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Histologic characteristics of the subscapularis tendon from muscle to bone: reference to subscapularis lesions

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Background: Although subscapularis tendon lesions seem to differ from those of the supraspinatus tendon, the features they have in common suggest that the subscapularis tendon may also have 2 distinct layers. Our aim was therefore to characterize the histologic structure of the subscapularis tendon from its humeral insertion point to the musculotendinous junction.

Material and methods: A histologic study was performed on 10 autopsy samples. The subscapularis tendon was extracted in one piece from the musculotendinous junction to the humeral insertion point and was prepared using standard (hematoxylin-eosin–saffron) staining.

Results: Histologic analysis revealed 2 fibrous layers, distinguishable by the orientation of the collagen bundles. The deep layer was thinner and composed of parallel longitudinal collagen fibers inserting onto the lesser tuberosity flush with the cartilage. The superficial layer was thicker and composed of interdigitated collagen bundles inserting onto the lesser and the greater tuberosity after splitting into 2 bands, 1 lining the floor of the bicipital groove, and the other extending over the long head of the biceps tendon across the groove. Each layer formed an independent musculotendinous junction in the subscapularis muscle.

Conclusions: The subscapularis tendon is composed of 2 distinct fibrous layers, just like the supraspinatus tendon, but arranged differently. The superficial layer of the subscapularis tendon passes across the bicipital groove and forms a fibrous ring around the long head of the biceps tendon that stabilizes the latter in the bicipital groove. These results explain some of the specific features of subscapularis tears described in the literature, namely, delamination and biceps subluxation.

Level of evidence: Basic Science Study; Histology; Cadaver Dissection

Keywords: Shoulder; rotator cuff; histology; subscapularis tendon; subscapularis tear; biceps tendon

Subscapularis tendon tears are among the more recently described rotator cuff tendon lesions. They can be isolated and often in this case traumatic,[10] but most are associated with typically degenerative superior rotator cuff tears.[22] Subscapularis tendon lesions differ from those of the supraspinatus and mostly begin in the upper part of the tendon,[3] as described in several classifications. The 5-stage Lafosse classification[15] associates the size of the tear with the degree of tendon retraction. The system, developed by the Société Française d'Arthroscopie (SFA; French Arthroscopy Society),[26] distinguishes traumatic ruptures of the entire subscapularis from degenerative tears with longitudinal splitting or tears, or both,

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of the deep face of the tendon with different degrees of retraction.

In terms of anatomy and pathology, subscapularis tears differ from those of the posterosuperior rotator cuff involving the supraspinatus and infraspinatus tendons, which are complete or articular or bursal, sometimes intratendinous, or partial tears.[8,15,21,26] One of the multiple factors that explains the nature of these tears is the histologic structure of the supraspinatus tendon, which has 2 distinct layers with different vascularization patterns.[7,9,19] Although the causes of the tears are multifactorial,[22] similar features (partial articular tears of the superior part, delamination) are also frequently observed for the subscapularis tendon,[3,22] which suggests that it may also have a 2-layer structure.

The objective of this study was to characterize the histologic structure of the subscapularis tendon from its humeral insertion point to the musculotendinous junction. We hypothesized that the subscapularis tendon would be composed of 2 histologically distinct layers.

Materials and methods

This was an autopsy study performed in March and April 2016 on 7 fresh cadavers conserved at 2°C to 4°C after an intra-arterial injection of an embalming solution (Safebalm; Hygeco, Garges Les Gonesse, France). The exclusion criteria were cutaneous scarring, substantial deformity, a previous dissection of the shoulder, or the discovery of rotator cuff tear involving the subscapularis tendon during the dissection. The 10 retained shoulders (7 left, 3 right) came from 5 female and 2 male donors who had died between February and April 2016 at a mean age of 80 years (range, 55-88 years). The causes of death were unknown.

Layered dissection of the shoulders was performed using a standard anterior deltopectoral approach. The deltoid muscle was removed completely by avulsion from the clavicle, the acromion, and the spine of the scapula. This revealed the lesser and the greater tuberosity, the bicipital groove with the long head of the biceps tendon (LHBT), and the different components of the rotator cuff (from front to back, the subscapularis, supraspinatus, infraspinatus, and teres minor tendons).

Humeral osteotomy was performed with a Lambotte chisel. The entire lesser tuberosity with the whole insertion of the subscapularis tendon and the anterior half of the greater tuberosity with the supraspinatus tendon were then excised, leaving the bicipital groove with the LHBT in place. The subscapularis and supraspinatus muscles were cut approximately 3 cm after the musculotendinous junction. The LHBT was cut through the joint proximal and distal to the bicipital groove (Figs. 1 and 2).

