehabilitation: range of motion of the forelimb during stair and ramp ascent. *J Small Anim Pract*, **54**, 409-413.

Cuddy, L., Lewis, D. & Kim, S. et al. (2012). Contact Mechanics and Three-Dimensional Alignment of Normal Dog Elbows. *Vet Surg*, **41**, 818-828.

Curtis, N., Witzel, U., Fitton, L., O'higgins, P., & Fagan, M. (2011). The Mechanical Significance of the Temporal Fasciae in Macaca fascicularis: An Investigation Using Finite Element Analysis. *Anat Rec*, **294**,1178-1190.

Evans, H. & deLahunta, A. (2013). *Miller's anatomy of the dog*. Elsevier. St. Louis, Mo.

Fechner, R., Stratmann, M., Gößling, R., & Sverdlova, N. (2012). The functional role of the ischiopubic membrane for the mechanical loading of the pubis in the domestic fowl (Gallus gallus). *J Anat*, **222**, 305-312.

Flato, R., Passanante, G., Skalski, M, Patel, White, E. & Matcuk, G. (2017) The iliotibial tract: imaging, anatomy, injuries, and other pathology. *Skeletal Radiol,* **46**, 605-622.

Fratzl, P. (2008). *Collagen: structure and mechanics*. Springer, New York.

Freund, K., Kieves, N., Hart, J., Foster, S., Jeffery, U., & Duerr, F. (2016). Assessment of novel digital and smartphone goniometers for measurement of canine stifle joint angles. *Am J Vet Res*, **77**, 749-755.

Galindo-Zamora, V., Dziallas, P., & Wolf, D. et al. (2014). Evaluation of Thoracic Limb Loads, Elbow Movement, and Morphology in Dogs Before and After Arthroscopic Management of Unilateral Medial Coronoid Process Disease. *Vet Surg*, **43**, 819-828.

Gerlach, U. and Lierse, W. (1990). Functional Construction of the Superficial and Deep Fascia System of the Lower Limb in Man. *Cells Tissues Organs*, **139**, 11-25.

Gillette, R. L., & Angle, T. C. (2014). 11: Canine Locomotion Analysis in Millis D., and Levine D. (eds), *Canine Rehabilitation And Physical Therapy*, Elsevier. PA, 201-210.

Goldner, B., Fuchs, A., Nolte, I., & Schilling, N. (2015). Kinematic adaptations to tripedal locomotion in dogs. *Vet J*, **204**, 192-200.

Goslow, G.E. Jr, Seeherman, H.J., Taylor, C.R., McCutchin M.N. & Heglund N.C. (1981). Electrical activity and relative length changes of dog limb muscles as a function of speed and gait. *J Exp Biol*, **94**, 15-42.

Govoni, V., Rahal, S., Agostinho, F., Conceição, R., Tsunemi, M., & El-Warrak, A. (2012). Goniometric measurements of the forelimb and hindlimb joints in sheep. *Vet Comp Orthop Traumatol*, **25**, 297-300.

Greene, L., Marcellin-Little, D., & Lascelles, B. (2013). Associations among exercise duration, lameness severity, and hip joint range of motion in Labrador Retrievers with hip dysplasia. *J Am Vet Med Assoc*, **242**, 1528-1533.

Huijing, P., Maas, H., & Baan, G. (2003). Compartmental fasciotomy and isolating a muscle from neighboring muscles interfere with myofascial force transmission within the rat anterior crural compartment. *J Morphol*, **256**, 306-321.

Jaeger, G., Marcellin-Little, D., DePuy, V., & Lascelles, B. (2007). Validity of goniometric joint measurements in cats. *Am J Vet Res*, **68**, 822-826.

Jaeger, G., Marcellin-Little, D., & Levine, D. (2002). Reliability of goniometry in Labrador Retrievers. *Am J Vet Res*, **63**, 979-986.

Künzel, W., Forstenpointner, G., & Skolek-Winnisch, R. (1993). Die laterokranialen Faszienstrukturen am Ober- und Unterarm sowie Unterschiede im Ansatz des M. *biceps brachii* bei Haussäugetieren. *Anat Histol Embryol*, **22**, 1-15

Lascelles, B., Dong, Y., Marcellin-Little, D., Thomson, A., Wheeler, S., & Correa, M. (2012). Relationship of orthopedic examination, goniometric measurements, and radiographic signs of degenerative joint disease in cats. *BMC Vet Res*, **8**, 10.

Leygonie, C., Britz, T. & Hoffman, L. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Sci*, **91**, 93-98.

Liljebrink, Y. & Bergh, A. (2010). Goniometry: is it a reliable tool to monitor passive joint range of motion in horses?. *Equine Vet J*, **42**, 676-682.

Mason, D., Schulz, K., Fujita, Y., Kass, P., & Stover, S. (2005). In vitro force mapping of normal canine humeroradial and humeroulnar joints. *Am J Vet Res*, **66**, 132-135.

Pfau, T., Garland de Rivaz, A., Brighton, S., & Weller, R. (2011). Kinetics of jump landing in agility dogs. *Vet J*, **190**, 278-283.

Preston, C., Schulz, K., & Kass, P. (2000). In vitro determination of contact areas in the normal elbow joint of dogs. *Am J Vet Res*, **61**, 1315-1321

Qi, J., Li, C., Chen, Y., Gao, F., Xu, X., & Zhou, G. (2012). Changes in meat quality of ovine *longissimus dorsi* muscle in response to repeated freeze and thaw. *Meat Sci*, **92**, 619-626.

Stecco, C., Machhi, V., Porzionato, M., Duparc, F., & De Caro, R. (2011). The fascia: the forgotten structure. *Ital J Anat Embryol*, **116**, 127-138.

Stecco, C., Porzionato, A. & Lancerotto, L. et al. (2008). Histological study of the deep fasciae of the limbs. *J Bodyw Mov Ther*, **12**, 225-230.

Thomas, T., Marcellin-Little, D., Roe, S., Lascelles, B., & Brosey, B. (2006). Comparison of measurements obtained by use of an electrogoniometer and a universal plastic goniometer for the assessment of joint motion in dogs. *Am J Vet Res*, **67**, 1974-1979.

Tokuriki, M. (1973a). Electromyographic and Joint-Mechanical Studies in Quadrupedal Locomotion: I. Walk. *Jpn J Vet Sci*, **35**, 433-446.

Tokuriki, M. (1973b). Electromyographic and Joint-Mechanical Studies in Quadrupedal Locomotion: II. Trot. *Jpn J Vet Sci*, **35**, 525-533.

Tokuriki, M. (1974). Electromyographic and Joint-Mechanical Studies in Quadrupedal Locomotion: III. Gallop. *Jpn J Vet Sci*, **36**, 121-132.

Williams, S., Wilson, A., Daynes, J., Peckham, K., & Payne, R. (2008). Functional anatomy and muscle moment arms of the thoracic limb of an elite sprinting athlete: the racing greyhound (Canis familiaris). *J Anat*, **213**, 373-382.

Zhang, M., Li, F., Diao, X., Kong, B., & Xia, X. (2017). Moisture migration, microstructure damage and protein structure changes in porcine longissimus muscle as influenced by multiple freeze-thaw cycles. *Meat Sci*, **133**, 10-18.

Tables

Table 1 Mean ± Standard error of the Mean (SEM) of the difference in flexion and extension of the greyhound elbow (n=12) 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.

