

Characteristics of Collagen Fibrils in the Entire Equine Superficial Digital Flexor Tendon

By

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Summary: The superficial digital flexor tendon (SDFT) is one of the longest tendons in the horse. In racehorses, disturbance of the locomotor functions of the SDFT occurs most frequently in the central area of the mid-metacarpal region. While many studies have investigated the equine SDFT, there are no reports to date of the morphological characteristics of collagen fibrils in the central and peripheral areas of each of the three regions that comprise the entire tendon: the myotendinous junction (MTJ), the mid-metacarpal region (mM) and the osteotendinous junction (OTJ). Mass average diameter (MAD), which provides functional information on the mean collagen fibril diameter and tensile strength of the tendon, was found to be smaller in the central area than in the peripheral area of all three regions. The MAD value was lowest in both the central and peripheral areas in the MTJ region, and tended to increase generally in a distal direction in the OTJ. The OTJ is important parts that unite with the bone.

We conclude that morphological structure suggested that it corresponds to biomechanical function in some region of the equine SDFT.

The superficial digital flexor tendon (SDFT) is one of the longest tendons in the equine forelimb. It is generally accepted that connective tissue such as tendons are primarily composed of collagen fibrils. The tendon not only transfers strength to joints but also contributes to the storage of energy to reduce muscle energy required for locomotion^{1,3)}. In general terms, the tendon is conventionally divided into three regions: the myotendinous junction (MTJ), the middle region and the osteotendinous junction (OTJ). However, no ultra-morphologic studies demonstrating regional differences in the tendon have appeared in the literature to date. We previously revealed variation in the distribution of collagen fibril diameter in different metacarpal regions of the equine SDFT and fusion of the collagen fibrils in the mid-metacarpal region (mM), proposing that large collagen fibrils are formed by the fusion of small fibrils¹⁾. However, the entire morphological structure of the equine SDFT remains to be clarified. To this end,

the present study sought to determine collagen fibril diameter, the collagen fibril index (CFI) and mass average diameter (MAD) among the aforementioned regions of normal equine SDFT using a morphological index.

Materials and Methods

Normal SDFTs of four Thoroughbred horses (aged 2–15 years) were used in this study. The experiment was approved by the Ethics Committee and the Institutional Animal Use and Care Committee of Rakuno Gakuen University, Ebetsu, Japan.

Each complete SDFT tissue sample was divided into the following three regions: the myotendinous junction (MTJ), mid-metacarpal region (mM) and osteotendinous junction (OTJ) (Fig. 1). Samples from each region were further subdivided into tissues of the central and peripheral areas, as defined

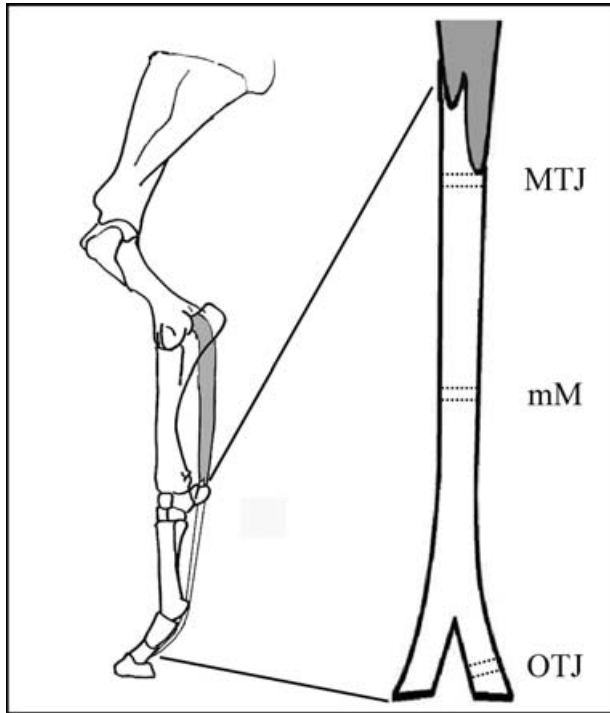


Fig. 1. Schematic drawing showing three regions of the equine superficial flexor tendon (SDFT): the myotendinous junction (MTJ), mid-metacarpal region (mM) and osteotendinous junction (OTJ).

by Birch *et al.*²). The samples were processed as follows: immersion in 3% glutaraldehyde in 0.1 M phosphate buffer, washing in phosphate buffer, post-fixing in 1% osmium tetroxide for 1 h at room temperature, dehydration through a graded series of ethyl alcohol, embedding in Quetol 812, sectioning with a diamond knife, and staining with 1% uranyl acetate and 1% lead citrate. After processing, the sections were examined by transmission electron microscopy (TEM; JEM-1220, JEOL, Tokyo, Japan) at an acceleration voltage of 80 kV. Seven hundred fibrils were randomly selected in photo-

graphs of each SDFT region. Mean collagen fibril diameter, collagen fibril index (CFI) and mass average diameter (MAD) were then calculated. Statistical analysis was carried out using the ANOVA test. Mean values, CFI and MAD of each region were tested with Scheffe's test. Significance was set at $P < 0.05$.

CFI is the percentage of area covered by collagen and represents a collagen-to-noncollagen ratio in the extracellular matrix^{7,10}. MAD provides information on the tensile strength of a tissue that is not represented by mean collagen fibril diameter^{4,8}. MAD is defined as the diameter of a fibril that contains the mean mass of the distribution⁵.

Results

TEM images of collagen fibrils and histograms of collagen fibril diameter are shown in Figs. 2 and 3. Comparison of collagen fibril diameter in the central and peripheral areas of each region revealed that within each region, there was a tendency for collagen fibril diameter to be smaller in the central area than in the peripheral area. Collagen fibril diameter in the MTJ and mM were distributed with a single peak at 40–60 nm. But the OTJ had a bimodal profile at 40–60 nm and 140–160. Collagen fibrils with the greatest diameter, ranging from 40–60 nm, were most abundant in all samples, particularly in the MTJ region, in which about 80% of the fibrils were in this diameter range. Mean collagen fibril diameter in the central area of each region ranged from 41.0 ± 1.1 to 67.9 ± 1.8 nm, while that in the peripheral area ranged from 43.9 ± 1.1 to 99.1 ± 2.2 nm. MAD values in the central area ranged from 95.4 ± 2.6 to 145.0 ± 2.6 nm, while those in the peripheral area ranged from 101.3 ± 3.0 to 151.1 ± 2.0 nm. The MAD value was lowest in the MTJ region and gradually increased toward the OTJ in both central and peripheral areas. Significant differences between the two areas were

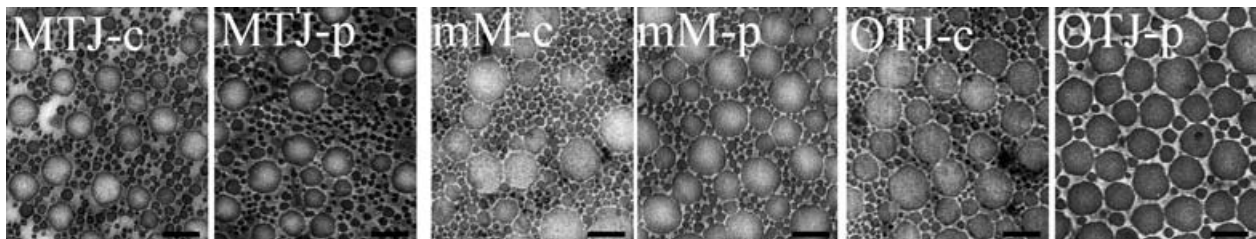


Fig. 2. Electron micrographs of the central (-c) and peripheral (-p) areas of a normal equine superficial digital flexor tendon at the myotendinous junction (MTJ), mid-metacarpal region (mM) and osteotendinous junction (OTJ). Bar = 200 nm.

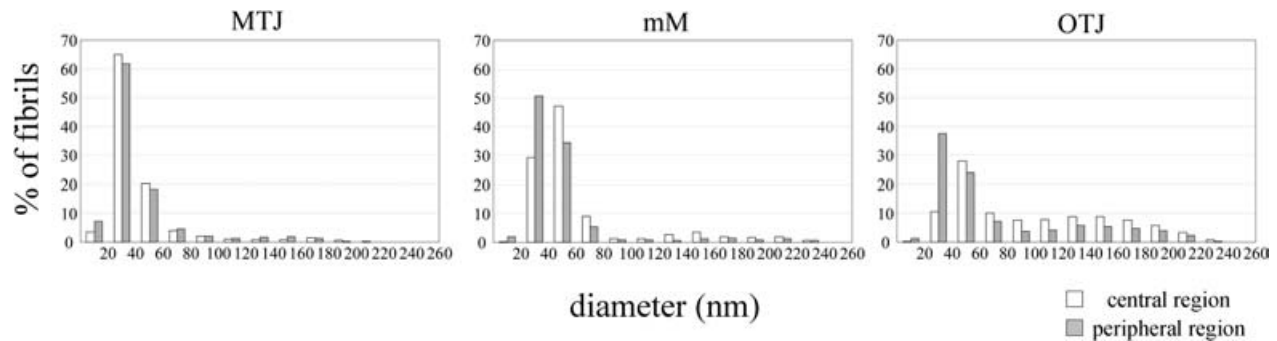


Fig. 3. Histograms showing percentage of collagen fibrils with different diameters in the central and peripheral areas of each tendon region.

MTJ: myotendinous junction, mM: mid-metacarpal region, OTJ: osteotendinous junction.

Table 1. Parameters of collagen fibrils in the equine superficial digital flexor tendon

area region	Central		
	MTJ	mM	OTJ
<i>Fibril diameter</i>			
Mean \pm S.E. (nm)	41.0 \pm 1.1	48.2 \pm 1.2*	67.9 \pm 1.8*
CFI \pm S.E. (%)	54.5 \pm 1.6	68.6 \pm 2.6	75.1 \pm 1.4
MAD \pm S.E. (nm)	95.4 \pm 2.6	128.0 \pm 2.4	145.0 \pm 2.6
area region	Peripheral		
	MTJ	mM	OTJ
<i>Fibril diameter</i>			
Mean \pm S.E. (nm)	43.9 \pm 1.1	60.8 \pm 1.6	99.1 \pm 2.2
CFI \pm S.E. (%)	59.2 \pm 1.9	74.2 \pm 1.8	76.1 \pm 2.0
MAD \pm S.E. (nm)	101.3 \pm 3.0	132.7 \pm 2.5	151.1 \pm 2.0

Significantly different from same region of another area. Significant difference () in mean fibril diameter was found in the central area of the mid-metacarpal region (mM) and osteotendinous junction (OTJ). There was no significant difference in the collagen fibril index (CFI). Significant difference in mass average diameter (MAD) values (*) was no significant difference in all area.

found between the mM and OTJ. The CFI value in the central area of each region ranged from 54.5 ± 1.6 to $75.1 \pm 1.4\%$, while that of the peripheral area ranged from 53.0 ± 2.6 to $74.3 \pm 2.4\%$. These results are summarized in Table 1.

Discussion

Morphological characteristics indicated that collagen fibril diameter increased from the MTJ in a

distal direction. Small-diameter fibrils in the MTJ have been reported to function to anchor the terminal ends of skeletal muscle fibers⁶. The mean collagen fibril diameter only in the metacarpal region of the SDFT has been reported to be larger in the distal part than in the proximal part¹¹. The present study is the first to report the mean diameter and MAD and CFI values of collagen fibrils in the entire SDFT. The higher the MAD value, the larger the load borne by a region. Similar to the results of fibril diameter, MAD values decreased from the MTJ to OTJ, suggesting that the OTJ region bears a larger load than other regions of the tendon. In addition, the MAD value in the central area was lower than in the peripheral area, indicating that different areas of the SDFT receive different loads. The findings of this study suggest that *in situ* and simultaneous load measurements in different areas of the tendon warrant study, although this is not an easy task. The CFI provides information on the collagen-to-noncollagen ratio in the extracellular matrix^{7,10}. With regard with size of collagen fibrils, a tendon having a higher CFI would show fibrils having larger diameter and would also span a larger area.

Our results clearly show that the mean collagen fibril diameter in both the central and peripheral areas in three regions of the tendon. Since the MTJ of the tendon has the additional function of an anchoring medium for muscle. The construction of MTJ was reported the terminal ends of skeletal muscle fibers had finger-like processes and small collagen fibrils of the tendons probably served as anchorages for those processes⁹. Indeed, the distribution of collagen fibrils in the mM region showed an intermediate distribution pattern between those in the MTJ and the OTJ in this study. It is worth noting that the tendon shows a graded

arrangement of collagen fibrils from one end of the tendon to the other. We speculated the graded material that continuously changes in arrangement and/or composition of its constituents has unique mechanical and functional characteristics. The graded structure in the tendon may have some mechanical functions, such as dissipating friction energy generated by distortion between bone and muscle effectively. Since bone is rather static compared to muscle, the OTJ could have more static property to accommodate differences in elasticity between bone and muscle. Larger fibrils in the OTJ may confer to greater stiffness and static property in this part of the tendon. We conclude that morphological structure suggested that it corresponds to biomechanical function in some region of the equine SDFT.

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