The autopsy specimens were fixed in 10% formalin saline. Three transverse sections were cut with a handsaw parallel to the superior horizontal edge of the subscapularis tendon within the upper first 10 mm of the tendon, below the biceps pulley at the top of the bicipital groove. An additional lower section was taken 15 to 20 mm from the superior border of the tendon (its middle portion), and a last section from the inferior part. Each transverse section consisted laterally to medially of the greater humeral tuberosity, the bicipital groove with the LHBT, the lesser tuberosity with the insertion of the subscapularis tendon, and finally, the subscapularis muscle.

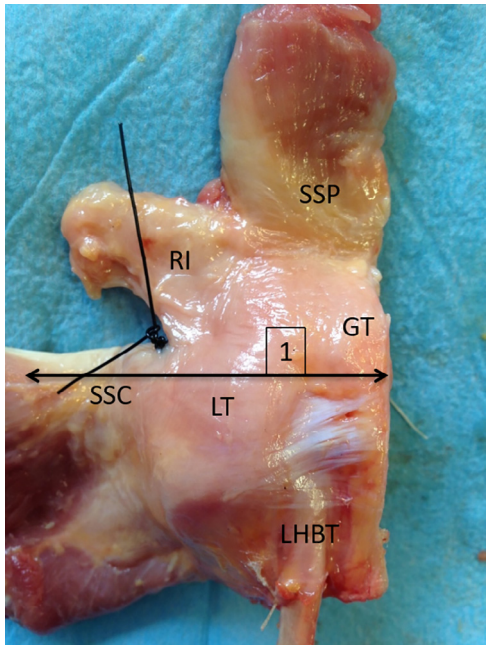

Figure 1 Macroscopic photograph of an autopsy sample. The thick extension of the subscapularis (*SSC*) tendon is visible in the upper part of the bicipital groove, where it interconnects with fibers from the supraspinatus (*SSP*) tendon and from the coracohumeral ligament stemming from the rotator interval (*RI*). The ↔ shows where the horizontal cut was made for the histologic study of the upper part of the subscapularis tendon. *GT*, greater tuberosity; *LHBT*, long head of the biceps tendon.

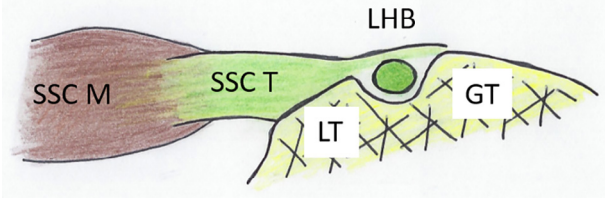

Figure 2 Freehand drawing of the section of the anatomic piece indicated by the black → in Fig. 1, which was used to analyze the subscapularis tendon (*SSC T*) from the musculotendinous junction to its humeral insertion. *SSC M*, subscapularis muscle; *LHB*, long head of the biceps; *LT*, lesser tuberosity of the humerus; *GT*, greater tuberosity of the humerus.

The sections were decalcified in a hydrochloric acid solution (DC2 QPath; VWR Chemicals, Fontenay-sous-Bois, France). Each specimen was dehydrated, embedded in paraffin, and 3-µm-thick sections were cut from each block and stained using hematoxylin-eosin–saffron.

The slices were analyzed by 1 anatomic pathologist, both macroscopically and histologically. The humeral enthesis and the subscapularis tendon in the bicipital groove, the substance of the tendon, and the musculotendinous junction were systematically analyzed in all 5 sections of the subscapularis tendon. The ligament structures in the vicinity of the subscapularis tendon were not investigated.

Results

Macroscopic results

The upper part of the subscapularis tendon was in each case clearly defined as a thick, horizontal tendon rounded on the articular face. The tendon shortened, and the insertion became purely muscular in the lower third of the subscapularis insertion.[13] A continuous stretch of tendon tissue extended laterally from the subscapularis tendon over the bicipital groove. This tissue, which surrounded the LHBT, was thick and well defined over the first 10 to 15 mm and became thinner toward the lower part of the subscapularis, revealing the LHBT underneath (Figs. 1 and 2). In its upper part, by the bicipital groove, descending vertical collagen fibers from the supraspinatus tendon merged with the superficial fibers of the subscapularis tendon. The LHBT thereby seemed enclosed in a tendinous sleeve in the bicipital groove.