	Mean difference and SEM (°)	Confidence Interval (95%) of mean difference (°)	P-Value
Step 1 vs Step 2			
Flexion	- 0.150 ± 0.118	(-0.411, 0.111)	0.231
Extension	+ 1.667 ± 0.293	(1.023, 2.311)	<0.001
Step 2 vs Step 3			
Flexion	- 0.550 ± 0.167	(-0.918, -0.182)	<0.05
Extension	0.000 ± 0.213	(-0.469, 0.469)	1.000
Step 3 vs Step 4			
Flexion	- 0.250 ± 0.171	(-0.626, 0.126)	0.171
Extension	+ 0.350 ± 0.171	(-0.026, 0.726)	0.065
Step 4 vs Step 5			
Flexion	+ 0.250 ± 0.162	(-0.106, 0.606)	0.150
Extension	+ 9.883 ± 0.262	(9.306, 10.460)	<0.001
Step 5 vs Step 6			
Flexion	+0.017 ± 0.171	(-0.361, 0.394)	0.924
Extension	+0.283 ± 0.155	(-0.057, 0.624)	0.094
Step 6 vs Step 7			

Flexion	-0.250 ± 0.533	(-0.589, 0.089)	0.133
Extension	+0.1167 ± 0.0968	(-0.0964, 0.1167)	0.253
Step 7 vs Step 8			
Flexion	-0.383 ± 0.211	(-0.848, 0.081)	0.097
Extension	+2.800 ± 0.344	(2.043, 3.557)	<0.001
Step 8 vs Step 9			
Flexion	+0.067 ± 0.242	(-0.465, 0.598)	0.788
Extension	+5.467 ± 0.407	(4.571, 6.362)	<0.001
Step 9 vs Step 10			
Flexion	+0.000 ± 0.174	(-0.383, 0.383)	1.000
Extension	+0.200 ± 0.178	(-0.191, 0.591)	<0.05
Step 10 vs Step 11			
Flexion	+0.417 ± 0.114	(0.166, 0.668)	0.548
Extension	-0.133 ± 0.215	(-0.607, 0.340)	0.548
Step 11 vs Step 12			
Flexion	0.383 ± 0.214	(-0.087, 0.854)	0.101
Extension	+0.117 ± 0.182	(-0.283, 0.517)	0.534
Step 12 vs Step 13			
Flexion	- 0.350 ± 0.152	(-0.685, -0.015)	<0.05
Extension	+0.383 ± 0.145	(0.065, 0.701)	<0.05
Overall Changes			
Step 1 vs Step 13			

Flexion	- 0.800 ± 0.337	(-1.541, -0.059)	<0.05
Extension	+ 21.133 ± 0.663	(19.673, 22.594)	<0.001

Table 2 Mean ± Standard Error of the Mean (SEM) are the effects of storage at 5-6°C on the flexion and extension of the greyhound elbow joint (n=4) over days 4, 5, and 6. The days correspond to the length of time since the limbs were moved from the freezer (on day zero) to be defrosted.

	Day 4	**Day 5**	**Day 6**	**P-Value**
Flexion (Mean ± SEM)	**22.700° ± 0.929**	**22.550° ± 0.900**	**22.650° ± 0.842**	**0.993**
Extension (Mean ± SEM)	**152.550° ± 0.340**	**151.550° ± 0.550**	**152.000° ± 0.707**	**0.471**

