

## On Tendinous Systems of Muscles - A Histology-US Correlation

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### Summary

**Aim of the study:** to determine histology of the striated muscles tendinous systems based on own histological specimens in transverse and longitudinal sections and its correlation to US images.

**Materials and methods:** transverse and longitudinal specimens of the triceps surae of the rat in Gomori and Azan stains, US images of chosen muscles.

**Results:** From analysis of the longitudinal sections of the muscle results that the tendinous system of the muscle is a continuous structure without any junctions at the end of the myofiber or anywhere else. Tendinous fibers detach from the tendon by easily defined bundles and on the short stretch (the “cone” of the myofiber) become from compact connective tissue (tendon) to loose connective tissue with a criss-cross pattern of built (endomysium and perimysium). Therefore endomysium is a sheath of the myofiber and covers the whole lot of it. On every level of Z-membrane of a sarcomeres myofibers attach to endomysium via extracellular matrix. Endomysium hosts capillaries. Perimysium is loose connective tissue bands strengthening the muscle structure and tightly bond to endomysium. Perimysium hosts vessels and larger nerves.

At the ends of myofibers no tendinous inserts are seen between myofibrils. Therefore it looks that there is no additional tendinous structure to parietal myotendinous junctions at the end of the myofiber.

**Conclusions:** Muscle structure is it's tendinous system (tendon, endomysium, perimysium) and myofibers. Myofibers are fully covered by endomysium and strengthened by perimysium every few myofibers. Tendinous system of the muscle is a continuous structure - tendon (compact connective tissue) gives away fibers which are transformed into criss-cross built pattern loose connective tissue (endomysium, perimysium), and then back to the tendon. Tendinous system of the muscle is a key to US diagnostics of the muscle stretch-type injuries of all muscles because it always tears first. US is the method of choice in the diagnostics of muscle tears. Knowledge of the tendinous system of every muscle can be beneficial to the surgeons, who repair muscles tears.

**Keywords:** Anatomy; Histology; Muscles; Rendons; US

### Definitions

**Muscle** - the whole myotendinous unit from origin/origins to insertion/insertions.

It is built of compact connective tissue (tendons) and loose connective tissue in the form of criss-cross oriented fibers (endomysium and perimysium) and myofibers tightly connected to endomysium on the whole length of the fiber (parietal myotendinous junctions).

**Tendon** - a fibrotic creation built of compact connective tissue of white-silver color. Tendons are structural parts of muscles and they are a source of fibers to create endomysium and perimysium which arise from tendons by unzipping the tendinous structure to criss-cross loose connective tissue.

Most tendons have a bare stretch (no myofibers) and a belly stretch (zone of distribution and transformation of tendinous tissue into endomysium and perimysium).

Belly level tendon is a stretch of a tendon where tendinous fibers detach from the tendon forming loose connective tissue which is tightly bond with myofiber at the whole length.

A belly level tendon may be located:

- on the belly surface and then we call it semipennate - distribution of tendinous system fibers on one side of the tendon only,
- within a belly, than we call it pennate - fiber distribution on all sides of the tendon.

**Muscle belly** - A part of the myotendinous unit where the myofibers strengthened by endomysium and perimysium are present. On the

surfaces or within the bellies of muscles semipennate or pennate tendons as well as secondary tendons are located.

**Belly level of tendon** - direct continuity of a bare stretch of the tendon at the level of a muscle belly or a tendon directly attached to the bone (no bare stretch). A distributor of the fibers converting to endomysium and perimysium and secondary tendon (by definition pennate).

Belly level tendon or for short **belly tendon** may be semipennate and pennate. Pennate tendons are visible after a belly dissection because they run within a muscle belly. Often tendon which are originally semipennate become pennate further on into the belly. In those cases the place where the tendon disappears from the surface of a muscle is not the end of the tendon. From that place the tendon runs within a belly and is not visible.

**Secondary tendon** - a band of tendon separating from the belly tendon, not being endomysium or perimysium. By definition pennate - the whole stretch runs within a belly. Distributor/collector of fibers for endomysium and perimysium.

**Endomysium** - loose connective tissue with a criss-cross fibers pattern arising from tendinous fibers or directly from the bony insertion via periosteum. Endomysium wraps up every myofiber together with its ends. Endomysium of Human striated muscles attaches to myofiber at 500 levels for every millimeter of length. It contains capillaries and nerves.

**Perimysium** - arises from tendinous fibers of the belly tendon or directly from bony insertions. It has a criss-cross loose connective tissue built - like endomysium and runs along and is tightly bond to endomysium. Perimysium hosts larger vessels and nerves.

**EP zone** - zone of endomysium and perimysium, that is myofibers within muscle belly - between belly tendons or EP insertions. Within this zone endomysium and perimysium have criss-cross pattern of built. Definition of EP zone is narrower to muscle belly. Muscle belly contains tendons that is compact connective tissue.

**EP insertion** - usually vast insertion of a muscle via direct endomysium, perimysium without forming a tendon. EP insert via periosteum.

**Tendinous system** - tendons, endomysium and perimysium.

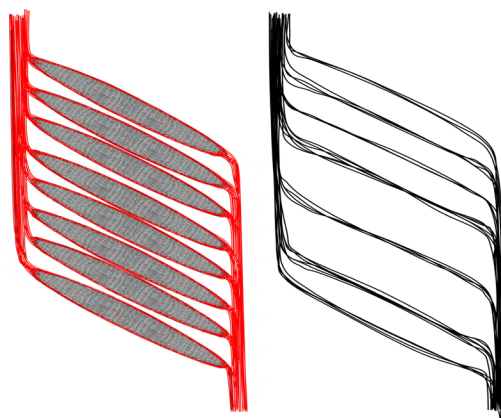
**Parietal myotendinous junction (PMTJ)** - a connection of myofibers to endomysium on every level of the Z-membranes of sarcomeres. Nociceptive zone of a muscle. Human striated muscle has 500 levels of parietal myotendinous junctions per every millimeter of length of a myofiber (every 2µm).