Histologic results

Upper part of the subscapularis tendon

No differences were observed between the 3 sections taken in the upper 10 mm of the subscapularis tendon. The substance of the tendon consisted of 2 layers, identifiable by the arrangement of the collagen bundles. There was no visible structure separating the 2 layers (Fig. 3).

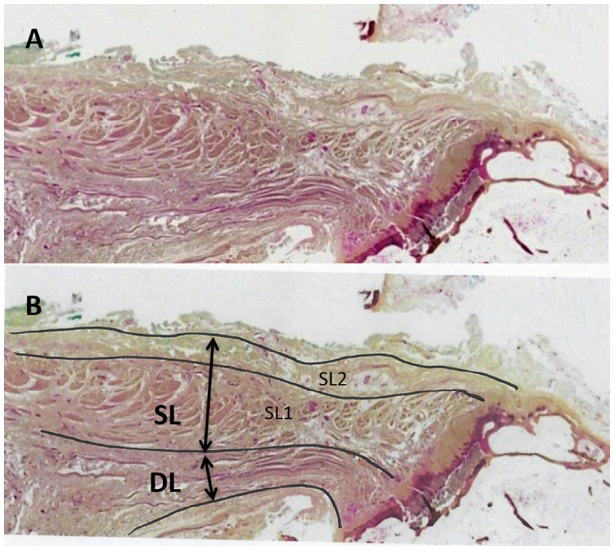

Figure 3 General view of the subscapularis tendon (hematoxylin-eosin–saffron staining, original magnification ×2.5) (**A**) without and (**B**) with annotations. A thin layer with parallel collagen fibers (deep layer, *DL*) is visible beneath a thicker layer with interwoven collagen bundles (superficial layer, *SL*) that accounts for most of the substance of the tendon and the humeral insertion. The layer with interwoven, differently oriented collagen bundles (*SL*) accounts for most of the thickness of the tendon and consists of a thicker sublayer with broad collagen bundles (*SL1*) and a very thin superficial layer with fine collagen fibers (*SL2*).

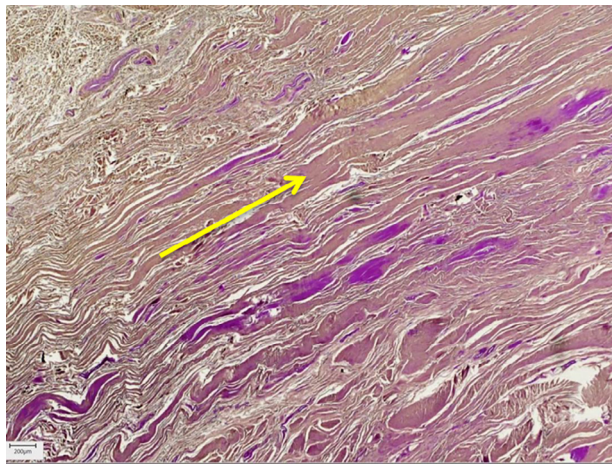

Figure 4 Photomicrograph (hematoxylin-eosin–saffron staining, original magnification ×10) of the deep layer of the subscapularis tendon with packing of the longitudinal collagen fibers. The → shows and points along the horizontally aligned collagen fibers in this layer.

The deep articular layer consisted of parallel collagen fibers with a strict longitudinal orientation along the tendon axis. These fibers were densely packed against one another, with no interstitial space (Figs. 3 and 4).

The superficial layer was composed of bundles of collagen fibers with different orientations, some perpendicular, some parallel to one another, separated by a sparse stroma (Figs. 3 and 5). Two sublayers were distinguishable from the diameter of the collagen fiber bundles. The outermost sublayer was thinner and contained finer bundles; the deeper sublayer was thicker and contained broader bundles (Figs. 3 and 5).

The average thickness of the upper part of the tendon was 9.7 mm for the male donors (range, 9-11 mm) vs. 7.4 mm for the female donors (range, 4.5-10 mm). The outer layer was