Figures

Figure 1

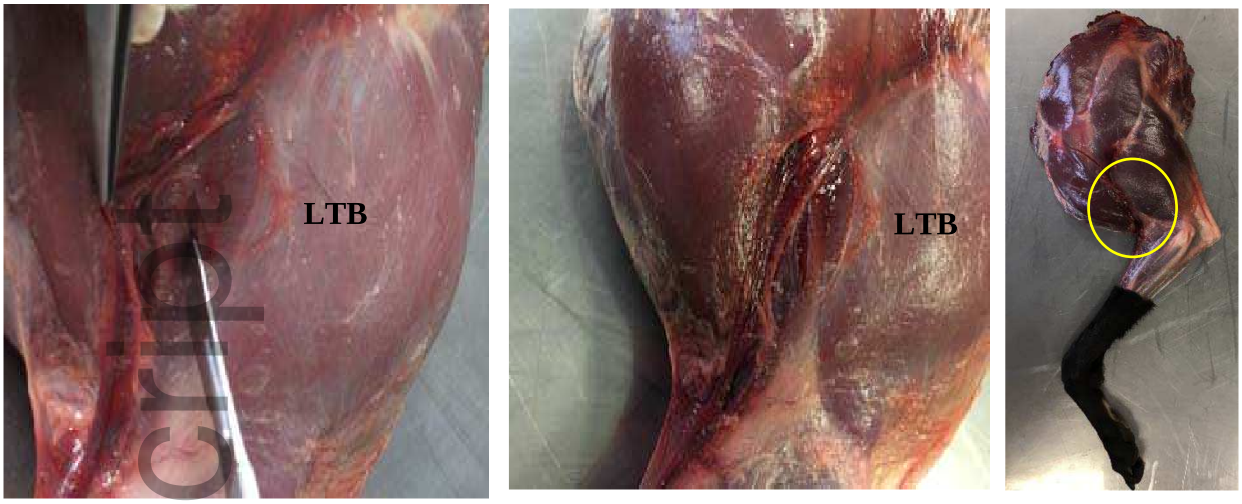


Figure 2

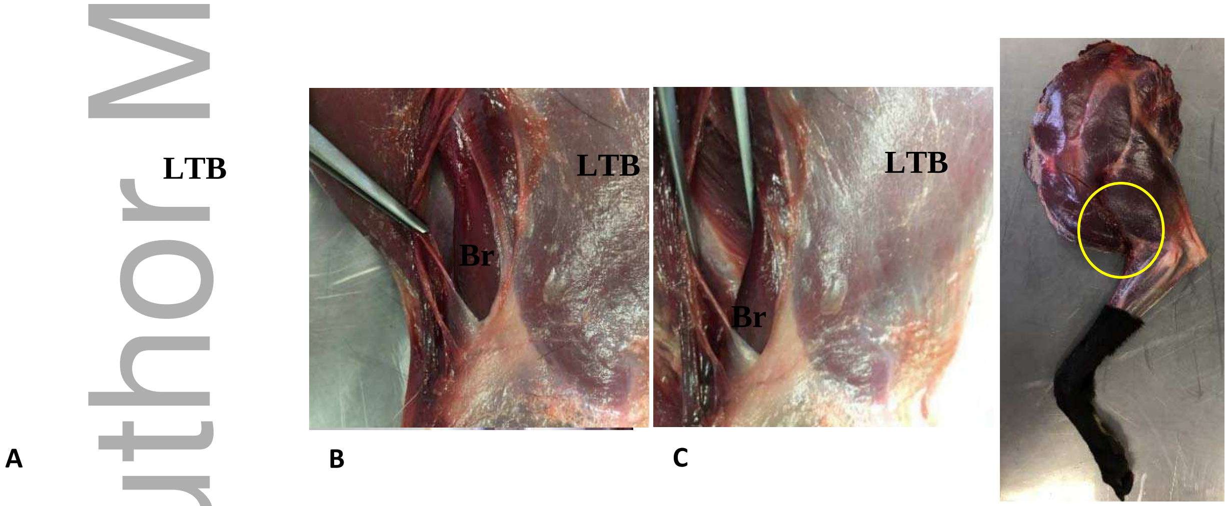


Figure 3

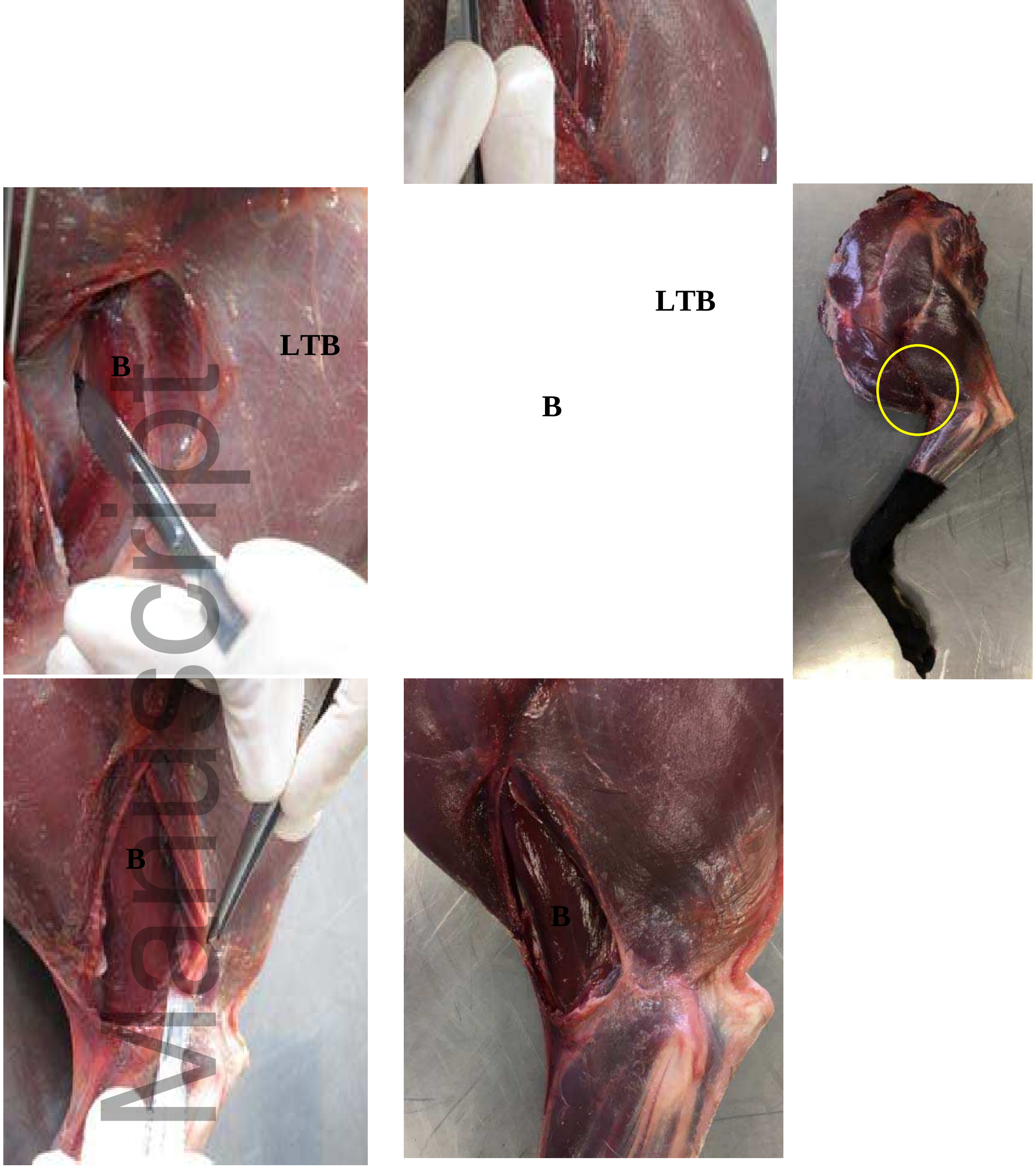
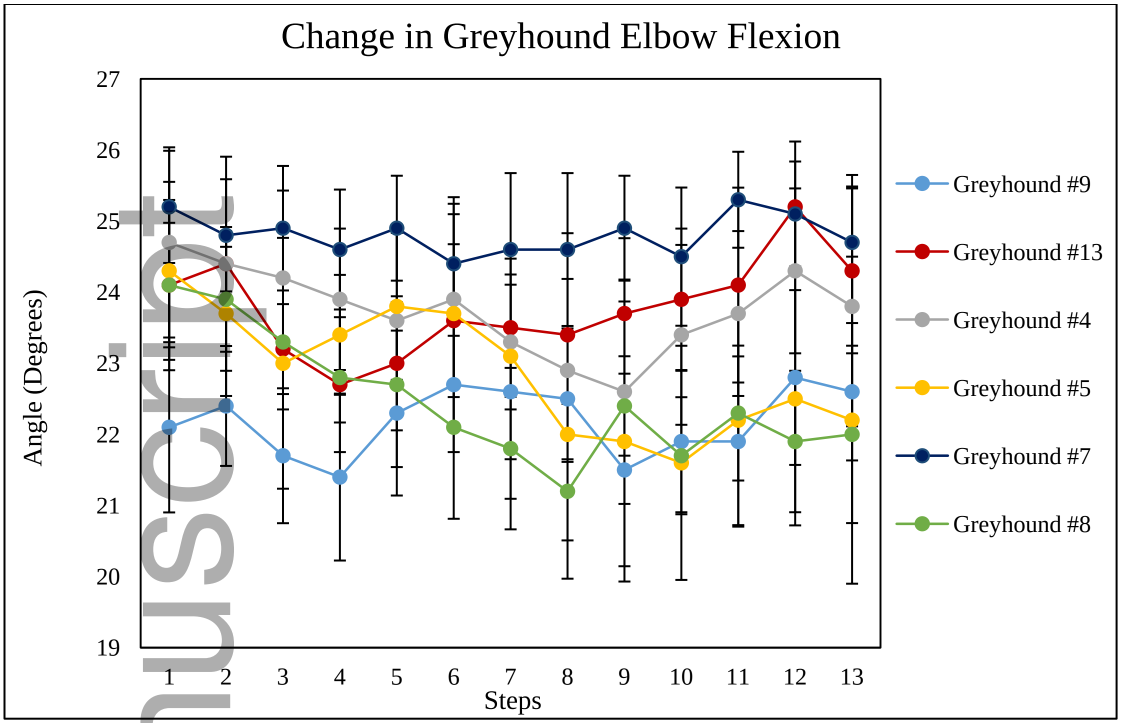
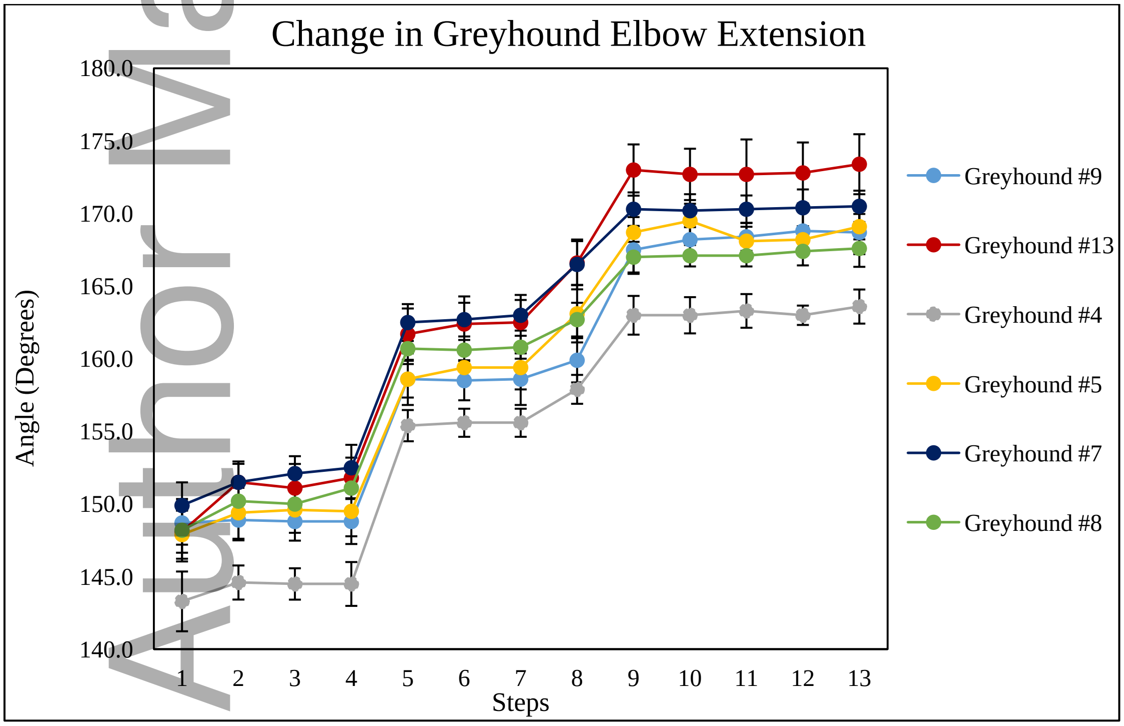