**The Cone of the myofiber** - at the end every myofiber narrows to become a 0mm thick structure + tendinous fibers which are formed from endomysium and perimysium. This is a transformation zone. The tendinous tissue changes here from the compact connective

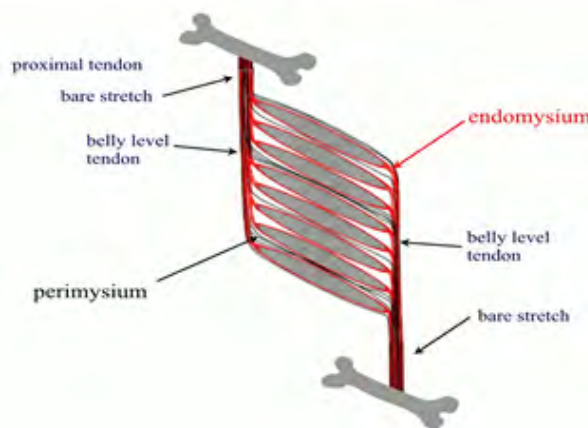
tissue to loose connective tissue and vice versa. The endomysium or endomysium+perimysium compress into bundles of compact connective (tendinous) tissue.

## Materials and Methods

Own longitudinal and transverse specimens of the rat triceps surae muscle Gomori and Azan stained and US images of normal muscles. They were analyzed to visualize and confirm or deny knowledge about striated muscles' tendinous system. Tendinous system consists of tendons, endomysium and perimysium (Figures 1,2). Within any striated muscle understood as a structure between origin and insertion there is no place where there wouldn't be a tendinous tissue. So muscle in fact is a tendon delaminated at the belly level to host myofibers (Figure 2).



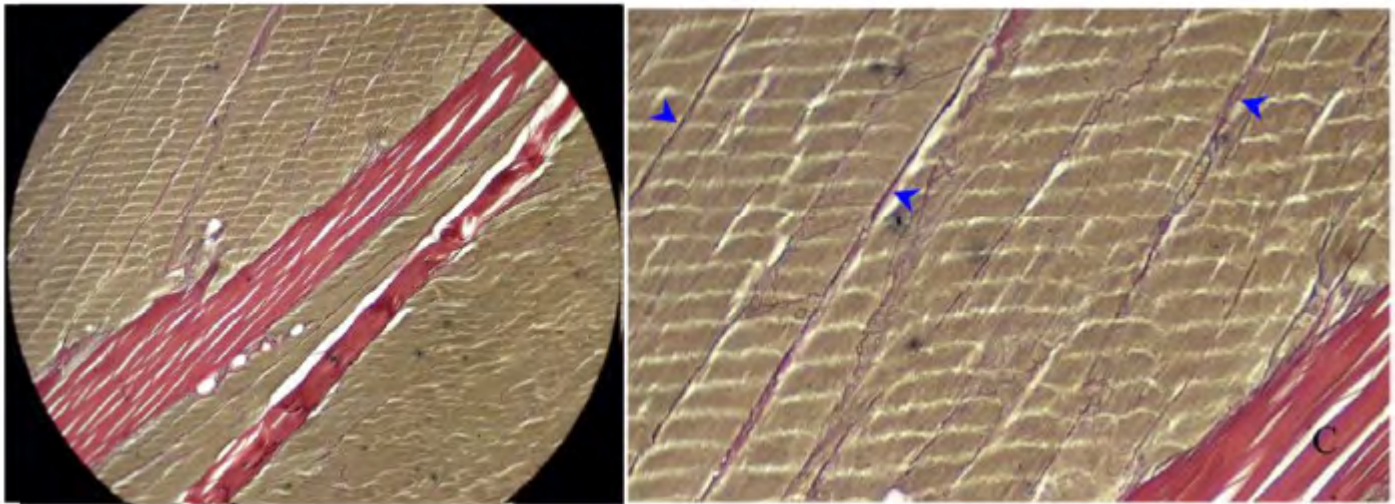
**Figure 1:** Red - endomysium and tendon, Black - perimysium and tendon. Purely theoretical separation. By permission [1].