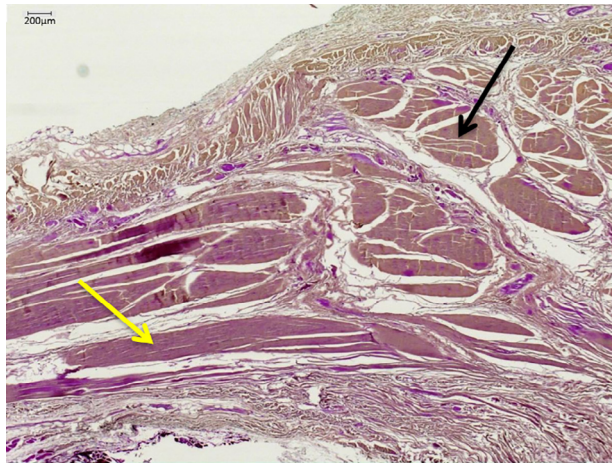

Figure 5 Photomicrograph (hematoxylin-eosin–saffron staining, original magnification ×10) of the superficial layer of the subscapularis tendon with interdigitated collagen bundles, broad in the deeper sublayer and thin in the outermost sublayer. The yellow → shows longitudinally oriented collagen bundles in this superficial layer, and the black shows → transversely oriented.

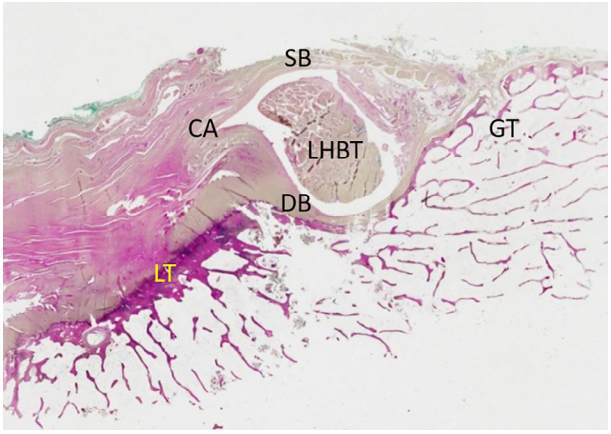

Figure 6 Photomicrograph (hematoxylin-eosin–saffron staining, original magnification ×2.5) of the subscapularis tendon in the bicipital groove. The tendon splits into 2 bands, the deep band (*DB*) lining the bicipital groove and the superficial band (*SB*) extending over the bicipital groove and the long head of the biceps tendon (*LHBT*). Inside the groove, the subscapularis tendon forms an annular sling that stabilizes the biceps tendon along with the medial edge of the groove. The canthus-like (*CA*) separation of the bands is a weak point in the sling. *GT*, greater tuberosity; *LT*, lesser tuberosity.

3- to 10-times thicker than the deep layer, which averaged 6.7 mm thick (range, 3.5-10 mm) vs. 1.3 mm thick (range, 1-2 mm), respectively. Similarly in the outer layer, the sublayer composed of finer bundles was always thinner (by a factor 2 to 7) than the sublayer with broader bundles, 1.1 mm on average (range, 0.5-2 mm) vs. 5.6 mm (range, 3-8 mm), respectively.

The deep longitudinal tendon layer inserted with a normal enthesis into the lesser tuberosity flush with the articular cartilage (Fig. 3). The superficial layer with interdigitated collagen bundles inserted in 2 separate sites: (1) onto the lesser tuberosity with a normal enthesis (for the sublayer with broad collagen bundles), and (2) onto the greater tuberosity after splitting into 2 bands passing one over and one under the LHBT in the bicipital groove. The band lining the bicipital groove beneath the LHBT consisted of the sublayer with broad collagen bundles. The band that extended over the LHBT consisted of both sublayers, the broad bundle sublayer and the fine bundle sublayer (Figs. 3 and 6). The 2 bands separated with an acute angle, forming a canthus- or drop-like sling around the LHBT. This separation occurred at no specific depth in the substance of the tendon in the layer with broad collagen bundles (Fig. 6).

The outer tendon layer with interwoven collagen fibers penetrated deep into the substance of the muscle, with the muscle and tendon fibers merging to form a normal musculotendinous junction. The layer with parallel longitudinal collagen fibers extended into the deep part of the subscapularis muscle without truly merging with the muscle fibers. The muscle seemed to insert flush with the deep band, which was always shorter (Fig. 7).