Figure 4

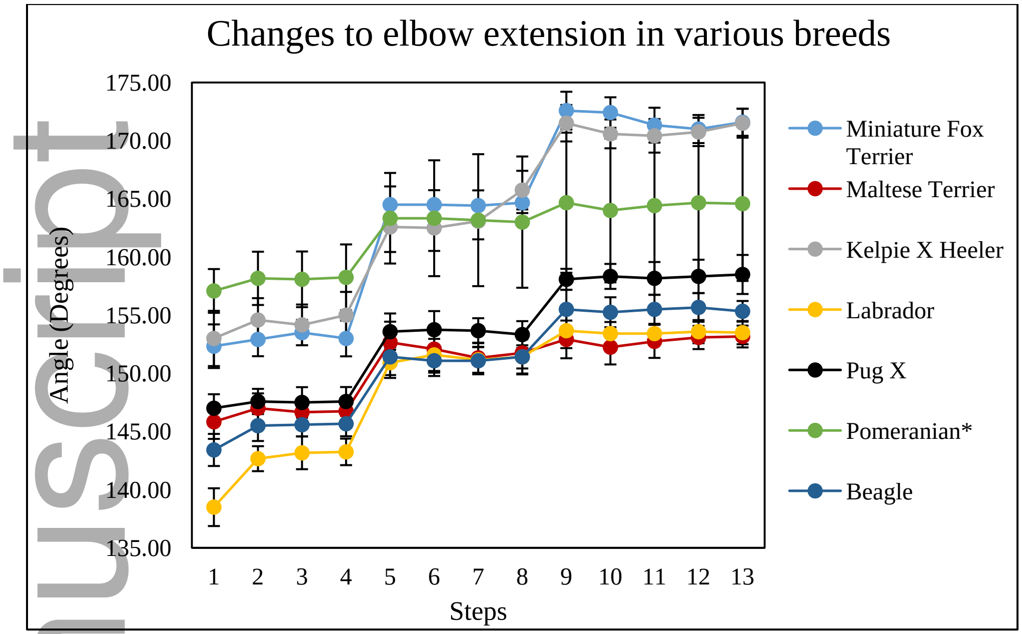
811 

812 **Figure 5**

813 

814 **Figure 6**

815



Changes to elbow extension in various breeds
175.00
170.00
165.00
160.00
155.00
150.00
145.00
140.00
135.00
Angle (Degrees)
1
2
3
4
5
6
7
8
9
10
11
12
13
Steps
Miniature Fox Terrier
Maltese Terrier
Kelpie X Heeler
Labrador
Pug X
Pomeranian*
Beagle

Figure 7

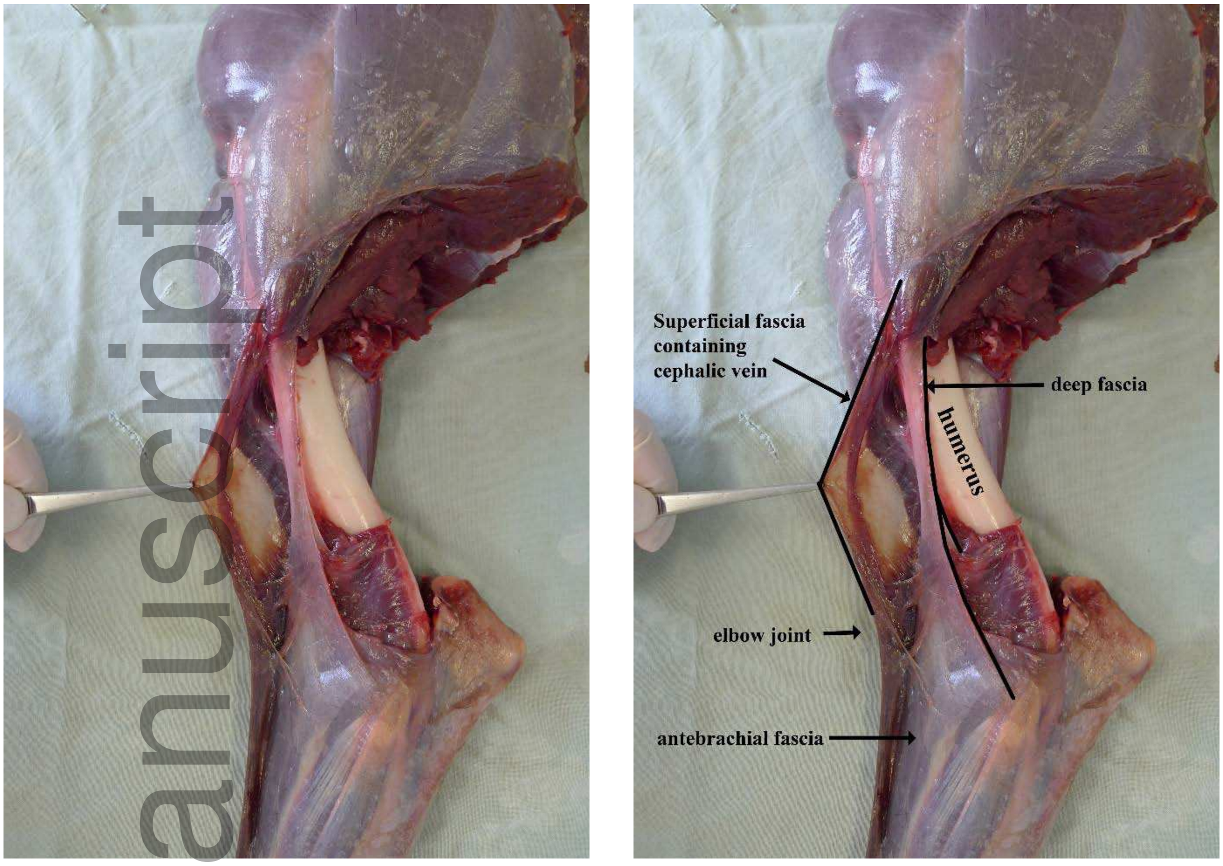

Superficial fascia containing cephalic vein
deep fascia
humerus
elbow joint
antebrachial fascia