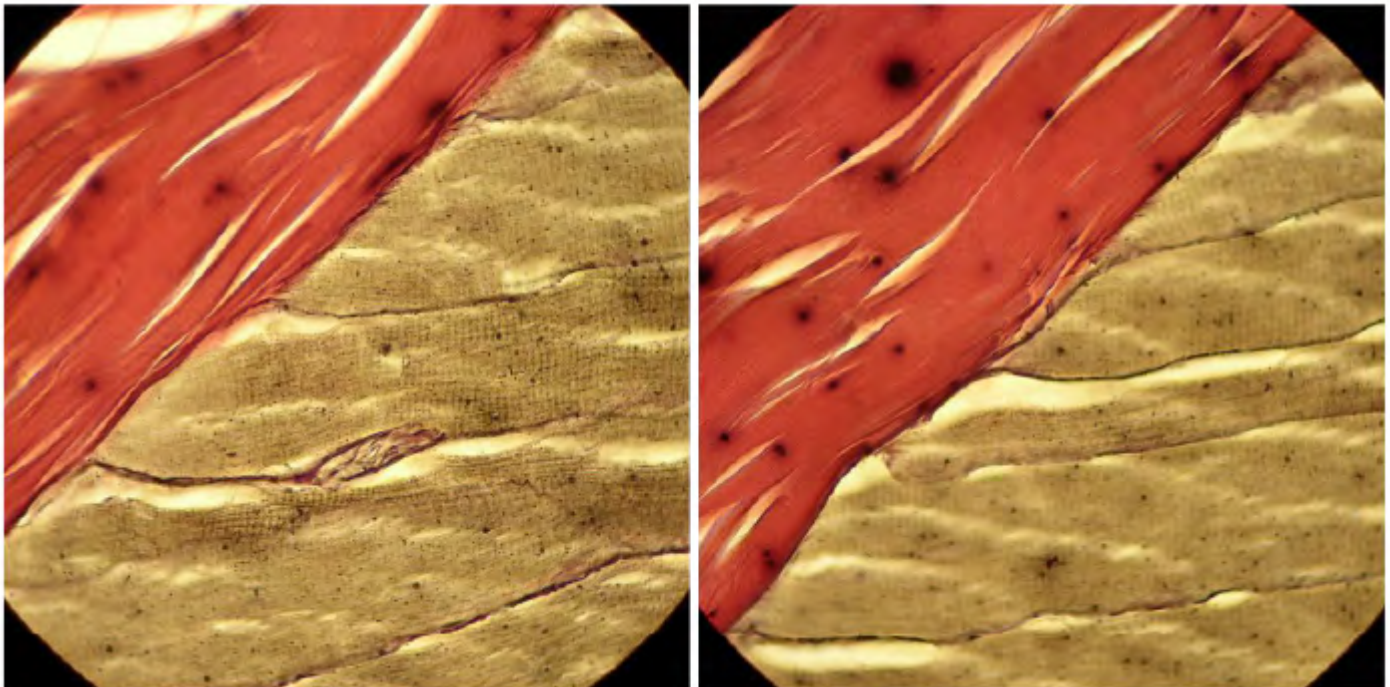
**Figure 2:** Scheme of a muscle. Red - endomysium, Black - perimysium. They arise from tendons and connect without any junction proximal and distal tendon. The scheme presents the simplest muscle. Both tendons are semipennate - run on the surface of the muscle distributing/collecting fibers on one side only. By permission [1].



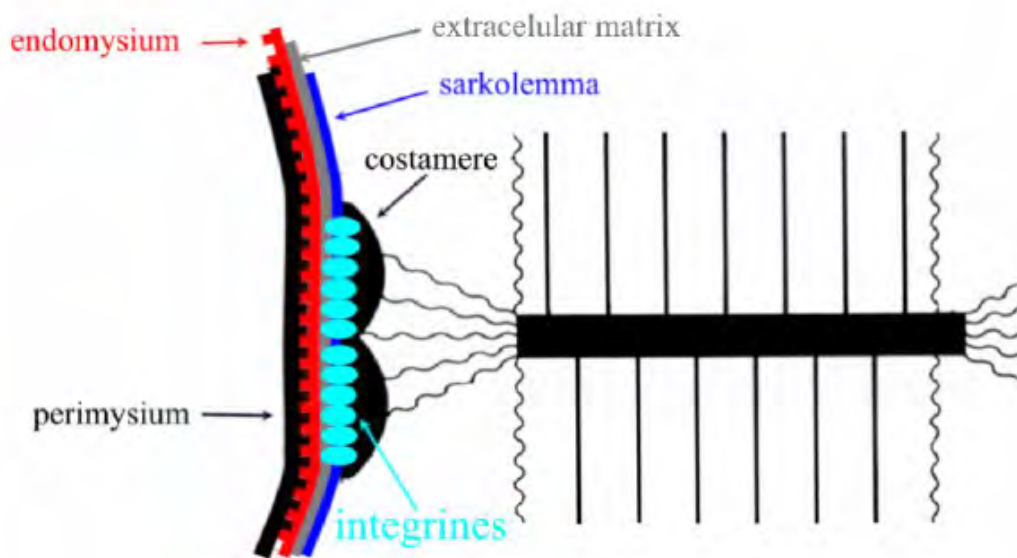
Histological specimens show that the tendons lose their mass as they go along the muscle belly (Figure 3). That is due to the fact that tendinous fibers detach from the tendon and without any junction cover the whole myofiber as loose connective tissue (Figures 4,5).



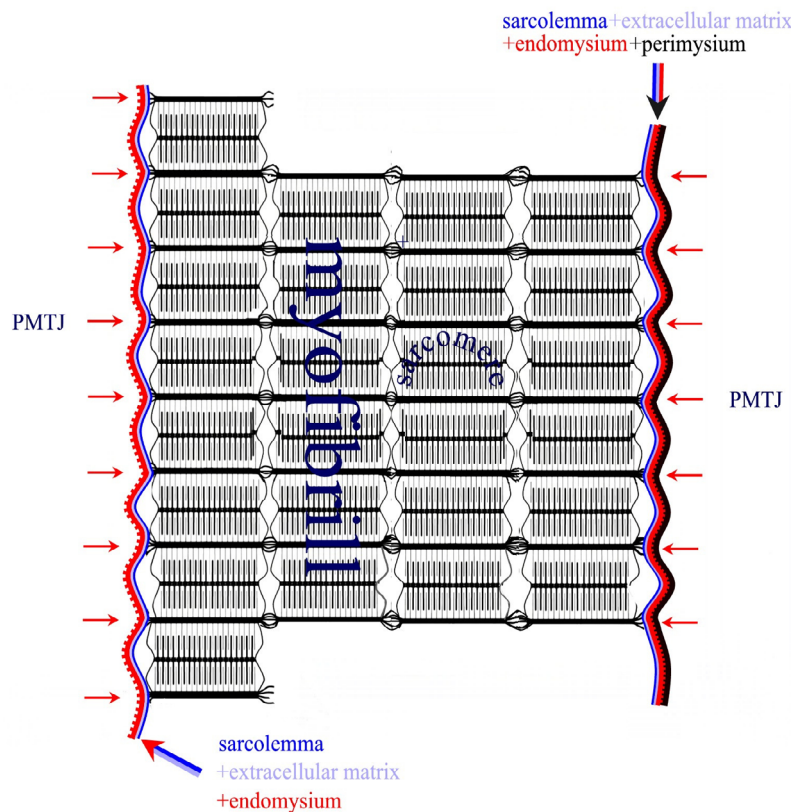
**Figure 3:** Longitudinal section of rat's triceps surae, Gomori stain. Tendinous system - red, myofibers - brown. The tendon becomes thinner due to loss of fibers which are transformed into endomysium and perimysium. Some thicker bands of perimysium indicated by blue arrowheads. By permission [1].