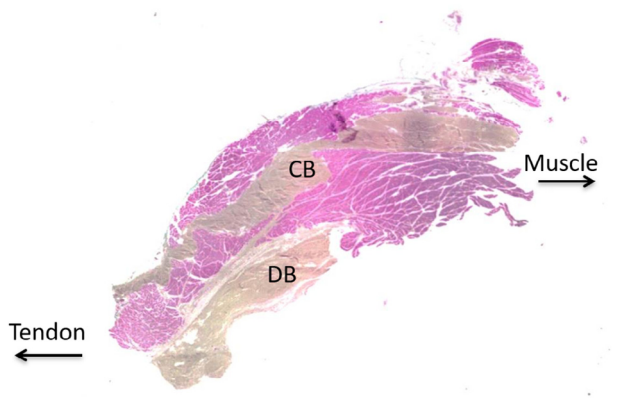

Figure 7 Photomicrograph (hematoxylin-eosin–saffron staining, original magnification ×2.5) of the musculotendinous junction of the subscapularis tendon. The short deep band (*DB*) corresponds to the deep layer with parallel collagen fibers. The central band (*CB*) inserts deep in the muscle and corresponds to the layer with interwoven bundles of collagen fibers.

Middle portion of the subscapularis tendon

No significant differences with the upper portion were observed for the middle portion of the subscapularis in the nature and arrangement of the humeral enthesis, in the structure of the tendon itself, or in the musculotendinous junction. The thickness ratios between the different layers and sublayers of the tendon were also similar. The superficial layer was thicker than the deep one in all cases: 6 mm on average (range, 3.5-8.5 mm) vs. 1.1 mm (range, 0.5-2 mm), respectively. Likewise, the sublayer with broad interwoven collagen bundles was always thicker than the one composed of finer bundles (5 mm on average vs. 1.1 mm). In the bicipital groove however, although this was difficult to quantify, the band passing over the LHBT seemed thinner than in the upper portion of the tendon (approximately 13% of the outermost tendon layer vs. 25% in the upper part).

Lower portion of the subscapularis tendon

The tendon was less well defined in this portion, the tendon fibers were sparser and interwoven with muscle fibers, which predominated. The thickness of the tendon could not be measured. The humeral enthesis was shorter, with a less clearly identifiable tendon/fibrocartilage/bone transition, and the muscle inserted directly onto the bone in most cases.

Discussion

Our histologic results confirm that there are 2 distinct layers in the subscapularis tendon. The deeper layer is composed of parallel longitudinal collagen fibers and inserts onto the lesser tuberosity. The superficial layer consists of interdigitated collagen fibers with various orientations and inserts onto the lesser tuberosity and the greater tuberosity after dividing into 2 bands, one lining the bicipital groove and the other passing over the LHBT. These 2 bands separate at an acute

angle, forming a canthus around the LHBT. Macroscopically, we also observed a dense, opaque extension of the upper portion of the tendon across the bicipital groove. This extension was thinner and transparent in the lower part of the tendon.[1]

The 2 layers of the subscapularis tendon seem to make separate junctions with the muscle, except in the lower part of the tendon where the muscle inserts directly into the bone. The superficial layer forms a classic musculotendinous junction, with deep interpenetration in the substance of the subscapularis muscle. The deep layer forms a shorter junction but also penetrates deep into the muscle.

There are histologic similarities between the supraspinatus and subscapularis tendons, both of which consist of 2 layers distinguishable by the orientation of the collagen fibers, parallel or interwoven with various orientations.[7,9,11,19] However, although the layer with parallel collagen fibers is thicker and superficial in the supraspinatus tendon,[7,19] it is thinner and deep in the subscapularis tendon. A 2-layer structure was described previously for the subscapularis[7,11] but without any description of the orientation of the collagen bundles.

Gleason et al[11] described the deep layer inserting onto the lesser tuberosity and lining the bicipital groove, with the superficial layer extending over the groove and the LHBT to insert onto the greater tuberosity. This is contrary to our results, which show that the deep layer with parallel collagen fibers inserts only onto the lesser tuberosity and does not approach the bicipital groove. The superficial layer with interwoven collagen fiber bundles inserts onto the lesser and the greater tuberosity after dividing into 2 bands that wrap around the LHBT in the bicipital groove.

The anatomic feature extending across the bicipital groove and the LHBT and commonly (erroneously) called the "transverse humeral ligament" is purely tendinous. Contrary to Clark and Harryman,[7] who describe it as an expansion of the supraspinatus tendon, most authors, in agreement with our results, report a superficial expansion of the subscapularis tendon that bridges the LHBT and inserts onto the greater tuberosity. This has been confirmed by several radiologic studies with magnetic resonance imaging,[6,11] autopsy,[2,5,11,18] and histologic studies.[5,11,22,25]