Figure 8

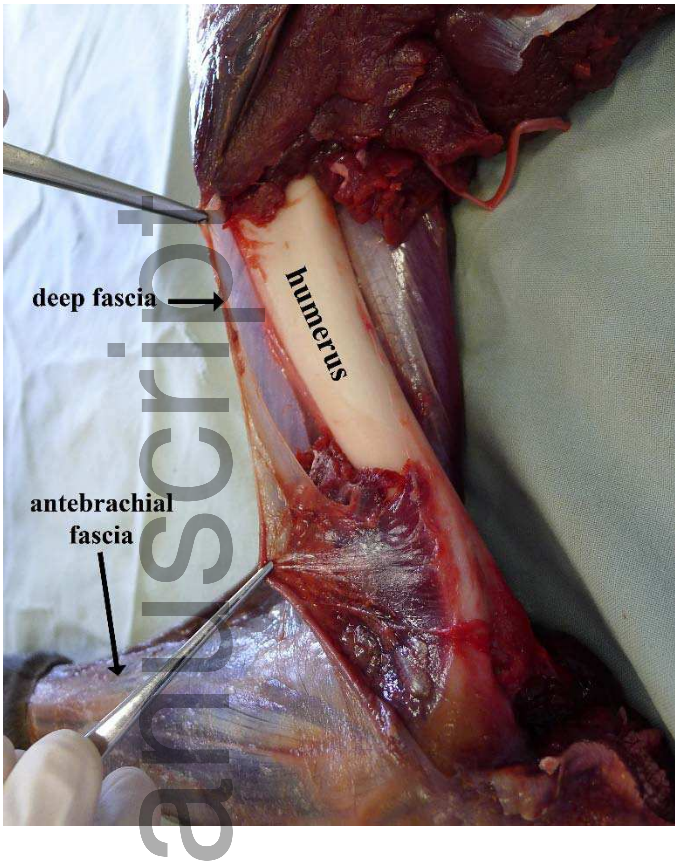


Figure 9

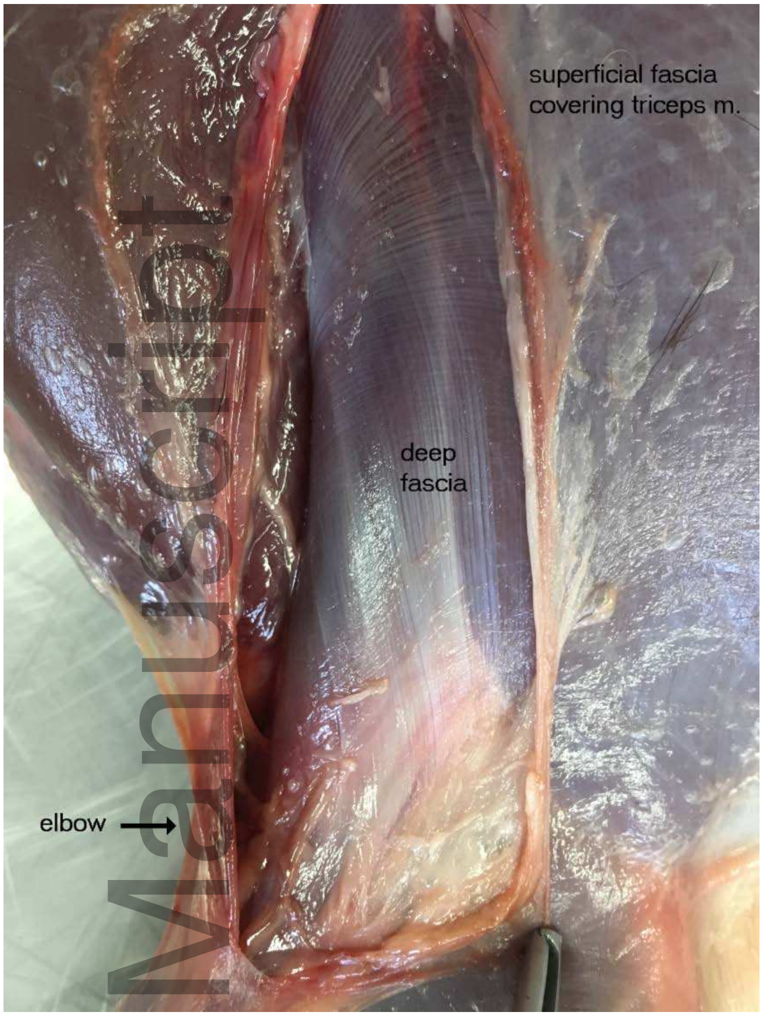


Figure 10

Figure Legends

Figure 1 An example of how the Liberty® 360 degree 25cm goniometer was aligned and used to measure the angle of a greyhound left forelimb during flexion (Top left) and extension (Top right) for each step of the dissection. The lateral aspect of the forelimb is facing up. An example of how the 66ffit^{TM} 360° 12cm goniometer was aligned with the forelimbs of small breed dogs during extension (bottom left) and flexion (bottom right) for each step in the dissection. The lateral aspect of this right forelimb is facing up.

Figure 2 The lateral aspect (slightly craniolateral) of a left forelimb showing the 3rd step of the dissection where an incision was made close to the lateral head of the triceps brachii m. (LTB) and superficial to the brachialis m. The yellow square on the orientation image (right) of the left forelimb indicates the region that was being dissected.

Figure 3 The cranial aspect (**A**), lateral aspect (**B**) and craniolateral aspect (**C**) of a left forelimb showing the 4th step of the dissection where an incision into the deep fascia was widened (**A**).Loose connective tissue was also separated from the fascia so that the fascia could be cut from the humerus (**B** and **C**). The brachialis m. (**Br**) and the lateral head of triceps brachii m. (LTB) are labelled for orientation purposes.. The yellow square on the orientation image (far right) of the left forelimb indicates the region that was being dissected.

Figure 4 The lateral aspect (slightly craniolateral) of a left forelimb showing the 5th step of the dissection where the deep fascial layers that connected along the distal half of the humerus were transected (Top Left). Then the rest of these fascial connections were transected (Top right and Bottom Left). The range of flexion and extension was then measured after this step (Bottom Right). The brachialis m. (B) and lateral head of the triceps brachii m. (LTB) are labelled for orientation purposes.

Figure 5 Multiple line graph showing the mean of repeated goniometer measurements (five repeats for each measurement of each limb) for the flexion of the elbow joint (degrees) from step 1 to step 13 for each pair of greyhound forelimbs (n=6) that was dissected. No consistent trends in the change in the flexion of the elbows could be identified. Greyhound ID # 7, 8 and 9 were female; and greyhound ID # 4, 5 and 13 were male. Error bars represent +/- one standard deviation.

Figure 6 Multiple line graph showing the mean of repeated goniometer measurements (five repeats for each measurement of each limb) for the extension of the elbow joint (degrees) from step 1 to step 13 for each pair of greyhound forelimbs (n=6) that was dissected. Significant changes in extension can be seen at Steps 2, 5, 8 and 9. Greyhound ID # 7, 8 and 9 were female; and greyhound ID # 4, 5 and 13 were male. Error bars represent +/- one standard deviation.

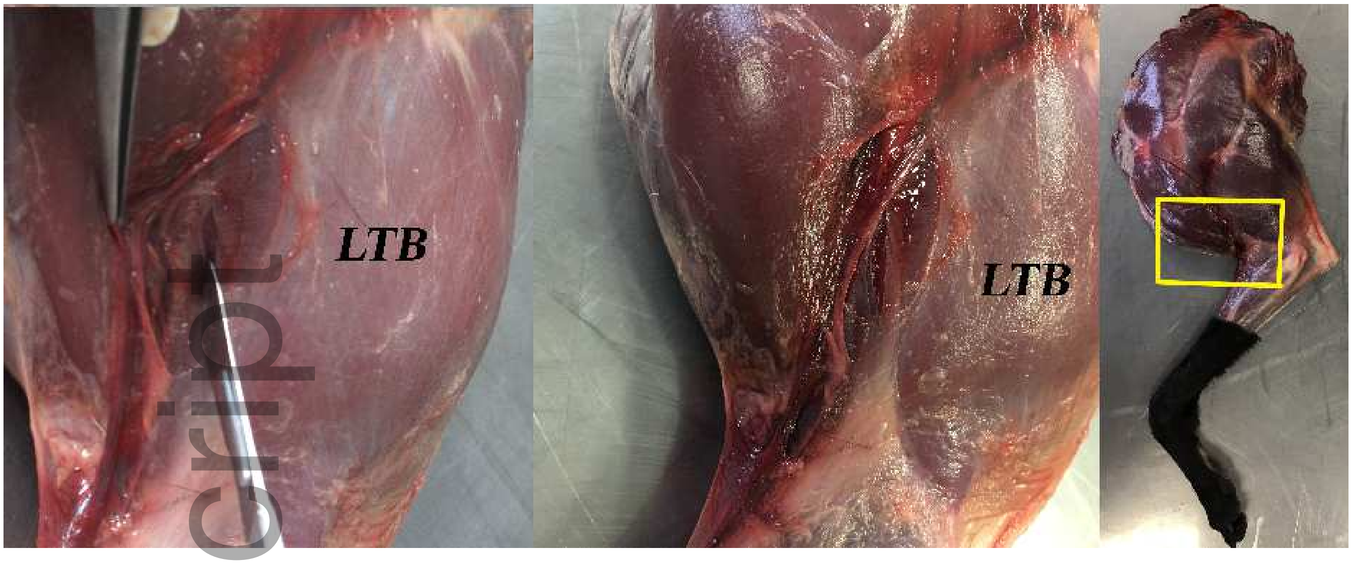
Figure 7 Multiple line graph showing the mean of repeated goniometer measurements (six repeats for each measurement of each limb) for the extension of the elbow joint (degrees) from step 1 to step 13 for each pair of canine forelimb (n=7) that was dissected. All breeds except for the Pomeranian were male. * Both Pomeranian elbows, especially the right elbow showed obvious signs of arthritic changes to the joint. Error bars represent +/- one standard deviation.

Figure 8 Lateral aspect of the brachial region of a left side greyhound forelimb. Muscles which include the triceps brachii m., tensor fasciae antebrachii m., anconeus m. and brachialis m. have been removed to show the attachment of the deep fascia to the humerus and its connections across the elbow. The deep fascia has also been cut.

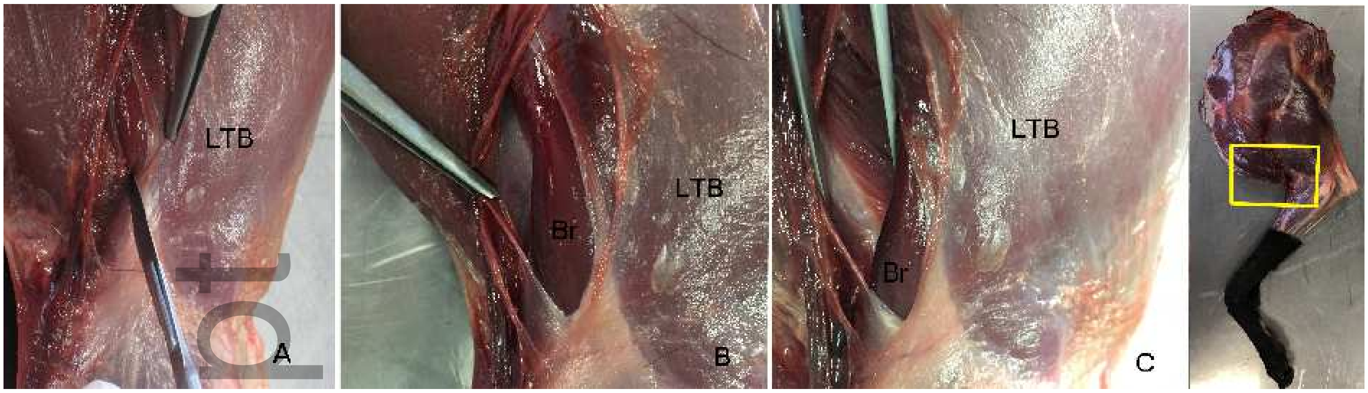
Figure 9 Image showing the lateral aspect of the left greyhound brachium. Muscles including the triceps brachii m., tensor fasciae antebrachii m., anconeus m. and brachialis m. were removed to show the connections and relative position of the deep fascia. The deep fascia has been cut but remains attached to the humerus and its distal connections.

Figure 10 Image of the lateral aspect of a left side forelimb, showing the deep fascia, superficial to the brachialis m., which restricts the elbow joint during extension. The superficial fascia has been cut and moved aside. In the proximal part of the deep fascia superficial to the brachialis m., the collagen fibres form a cross-hatched arrangement. Distally, along the brachialis m. and close to the extensor carpi radialis m., collagen fibres run along the length of the muscle.