**Figure 4:** Longitudinal section of rat's triceps surae, Gomori stain. Tendinous system - red, myofibers - brown. Endomysium and perimysium contribute their fibers to the tendon. This is the longitudinal section right through the middle of the myofibers. On these two images it is clear that there is no additional to parietal MTJ myotendinous junctions at the end of the myofiber. The tendon gives away bundles of compact fibers to form a criss-cross loose connective tissue of the endomysium and perimysium without any junction. By permission [1].

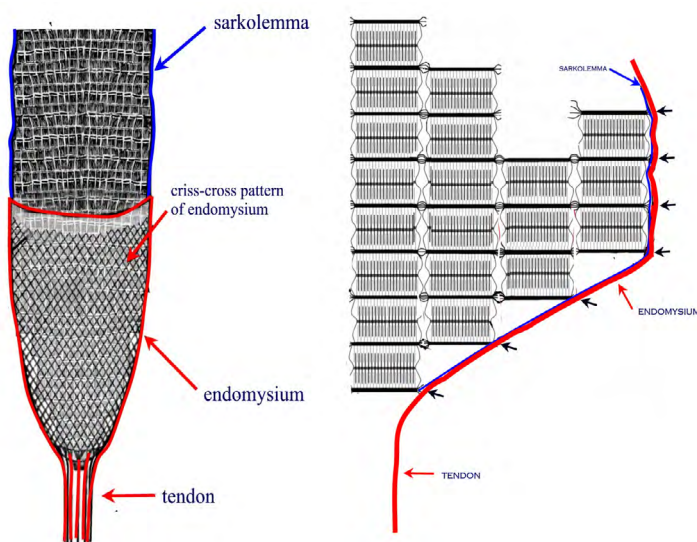


**Figure 5:** Scheme of the parietal myotendinous junction (PMTJ) at the level of the Z-membrane of the sarcomere. The only way the myofiber attaches to tendinous system is a parietal myotendinous junction (PMTJ) (Figures 5,6,7).



**Figure 6:** Myofiber, Parietal Myotendinous Junctions (PMTJ) at every level of the Z-membrane of sarcomeres (red arrows). By permission [1].

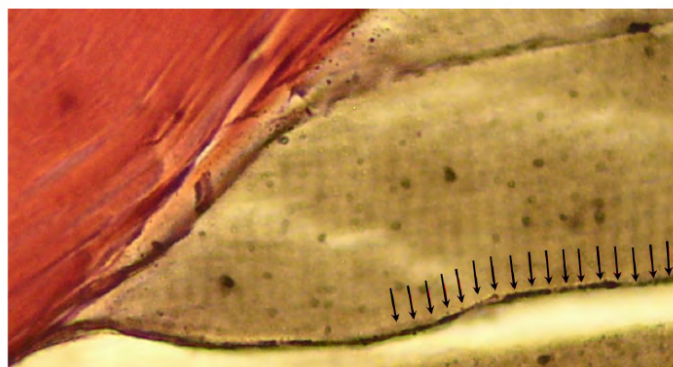




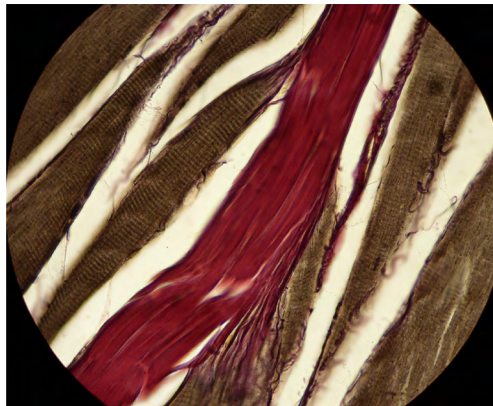
**Figure 7:** Tendinous system of the cone of myofiber - endomysium turning into a tendon at the narrowing end of the myofiber. Transition zone from tendon to endomysium. It narrows to allow sarcomeres of the internal myofibrils attach to endomysium via sarkolemma and extracellular matrix - parietal myotendinous junctions (black arrows).

The basic contractile element of the myofiber is a sarcomere. When myotendinous junctions are a “transmission” (transfer of the generated force onto the tendinous system), sarcomeres are the “engine” of the muscle. In order to attach a myofibril to sarkolemma and further on to endomysium a complex of transverse intermediate filaments attaches Z-membrane of the sarcomere to a protein complex called costamere which directly attaches to sarkolemma. In turn costameres are attached to a protein complexes called integrins which reach out of sarkolemma and anchor in the extracellular matrix [2]. This is in fact a system of myotendinous junctions called in this paper the Parietal Myotendinous Junction (PMTJ). These junctions are in fact a transmission by which the force generated by myofibers is transmitted onto the tendinous system by means of endomysium. So sarcomere shortening during contraction transmits the force on every level of the Z-membrane onto tendinous endomysium and combined with it perimysium and further on to belly tendon and finally tendon or via a direct EP insertion. It is clear that the only myotendinous junctions are on the whole surface of the myofiber at each level of the Z membrane of the sarcomere - Parietal MyoTendinous Junction (PMTJ).