In agreement Gleason et al[11] and Boon et al,[5] we observed that the subscapularis tendon completely encloses the bicipital groove and forms part of its medial edge up along the lesser tuberosity. The existence of a layer with interdigitated collagen bundles in both the supraspinatus and the subscapularis tendon suggests that the 2, along with the coracohumeral ligament, are connected,[2,5,6,11,14] an arrangement that is strengthening according to some authors.[2,7] This tendinous connection may be the "comma sign" described by Lo and Burkhart,[16] whose observation requires detachment and retraction of the subscapularis tendon.[27]

The teardrop-shaped ring formed by the subscapularis tendon around the LHBT in the bicipital groove[10,23] stabilizes the LHBT through the whole length of the bicipital groove,[3] as does the biceps pulley formed by the coracohumeral and superior glenohumeral ligaments at top of the groove.[4,28,30] Several authors report that dislocations or subluxations of the LHBT are always accompanied by subscapularis tendon tears.[3,12,22,24] According to Sakurai et al,[22] most subscapularis tears occur in the articular side of the tendon, reducing the height of the medial edge of the bicipital groove and thus increasing the risk of LHBT subluxation, which in turn aggravates the subscapularis tear.

Altogether, these histologic observations may explain the particular anatomic presentation of subscapularis tendon tears. For instance, several authors report that dislocations or subluxations of the LHBT are always accompanied by subscapularis tendon tears.[3,12,22,24] Partial tears (type I in the Lafosse[15] and SFA[26] classifications) typically begin deep in the upper portion of the tendon, which corresponds to the deep layer with parallel collagen bundles.[3,11,22] Other tears (type II in the SFA classification.)[26] begin on the medial edge of the bicipital groove, probably in the canthus, with delamination in the substance of the tendon extending in some cases to a full-length split. These tears most often occur, whether as a cause or an effect is unclear, with dislocation or subluxation of the LHBT into the subscapularis tendon.[3,12,28]

Type III tears[15,26] involve complete detachment of the tendon from the lesser tuberosity, leaving the footprint of the tuberosity devoid of any tendon insertion but with the superficial subscapularis band extending over the bicipital groove remaining in place. Arthroscopically, the observation of this outer band can misleadingly suggest that the subscapularis tendon is in place and intact.[11] This feature may also be the explanation for the "hidden tears" described by Walch et al[29] for open approaches, whether the LHBT is in place or dislocated. Finally, full-thickness tears of the subscapularis (type IV),[15,26] with retraction to the glenoid, involve complete detachment of both layers from the lesser tuberosity and of the superficial layer from the greater tuberosity.

The 2 separate musculotendinous junctions described here for the first time may explain the specific type of deep fatty infiltration observed in the upper portion of the subscapularis muscle,[20,23] which would be consistent with a tear in the deep layer of the tendon with parallel collagen bundles. Repair in these cases should involve reattachment of both layers to the lesser tuberosity, the deep layer often being retracted.[26]

The old age of the donors raises the question of whether the subscapularis tendon could have been damaged by mechanisms such as the roller-wringer effect,[17] which causes partial tears in the deep part of tendon. This is unlikely because the present study considered only healthy tendons, and those with even partial tears were excluded. Furthermore, there is no evidence to suggest that the roller-wringer mechanism modifies the histologic structure of the tendon. Finally, the 2 layers we describe have differently oriented collagen bundles and

independent musculotendinous junctions, which is strong evidence that this arrangement is primitive and not the result of an external mechanism.

The limitations of this study are the small number of samples included and that we only investigated the tendon and not the surrounding ligaments or the blood supply of the different structures. Immunohistochemical analysis of smooth muscle cells was not performed because the samples had been decalcified. Our best efforts notwithstanding, the reproducibility of the slices cannot be guaranteed because of size variations between individuals. Nonetheless, the same results were obtained for all the samples, regardless of the part of the tendon concerned, providing strong support for our conclusions.

Conclusion

Histologic analysis shows that the subscapularis tendon is composed of 2 layers, distinguishable by the orientation of the collagen bundles. These are similar to those of the supraspinatus tendon but are arranged differently. The thinner deep layer is composed of parallel collagen fibers and inserts onto the lesser tuberosity. The superficial layer is thicker, composed of interwoven bundles of collagen fibers, and splits into 2 bands that form a stabilizing sling around the LHBT. These 2 bands insert onto the greater tuberosity, whereas another part of the superficial layer inserts onto the lesser tuberosity. The 2 layers form separate musculotendinous junctions deep in the subscapularis muscle. The superficial layer covers the bicipital groove and connects with the supraspinatus tendon and the coracohumeral ligament. These histologic characteristics may explain the specific anatomic characteristics of subscapularis tendon tears. Further studies are required to test this hypothesis.

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