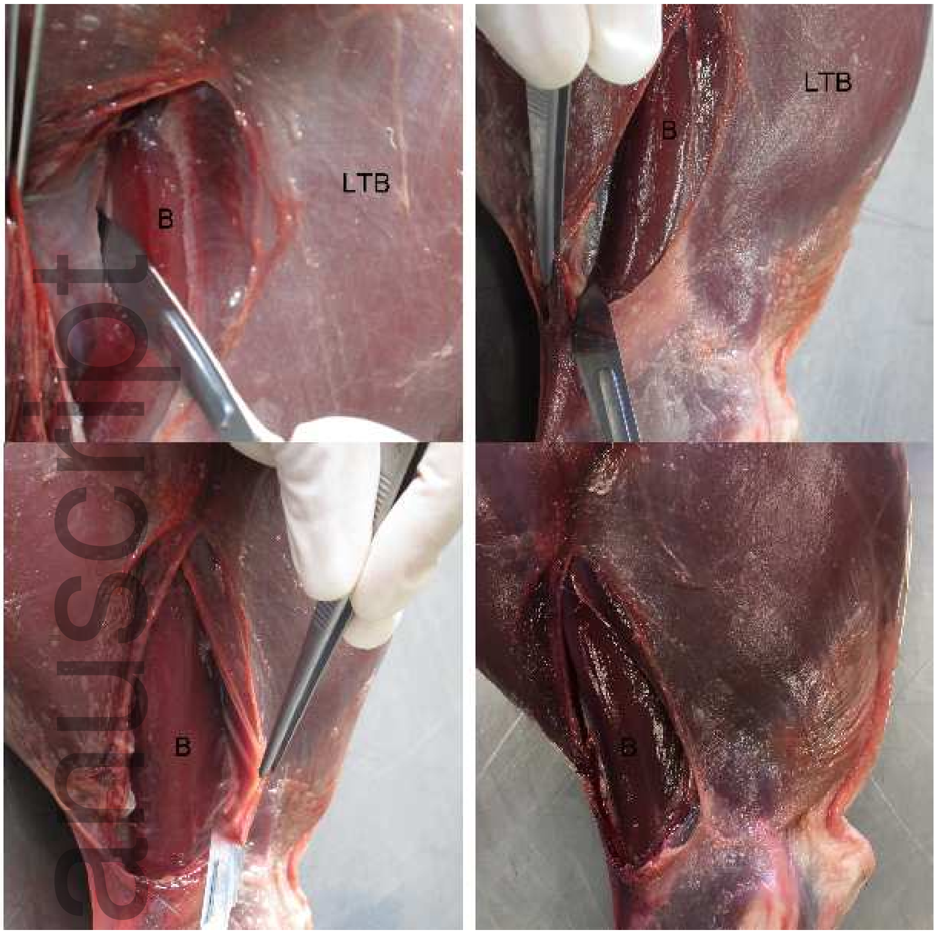
joa_12763_f1.tif



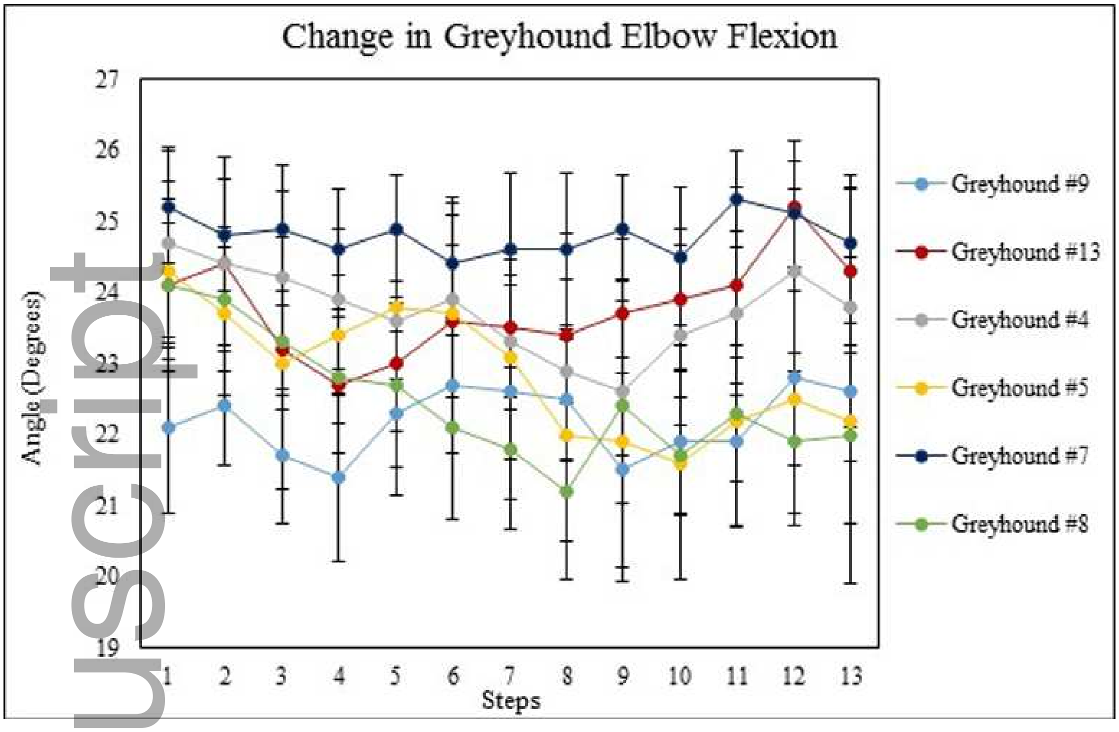
joa_12763_f2.tif



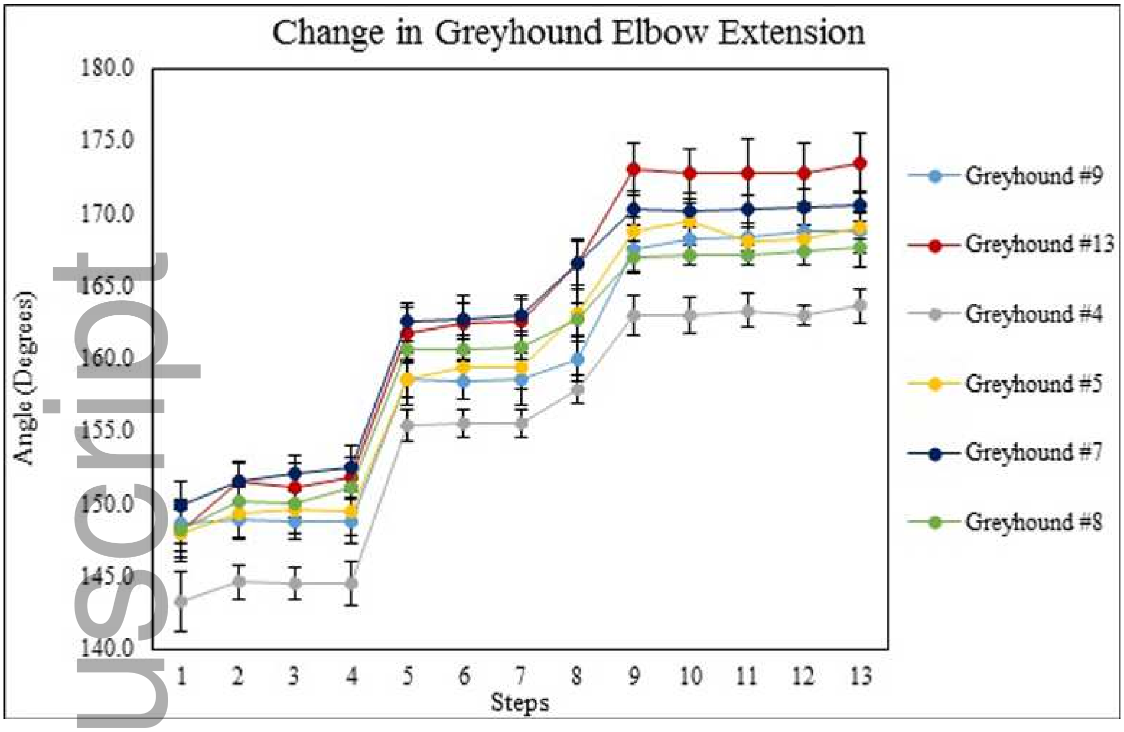
joa_12763_f3.tif



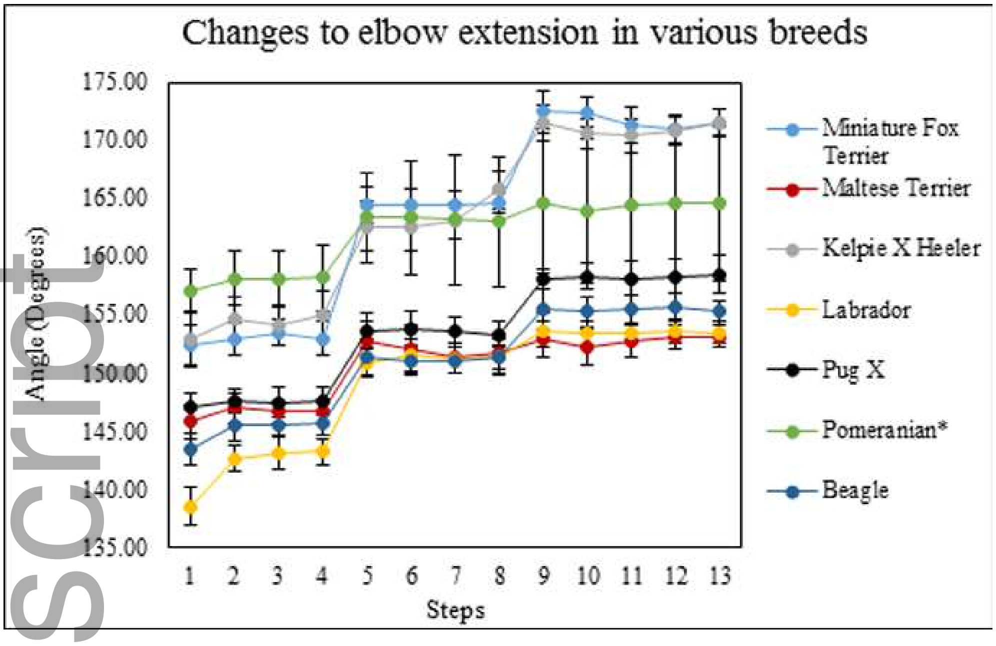
joa_12763_f4.tif