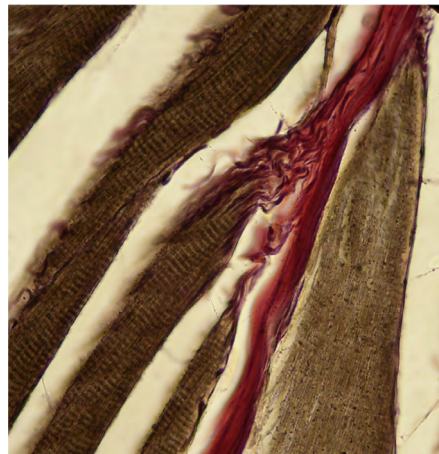
It is also clear that the tendinous system (tendons, endomysium and perimysium) is a continuous structure with no junctions. It is important to notice that the tendinous system is the structure that tears first in stretch-type injuries therefore one should follow the tendinous system to detect a tear. Ultrasound is a method of choice in this type of diagnostics. It only takes some effort to learn all the muscles tendinous systems anatomy. The end (cone) of the myofiber shows interesting feature of the tendinous system. At the level of the cone longitudinal striations can be seen on longitudinal/oblique sections (Figures 8,9,10). This phenomenon can be explained in only one way. This is where the loose connective tissue covering the whole myofiber must compress to single fibers of compact connective tissue and further on the tendon. It looks as they compress forming thin bands of tendon which gradually become a solid tendon. Therefore the end of the myofiber (the cone) is a transition zone from loose to compact connective tissue. At the cone level despite the parietal myotendinous junction there is another myotendinous junction mechanism. Actin filaments are anchored from one side to the Z-membrane, from the other side instead of interlacing with miosin penetrate through sarkolemma to the extracellular matrix just like integrines - also a parietal MTJ. Transverse sections of the muscle show that bands of endomysium are every few myofibers strengthened by bands of perimysium (Figures 11,12).



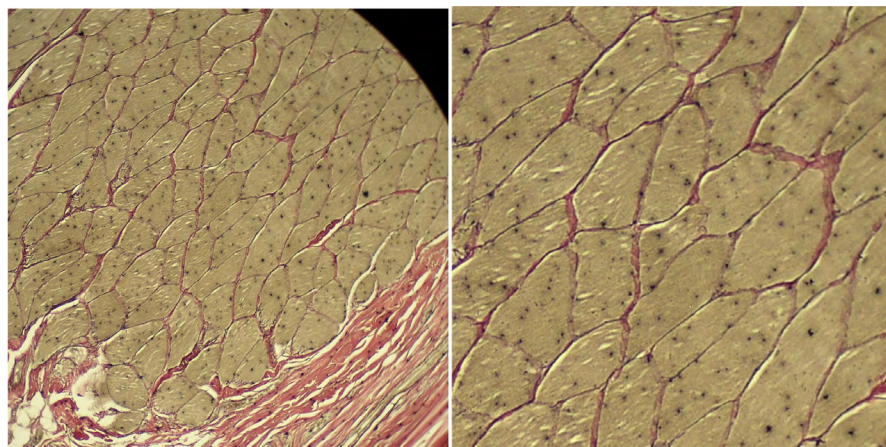
**Figure 8:** Longitudinal section of rat's triceps surae, Gomori stain. Enlargement of Figure 4. Arrows indicate some PMTJ's. This image shows clearly that myofibrils attach to the endomysium directly without any additional myotendinous junction at the end. No tendinous inserts between myofibrils are seen in light microscope. Myofiber's ends are narrowing gradually forming a “cone” to which all internal myofibrils can attach. By permission [1].



**Figure 9:** Longitudinal/oblique section of rat's triceps surae, Gomori stain. Tendinous system - red, myofibers - brown. Endomysium and perimysium of the narrowing zone of the myofiber present longitudinal red/bright brown striations which turn into bands of tendinous (compact connective) tissue contributing their fibers to the tendon. By permission [1].

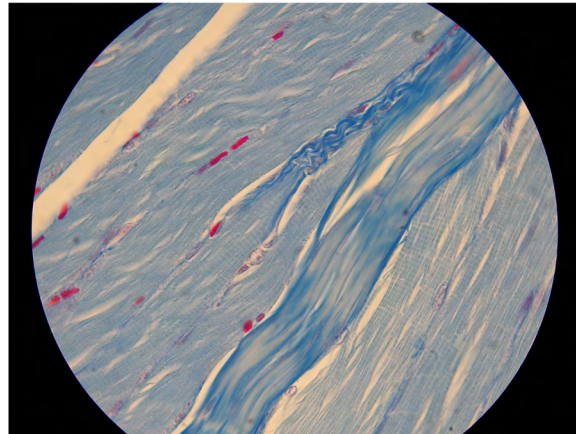


**Figure 10:** Longitudinal/oblique section of rat's triceps surae, Gomori stain. Tendinous system - red, myofibers - brown. Endomysium and perimysium contributing their fibers to the tendon. There is also as in the previous figure a striated zone of the myofiber at its end - the cone of the myofiber, a transition zone from loose connective tissue and compact connective tissue. By permission [1].



