A Micro-CT quantitative analysis of tendon enthesis


Introduction
In the field of orthopaedic research, X-ray micro-tomography has became a frequently used method for the investigation of bone specimens. The reason is that a µCT system permits the non-destructive investigation of trabecular and cortical bone specimens (for example tendon enthesis) without any special preparation of the sample. Tendon is a highly organized connective tissue joining muscle to bone, capable of resisting high tensile forces while transmitting forces from muscle to bone (1). Entheses is the point at which a tendon or ligament or muscle inserts into bone, where the collagen fibers are mineralized and integrated into bone tissue. These insertion points are commonly called Sharpey’s fibers (2-3) (Fig.1). The enthesis consists of four zones: the tendon, unmineralized fibrocartilage, mineralized fibrocartilage and lamellar bone. Entheses are metabolically active and are nourished by blood supply from the peritenon, perichondrium and periosteum. In the context of a tendon, it ensures that the contractile forces generated by the muscle belly are transmitted to the skeleton (1).

Bone sample can be examined with a spatial resolution in the micrometer range and this is not possible with the standard procedure.

Fig.1: Anatomy of the enthesis in Achilles tendon.
Aims
Little is known about tendon adaptations induced by mechanical loading; a variety of conditions, might include immobilizations, training, detraining, aging, medications, etc may alter the normal characteristic of tendon and entheses.
The aim of current experimental study was to make a quantitative measure of entheses thickness of patellar tendon (the central portion of the common tendon of the quadriceps femoris, which is continued from the patella to the tuberosity of the tibia) in rat and, besides, to asses the effect on entheses of a moderate exercise in a group of trained animal, a group of de-trained and a group of not trained.

Materials and methods
In our study we used six male albino Sprague-Dawley rats aged 9 weeks. Four rats were randomly chosen to run on a six-lane rodent treadmill 1 h a day, three times a week. Two control animals were placed on a non-moving treadmill during the training session (control group). At the end of the 10-week training, two rats were randomly chosen for being sacrificed (trained group), while two of them were caged without exercise 4 more weeks before being sacrificed (detrained group).
Animal handling, training protocol and mode of sacrificing were approved by the Ethical Committee and following D.L 116/92. The rats were anesthetized and the knee articulation was removed and immediately fixed in 4% paraformaldehyde for 48 hours. The samples were dehydrated in different concentrations of alcohol (EtOH 75%, 95%, 95%, 100%, 100%). The bone specimens were put in parafilm, to prevent drying, and they were scanned by the X-ray Micro-tomograph Sky Scan 1172. Each X-ray projection was acquired a 12-bit grey level image, 2000x1150 pixel, 10.97 µm image pixel sizes, with Al filter and for a time of 35 minutes. After acquisition the projection images were reconstructed (NRecon 1.4.4), and all the sections were cut for a longitudinal plane (CTAn 1.7) (Fig 2-3). After this a region of interest (ROI), at the entheses level, was selected. ROI was selected inside the volume of the bone area linked to the tendon. The two lateral extremes were detected by the investigator and these extremes dovetail with the tendon lateral extremes visible in the sections that were analyzed. The other two extremes follow the bone profile (Fig 2b). This operation was repeated for different sections arranged significantly along the volume and consequently the ROI was interpolated. To compare the same number of sections for each sample, ROI dataset of every examining sample was reduced at the 70 most central sections. Subsequently the images inside the selected ROI were binarized considering the bone as white and the quantitative 2D parameters were saved in a Microsoft Excel file. After this all the sections were analyzed in 3D and, also in this case, quantitative parameters were saved in a Microsoft Excel file.

Results
Data concerning Bone Area (B.Ar) were plotted in a graphic. The curves trend shows the profile of bone area of entheses along the 70 sections above selected (Fig 4, 5, 6).
Moreover after the 2D investigation, was carried out a 3D analysis and the bone volume (B.V) was calculated (Table 1).
Tab.1: Mean Bone Area (B.Ar) and bone volume (BV) of right and left entheses in control group, trained group and detrained group.

In our study the structural changes (detected by the Bone Area analysis and the Bone Volume analysis) were analyzed on the entheses patellar tendon of trained, de-trained and not trained rats.

The profile of the Bone Area (B.Ar) along the three Groups had not showed any significant difference. Moreover, the entheses Bone Volume, along the three Groups, had showed changeable values; the Control Group has the high values compared to the Trained and the Detrained Group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Media Bone Area</th>
<th>Bone Volume</th>
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<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>CONTROL</td>
<td>1.30 mm²</td>
<td>1.40 mm²</td>
</tr>
<tr>
<td>TRAINED</td>
<td>1.10 mm²</td>
<td>1.07 mm²</td>
</tr>
<tr>
<td>DETRAINED</td>
<td>1.17 mm²</td>
<td>0.99 mm²</td>
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Fig.2: a) longitudinal plane of knee articulation; T: tendon; E: entheses; Ti: tibia; F: femur. b) entheses, region of interest (ROI).

Fig.3: MIP (maximal intensity projection) of knee articulation
Conclusions
In previous studies the bone area was evaluated by histology and histomorphometry. Using the classic histomorphometry, the entheses thickness was calculated measuring the mean length of three points picked out along the entheses thickness and considering only three consecutive sections. However this is a destructive (the samples must be cut and stain) and slow (the samples must be embedded) investigation. The µCT analysis is very important for this kind of study because is a fast method and consent to have 3D measures that are impossible to obtain with normal histomorphometry. With the help of µCT we can reconstruct the profile of the area along the curve, observe the thickness of the entheses at all the different heights of each sample and compare them. These data of the three groups (trained, detrained and control) allow detecting the bone behaviour, the bone state and how the physical activity affects on the bone welfare.

The aim of work was to evaluate the structural modifications, through µ-CT analyses, of entheses calculating its Bone Area and its Bone Volume in trained, untrained and detrained rats. Although some differences between the three Groups, trained, detrained and untrained, are present, the number of data that we have examined are still not enough to confirm and discuss these differences between the groups. At the moment we are examining a larger number of case in our laboratory to confirm all the results.

References: