In vitro DTI assessment of muscle architecture in osteoporotic and osteoarthritic subjects: a preliminary study.

Giulia Di Pietro[1,2], Marco Palombo[2,3], Jacopo Baldi[4], Eleonora Piccirilli[4], Monica Celi[4], Umberto Tarantino[4], and Silvia Capuani[2,3]

[1]IIT@Sapienza, Physics Department, "Sapienza" University, Roma, Rome, Italy, [2]Physics Department, "Sapienza" University, Rome, Rome, Italy, [3]CNR IPCF UOS Roma, Physics Department, "Sapienza" University, Rome, Rome, Italy, [4]Department of Orthopaedics and Traumatology, University of Rome "Tor Vergata", Roma, Roma, Italy

Target audience. Translational researchers interested in noninvasive assessment of muscle microstructures and musculoskeletal disorders.

Purpose. Osteoporosis and osteoarthritis are the most common diseases of musculoskeletal system. Osteoporosis is a metabolic disease characterized by low bone mass and microarchitetural deterioration of bone tissue[1]. Moreover, it is also associated with sarcopenia, loss of muscle bulk and power[2]. Osteoarthritis consists in a progressive articular cartilage loss with concomitant changes in the bone underneath the cartilage. Soft-tissue structures (synovium, ligaments, and bridging muscle) in and around the joint are also affected by osteoarthritis[3].A recent study showed that in osteoporotic subjects (OP) a preferential and diffuse type II fibers atrophy occurs compared to osteoarthritic patients (OA). In OA the muscle atrophy involves both type I and II fibers, and this status seems to be caused by disuse and pain[4].

The aim of this study was to investigate the microstructural features in muscles of osteoporotic and osteoarthritic women by using diffusion tensor imaging (DTI)[5]. Toward this goal we examined in vitro at 9.4T the vastus lateralis biopsy of osteoporotic and osteoarthritic subjects (extracted during the surgical operation of femoral head replacement) by measuring mean diffusivity (MD), fractional anisotropy (FA), the three eigenvalues (λ_1 λ_2 λ_3) of muscles and assessing associations between DTI parameters, subjects age, subjects bone mineral density (BMD) and subjects body mass index (BMI).

Methods. Vastus lateralis biopsy was performed in 5 women with osteoporosis (mean age = 82.3±3.5) undergoing surgery for hip fracture and in 5 age matched women (mean age = 75.0±5.5) undergoing arthroplasty for hip osteoarthritis with no significant functional limitations. This study was approved by the local Ethics Committee and written informed consent was obtained in all cases before study initiation. A Magnetic Resonance (MR) system operating at 9.4T and equipped with a micro-imaging probe with a maximum gradient strength of 1200 mT/m (rise time of 100 μs) was used to investigate muscle samples. Each muscle of 2 cm in length was stored in a 4% paraformaldeyde and PBS immediately after being extracted from the patient and then placed in a 8mm NMR tube. The DTI protocol consists of one b_0 image and diffusion weighted (DW) images obtained with b-values equal to 400 and 700 s/mm^2 along six no-coplanar directions. A Pulsed Field Gradient Stimulated Echo (PGSTE) imaging sequences was used (TE/TR=14.5/2500 ms, diffusion gradient pulse delay Δ=40 ms, diffusion gradient pulse duration δ=2 ms, field of view FOV= 0.75 cm and number of average NS=4). Twelve axial slices (slice thickness ST=1mm) were acquired. We evaluated the FA, the MD and the three eigenvalues (λ_1>λ_2>λ_3) in each slice and these quantities were averaged over all slices within the muscle. The muscle was identified through a threshold mask on MD maps. All computation was made using an homemade script in MATLAB®. Mean values and standard deviation were obtained for each variable for OP and OA subjects.

Between-group comparisons to assess group differences and Pearson correlation analysis were performed. P values<0.05 were considered statistically significant.

