

Macromolecular structure of myotendinous junction in hamstring muscle from human subjects following a single bout of strenuous exercise.

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The purpose of this pilot study was to examine the structural adaptation of the myotendinous junction of the humane hamstring muscle after one bout of strenuous exercise.

Background.

The myotendinous junction (MTJ) is the site where the force generated by muscle contraction is relayed to tendon. Electron microscopic examinations of MTJ reveal invaginations of the adjoining musclecell and tendon forming so called interdigitations and invaginations like folded hands.

In this fashion the contact surface between the two types of tissue is markedly increased and so is the potential upper limit for transmission of force (Jozsa et al. 287-97; Jozsa et al. 306-12).

On a macromolecular level, the basis of the mechanotransduction are transmembrane glycoproteins called integrins distributed in the cellular membrane as adhesion receptors

The basement membrane is a sheet-like layer of ECM predominantly composed by:

collagen type IV, laminin, fibronectin and several proteoglycans such as perlecan and nidogen (Timpl and Brown 123-32).

Fibronectin and laminin, both adhesive glycoproteins, are of special interest as they constitute some of the important ligands for integrins. The ECM serves as a strong scaffold for the cells in the tissue but also as a grid where mechanical stimuli can be relayed throughout the matrix and to the cells.

Material and method.

Subjects

14 healthy human subjects age 25+/- 3 (mean +/- SD) with torn anterior cruciate ligaments were recruited and included for this pilot study. Individuals with severe meniscus og cartilage injury revealed on MRI were excluded with regards to the planned strenuous exercise preoperatively.

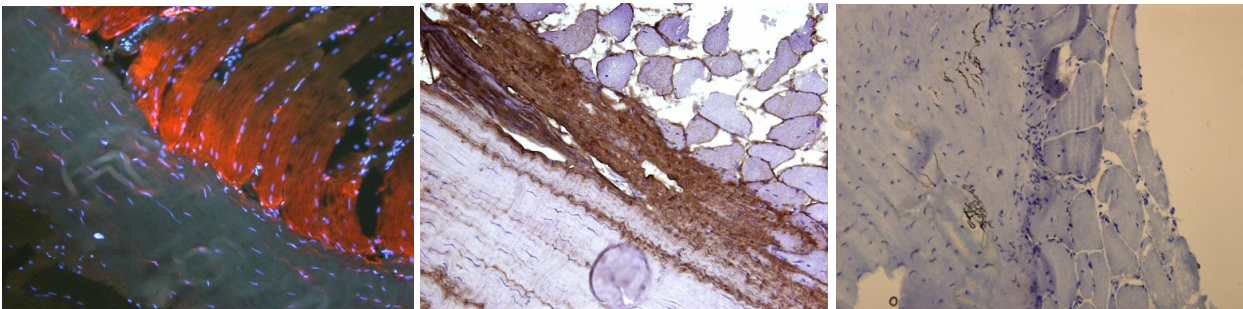
Verbal and written informed consent was obtained from the subjects prior to inclusion. The study was conducted according to the Declaration of Helsinki and approved by the local ethics committee (H-B-2008-066). The subjects were randomized into two groups, one group performed one bout of exercise 18+/- 2,5 hours prior to surgery. The exercise was performed by loading the hamstrings on the sick side and performing three sets of 80 % maximal performance (RM). The other group merely rested and was restricted to light weigthbearing 24 hours up to surgery.

Procedures

Tissue with tendon, myotendinous junction and muscle from was secured by the surgeon during harvesting for preparation of the anterior cruciate ligament graft. The sample was then swiftly divided up in tissue types, embedded in mounting medium and frozen in isopentane. Serial transverse sections of the tissue were cut in 10 um slices using a myotome at – 20 degrees Celsius. To map out the myotendinous junction a wide variety of antibodies were used, specific for the structural hallmarks of the MTJ.

Results.

The stainings of the myotendinous junction performed included the aforementioned laminin, fibronectin, collagens, integrins, dystroglycans, fibertypes. The staining for integrins with the antibody we used in this study did not succeed. The other elements of the myotendinous junction were represented as expected. No ascertainable structural change was seen in the stainings from the group that performed preoperative excercise as there was no significant difference between the two groups. The images below are representative of the different samples analysed.



Images: Muscle fibres (upper/right of images) meet tendon tissue (lower/left side) at the myotendinous junction (MTJ). From left to right: 1) NCAM staining (red) of fibres close to the MTJ; 2) Collagen type III staining (brown) only of the part of the tendon closest to the MTJ; 3) a high density of nuclei (blue) around the MTJ.

Conclusions.

To better examine the myotendinous junction, we will in future studies focus on other analysis methods such as electromicroscopy. Furthermore excercise done preoperatively should probably be of higher intensity and duration as to provoke structural change in the myotendinous junction.