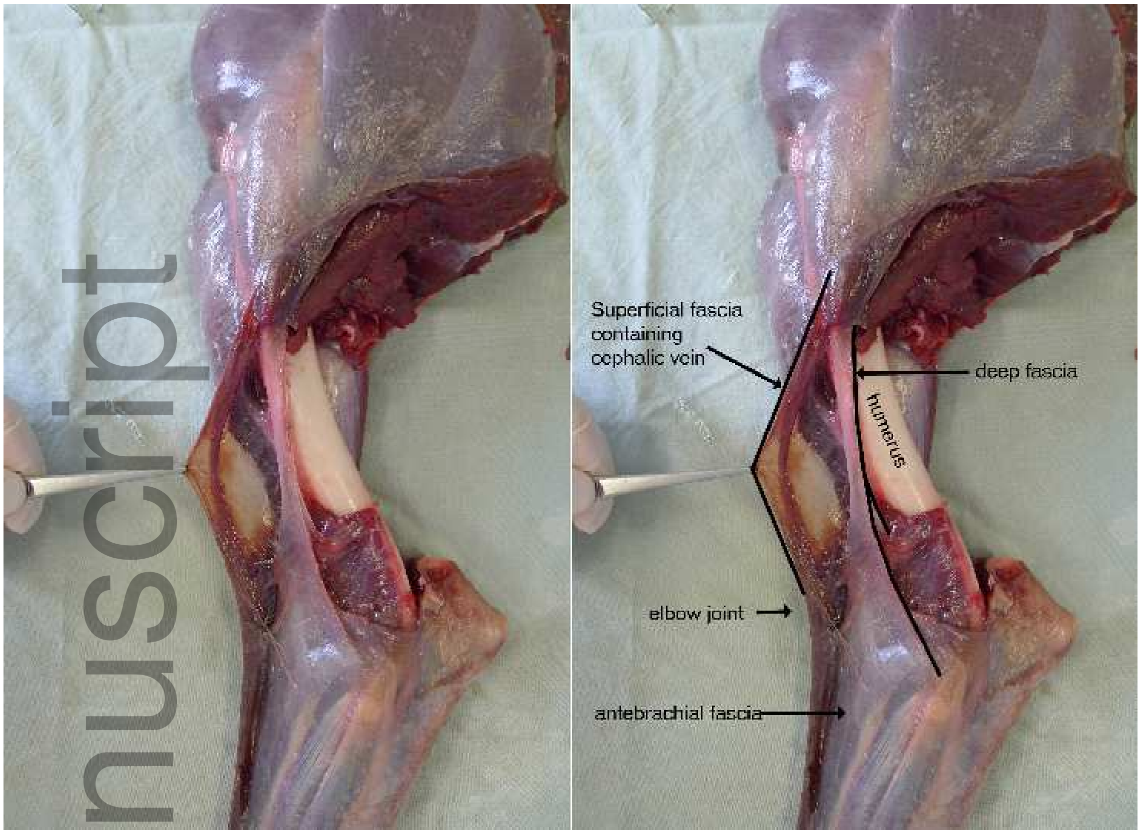
joa_12763_f5.tif



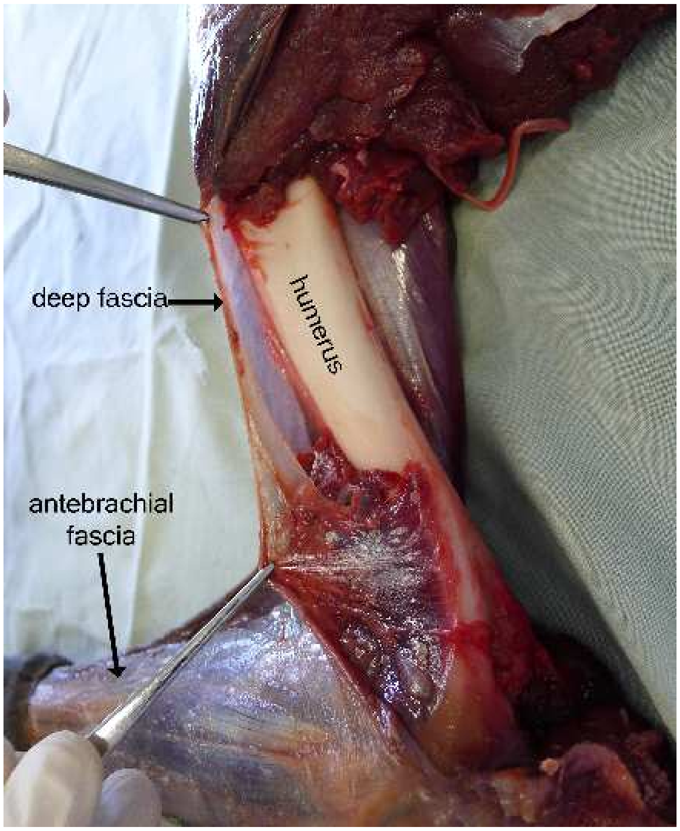
joa_12763_f6.tif



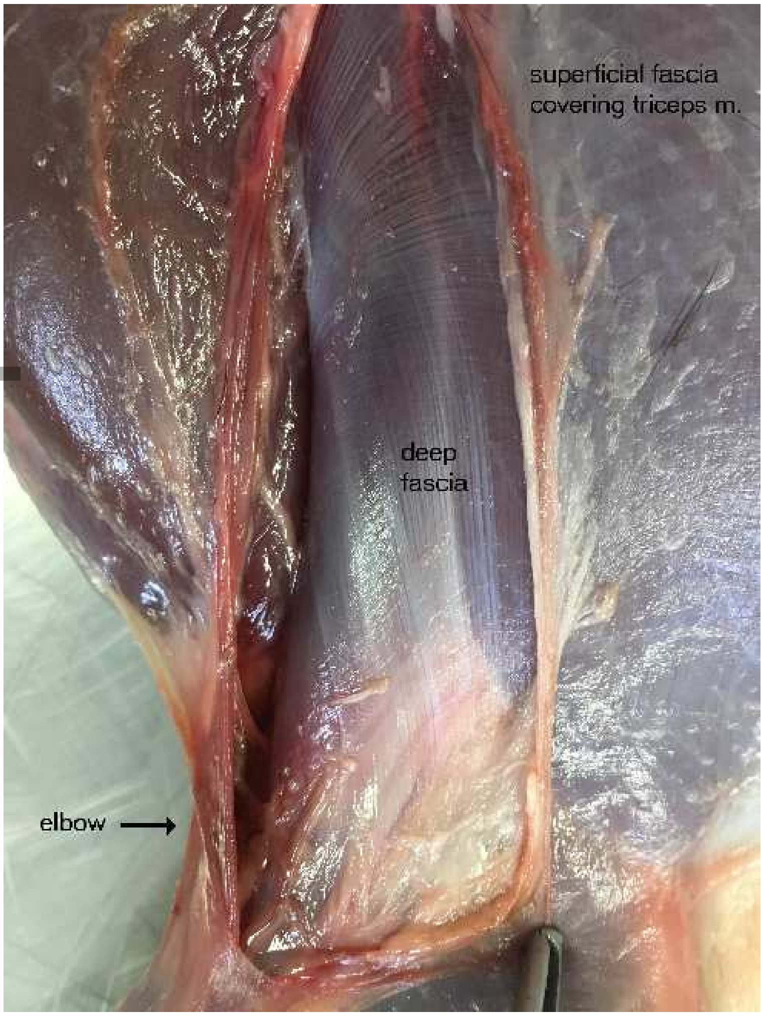
joa_12763_f7.tif



joa_12763_f8.tif



joa_12763_f9.tif



joa_12763_f10.tif