Results. No significant age difference was found between OA and OP (P=0.126). FA was significantly higher in OA compared to OP (P=0.022, **Fig.1A**) while MD, λ_2 and λ_3 were lower in OA compare to OP(P=0.039 **Fig.1B**, P=0.040 **Fig.1C**, P=0.022, **Fig.1C**, respectively). No significant difference in λ_1 was found between OP and OA (P=0.063 **Fig.1C**). A significant (P=-0.936) linear correlation was found between FA and BMI in osteoporotic subjects only. No significant correlation was found between DTI parameters, BMD and age.

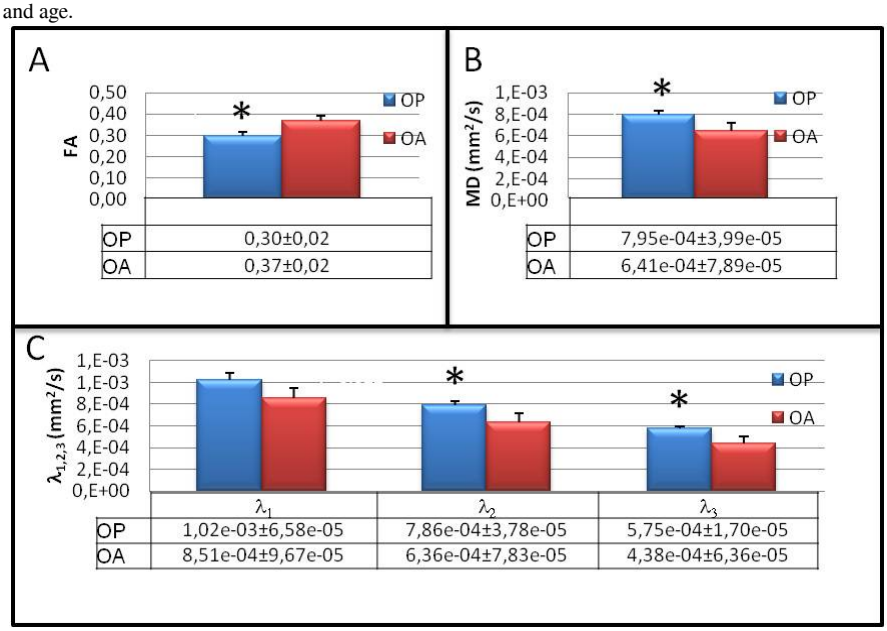

Fig.1 Histograms of osteoporotic (OP) and osteoarthritic (OA) subjects. **A**) FA. **B**) MD. **C**) λ1, λ2 and λ3.
* Statistically significant differences between OP and OA subjects (P<0.05).

Discussion. Our in vitro preliminary results highlight differences in DTI parameters between OP and OA muscles. FA is lower in OP when compared to OA. This outcome suggests that OP muscle has a more isotropic microstructure compared to that of OA muscle. Moreover MD, λ_2 and λ_3 are lower in OA compare to OP, while no differences was found in λ_1 between the two groups. These results indicate that the microarchitetural alteration due to osteoporosis disease causes a higher radial diffusivity. Because in vastus lateralis muscle type II fibers are more frequent compared to those of type I[6], osteoporosis induces type II fibers atrophy[4] with a consequent enlargement of water space between fibers in muscle. This microstructural scenario results in a decrease of FA together with an increase of radial diffusivity (λ_2 and λ_3). Our preliminary findings in OP muscles, based on DTI parameters measurement, confirm previous histological evidences[4].

Conclusion. The analysis of osteoporotic and osteoarthritic muscle shows that DTI measurement is a powerful tool to detect differences in the muscle structure due to different musculoskeletal pathologies. This data seems to be encouraging further analysis of muscle microstructure by means of diffusion techniques at high resolution and high magnetic field. For this reason a larger number of human muscle samples will be investigated to study the pathophysiological markers associated to osteoporosis and osteoarthritis diseases.

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