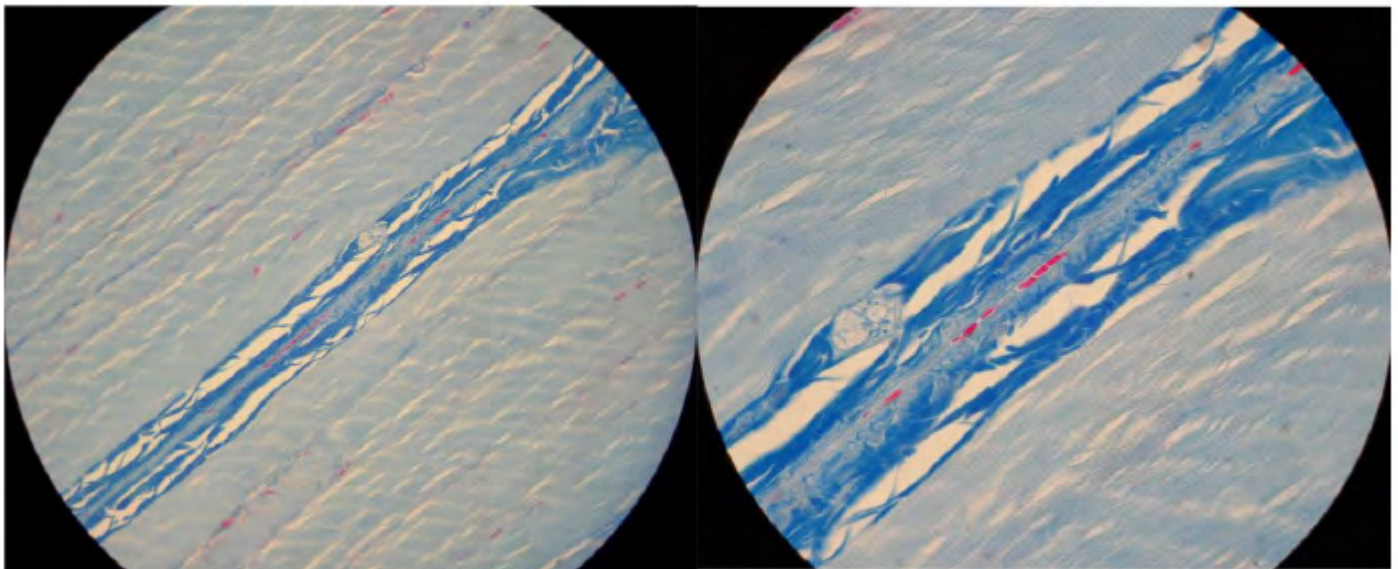
**Figure 11:** Transverse section of rat's triceps surae, Gomori stain. Tendinous system - red, myofibers - brown. Every myofiber is surrounded by a tendinous tissue. Thicker tendinous tissue means presence of perimysium. Right image - enlargement of the central section of the left image. By permission [1].



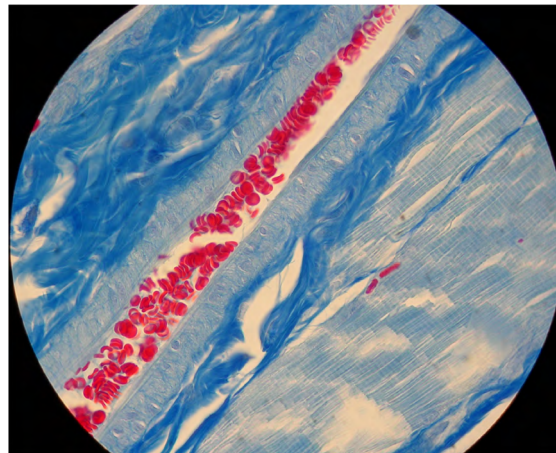


**Figure 12:** Longitudinal section of rat's triceps surae, Azan stain. Tendinous system, myofibers, vessels - blue. Endomysium and perimysium contributing their fibers to the tendon. Red objects - erythrocytes in the capillaries (endomysium and tendon). By permission [1].

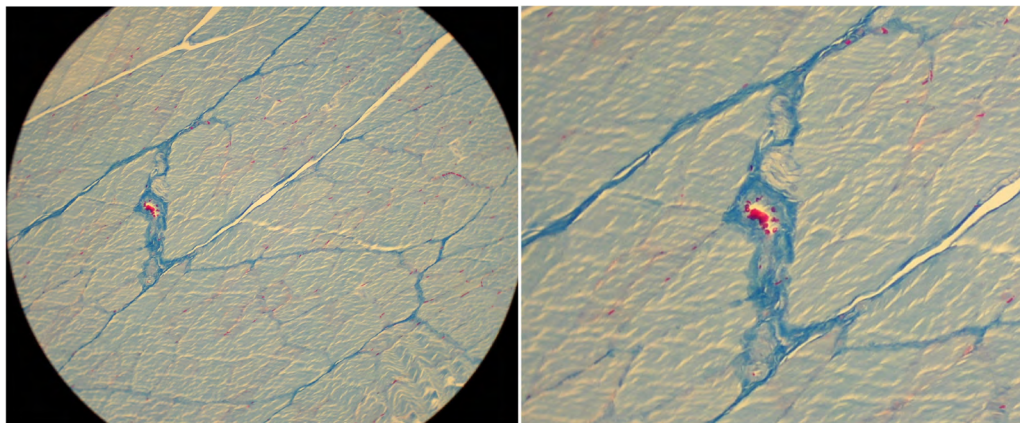
On Figures 13,14,15 examples of Azan stained specimens showing what perimysium does to the muscle's tendinous system structure. During the process of contraction myofiber ripples as do many worms (Figure 16). The difference between a worm and the myofiber is that the worm contracts and relaxes its segments to crawl and the myofiber contracts at the whole length to shorten and bring the origin to insertion generating movement or create a desired position. It is worthy to notice that according to Gray's anatomy [3] a human sarcomere is approx. 2 mm long in the relaxed state ( $1\text{mm}=1\times10^{-6}\text{m}=1\times10^{-3}\text{mm}$ ). That means that a human myofiber has 500 levels of PMTJ for every millimeter of its length. An interesting feature of a myofiber is that the only myofibrils that transmit force directly onto endomysium (that is forming a PMTJ) are those which are peripheral. All other myofibrils transmit their force onto PMTJ indirectly through neighboring myofibrils and at the ends (the cone) of the myofiber. At the cone of myofiber all four types of nerve endings are seen: Ruffini and Vater-Paccini corpuscles, Golgi apparatus and free nerve endings [4] This fact proves that the cone is a key proprioceptive zone of the myofiber.



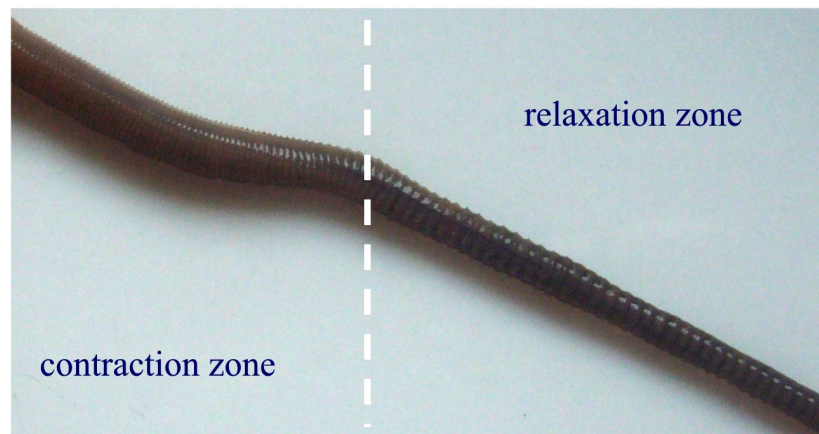
**Figure 13:** Longitudinal section of rat's triceps surae, Azan stain. Tendinous system, myofibers, vessels - blue. There is no tendon on these images. Centrally there is a vessel wrapped up in perimysium and it runs parallel to myofibers. Red objects - erythrocytes within a vessel and capillaries. Right image is an enlargement of the left image. By permission [1].



**Figure 14:** Longitudinal section of rat's triceps surae, Azan stain. Tendinous system, myofibers, vessels - blue. Image of a bigger than on the previous figure vessel filled with erythrocytes and wrapped up in thick band of perimysium. By permission [1].



**Figure 15:** Transverse section of rat's triceps surae, Azan stain. Tendinous system, myofibers, vessels - blue. Red - erythrocytes within a vessel and capillaries. Thick, darker blue bands - perimysium, the thickest hosting a large vessel. A band of perimysium appears every 4-6 myofibers. Right image is an enlargement of the left image. By permission [1].

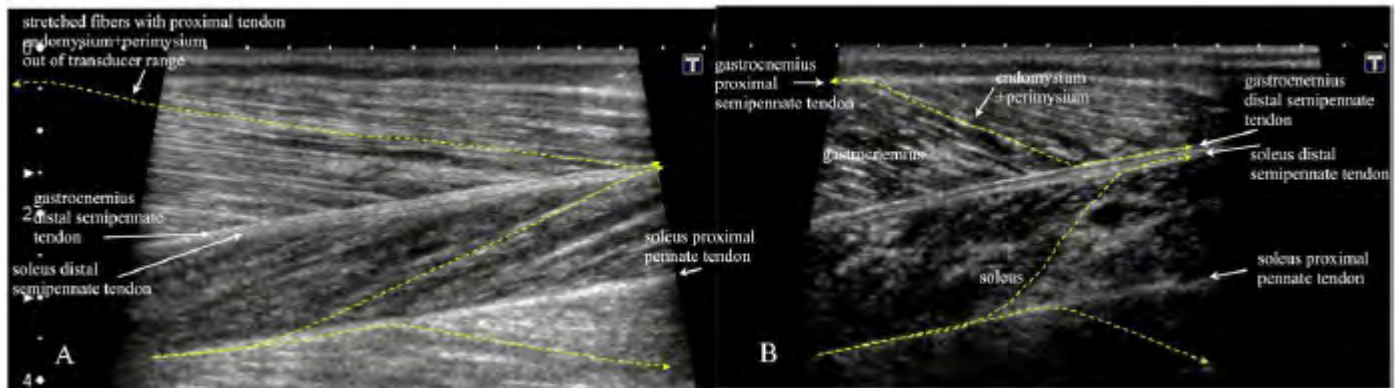


**Figure 16:** A photograph of a red worm - an ideal model of myofiber. In the relaxation zone the rings are long and narrow (reflect the US beam well), In the contraction zone the rings are short and folded (reflect US beam poorly). By permission [1].

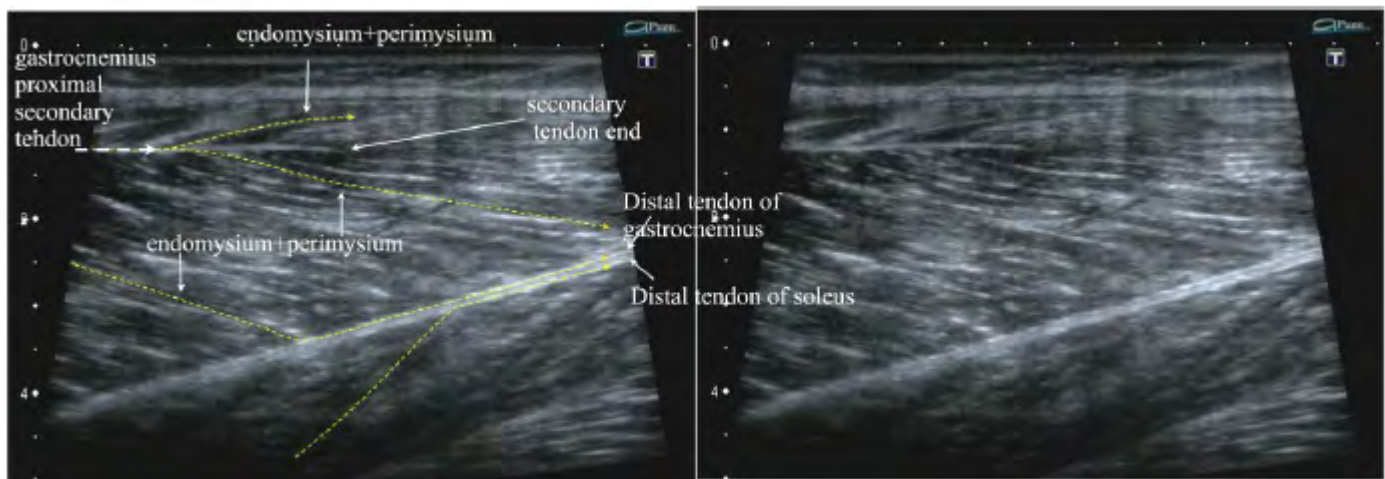


## Histology-US Correlation

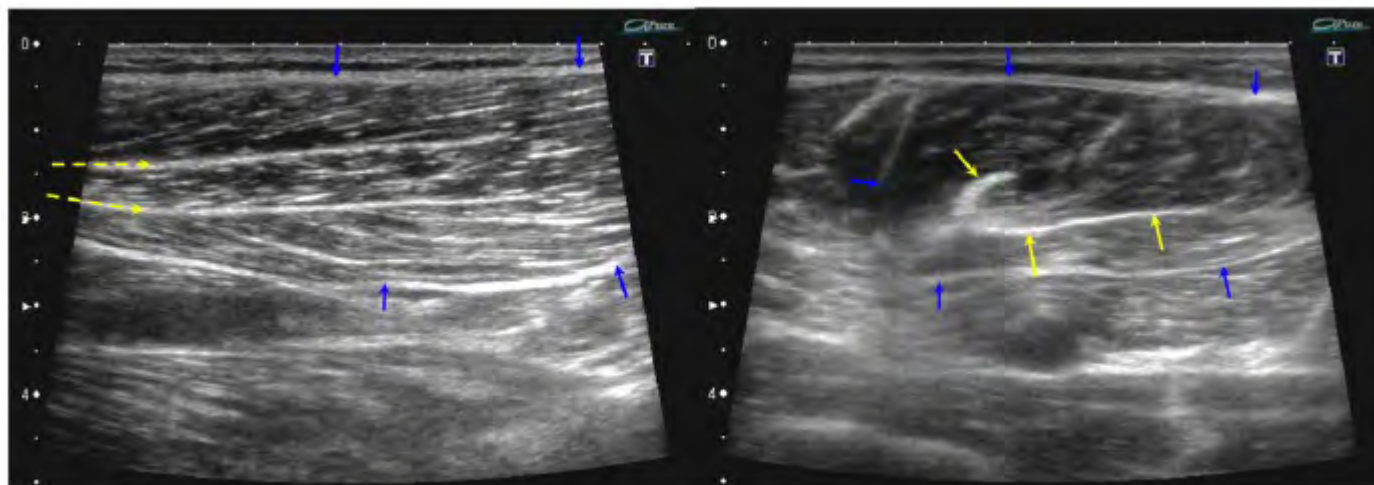
The whole tendinous system can be clearly visualized by ultrasound. The tendons as well as perimysium+endomysium show in a relaxed muscle as hyperechogenic lines of different thickness. Skeletal muscles show 0,1-0,4mm of anechoic space between the bright lines and that is 4 to16 myofibers (depending of the size of myofibers) per dark space between the lines of endomysium + perimysium in a relaxed state, neutral position. Figures 17,18,19 show the US images of selected muscles.



**Figure 17:** Longitudinal US image of the triceps surae. When muscles are stretched they appear as a structure of multiple bright straight lines of endomysium and perimysium connecting tendons. Between them there are dark stripes of myofibers. When muscle is or contracted the bright lines of EP are fewer and they are thicker, in places endomysium and perimysium are not seen. That is due to change of the myofibers surface from stretched/smooth to contracted irregular and thicker.



**Figure 18:** Longitudinal image of the medial head of gastrocnemius muscle. Anatomical variant of secondary proximal tendon. A part of the distal tendon runs within muscle belly - pennate.



**Figure 19:** Longitudinal (left) and transverse (right) image of the distal stretch of the proximal tendon of semimembranosus muscle. Blue arrows - outline of the muscle belly, yellow arrows - proximal tendon. The tendon and myofibers around it are most frequently torn in stretch-type contusions.

## Results

When looking at the histological specimens it is obvious that a tendinous system of a muscle is a continuous structure without any junctions. At the ends of myofibers (the cone) there is no additional to Parietal Myotendinous Junction (PMTJ) and there is a transformation zone for the tendinous tissue. At the belly level the tendon delaminates its superficial portion in a form of well defined bands of collagen fibers and covers to the whole myofiber attaching to it without any additional to parietal myotendinous junction.

## Discussion

It has been widely accepted in the world literature and lectures that the striated muscle is build as follows: tendon-myotendinous junction at the ends of myofibers-muscle-myotendinous junction-tendon [5]. My research shows that the tendinous system of the muscle is a continuous structure without a junction. There is no other than PMTJ myotendinous junction within muscles. The structure of the tendinous system of muscles should be studied by the surgeons who repair tendinous system tears. Knowledge of every muscle anatomy will be beneficial to the choice of suturing technique. It is the tendons which should be primarily found and sutured. Their location can be well visualized by ultrasound.

## Conclusions

The tendinous systems of muscles is a continuous structure and is a key to understanding of stretch-type muscle tears. The tendinous system of muscles is perfectly shown by ultrasound examination. Knowledge where what kind of tendon is, will lead the way to good diagnosis. Ultrasound is the best of all imaging methods to show muscles' anatomy and injuries. Knowledge of the tendinous systems anatomy will be also beneficial for the surgeons who repair muscle tears because it is the tendon that must be repaired first.

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