Novel Muscle Imaging Biomarkers to Characterize Progression of Osteoarthritis in a Post-Traumatic OA Mice Model

Usha Sinha[1], Sheronda Statum[2], Sophia Dwek[2], Kevin Burt[3], Yajun Ma[2], Aurea Mohana Borges[2], Carla Scanzello[4], Shantanu Sinha[2], Christine B. Chung[2]
[1]San Diego State University, San Diego, CA [2]University of California SD, La Jolla, CA, [3]University of Pennsylvania, Philadelphia, PA, [4]Corporal Michael J Crescenz VA Medical Center, Philadelphia, PA. Presenter email: (chchung@health.ucsd.edu)

INTRODUCTION: The socioeconomic impact of osteoarthritis (OA) is substantial, estimated to approach $60 billion per year, with no disease-modifying treatments yet available in the pharmaceutical market[1,2]. Advanced age and female sex are among the most common risk factors for knee OA (kOA). Although kOA is defined and diagnosed as a loss of hyaline cartilage within the joint, muscle impairments associated with the disease may be the primary underlying cause of functional impairments, and muscle dysfunction may actually precede and expedite the cartilage deterioration[3]. Dynamic stabilization, driven by neuromuscular coordination and periarticular muscle strength, is increasingly recognized as a critical factor in mitigating joint loading abnormalities following trauma. Targeting muscle dysfunction early may offer a novel, modifiable pathway to slow or prevent the progression of post-traumatic OA[4]. The **overall objective** of the research behind this preliminary report is to implement and validate state-of-art muti-parametric quantitative structural and functional MRI biomarkers for comprehensive muscle characterization that will subsequently inform individually tailored, precision intervention optimized for subjects with post-traumatic osteoarthritis (PTOA). To that end, novel, state-of-the-art MR imaging biomarkers, including Ultrashort Echo Time (UTEs), high-resolution MRI, Diffusion Tensor Imaging and Fiber Tracking was utilized to gain insight on the impact of different musculoskeletal factors, including inflammation markers, adipose and fibrosis quantification and fiber characteristics on the longitudinal progression of OA in murine models.

METHODS: This study was approved by IACUC. PTOA was induced in five female mouse model using partial meniscectomy (PMX). Female animals were used since advanced age and female sex are among the most common risk factors for knee OA. Both sexes will be compared at a later stage. Two animals were sacrificed after 1 week and three after 3 weeks. Imaging was performed of the injured limb and compared to the contralateral normal limb, on a 3T Bruker BioSpin, Ver. ParaVision 5.6 animal MRI scanner, at 1 and 3 weeks, to evaluate the longitudinal progression of the following: (1) **Atrophy**: Muscle volume was determined from manually outlining of 3D high resolution morphological images of the quadriceps on the injured and contralateral side, acquired with a T1-weighted 3D-FLASH sequence, yielding isotropic voxels of 100 micron, 128-170 contiguous slices to cover the entire length of the femur, covering about 3 cm along the Z-axis of the sample) (Fig.1). Slicer 2.8.1 software was used for manual segmentation and volume rendering of the quadriceps. **(2) Fat Fraction/ Intra-**muscular Adipose Tissue (IMAT): A 3D RARE variable-flip-angle Fat-Water Separation sequence based on the Dixon method was used to generate fat-only and water-only images for quantification of fat fraction. ~128~170 contiguous slices (i.e. no slice gap), an in-plane resolution of ~100 microns, covered the entire length of the femur. **(3) Fibrosis/Macromolecular Fraction**: A 3D Ultrashort Echo Time Magnetization Transfer (UTE-MT) with fat suppression sequence was used with modeling (two-pool model) to extract the macromolecular fraction in ROIs placed in the injured and contralateral quadriceps (Fig.2). **(4) Quantitative T2 imaging:** T2 maps were computed from data acquired with a MSME sequence with 10 echoes, yielding in-plane: 250x250 microns, slice thickness: 500 microns. (5) **Muscle Fiber Integrity**: The DTI data was acquired with a Spin echo planar imaging sequences with 5 baseline images, 30 non-collinear gradient directions, with b value of 400s/m^2. The DTI data was processed to extract DTI metrics at each voxel and track fibers in the quadriceps. Fibers were tracked bilaterally using manually placed regions in the injured and contralateral quadriceps (Fig. 3), using DSI-Studio.

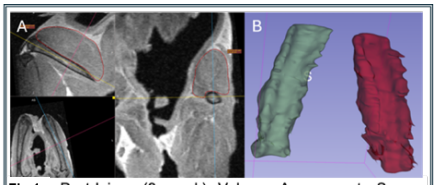

Fig.1. Post-Injury (3 week) Volume Assessment: Source images (A) and volumetric evaluation (B) show significant volume loss (green – 120mm3 in injured versus uninjured (red – 156 mm3) thigh muscles.

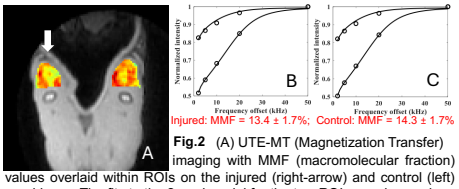

Fig.2 (A) UTE-MT (Magnetization Transfer) imaging with MMF (macromolecular fraction) values overlaid within ROIs on the injured (right-arrow) and control (left) quadriceps. The fits to the 2-pool model for the two ROIs are shown along with the MMF values, showing a (B) decrease in MMF with injury compared with (C) control.

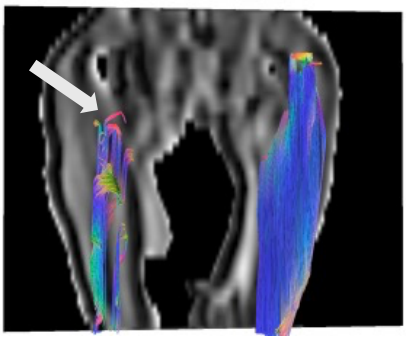

Fig.3. : DTI of injured right (arrow) thigh versus normal left thigh shows fewer (15744 v 58857) and shorter (13.3 v 13.8 mm) tracts, respectively. Differences in fractional anisotropy, mean diffusivity and radial diffusivity were also noted and presented in the text.

RESULTS: Changes were seen in the multiparametric images in mice cohort images acquired at 1 week and at 3 weeks after injury. Volume of the quadriceps muscle of the injured leg showed an average decrease of 10% at 3-weeks post injury compared to the contra-lateral leg (Fig. 1). T2 values increased on an average by 10% in the quadriceps muscle of the injured leg (3-week post-injury). The 1-week post injury showed a surprising decrease in T2 compared to the contralateral quadriceps. Macromolecular fraction decreased on an average by 10% in the injured quadriceps in both the 1-week and 3-week mice cohorts (Fig.2). Fat fraction increased by 20% in 3-week post-injury quadriceps muscle compared to the contralateral muscle, whereas in the 1-week post-injury there was a surprising decrease in fat fraction. Diffusion tensor imaging (DTI) yielded reduced fiber length (Fig. 3), accompanied by a decrease in Fractional Anisotropy (FA) and Mean Diffusivity (MD) in the injured quadriceps compared to the contralateral muscle in both mice cohorts.

DISCUSSION: Disuse of the injured leg would lead to muscle atrophy of the quadriceps on the injured side compared to the normal side and this is confirmed in our study by the decrease in volume and in fiber length from morphometric and DTI fiber tracking respectively. T2 is an important marker for inflammation/edema; muscle degradation in several MSK conditions is accompanied by an increase in inflammation. The increase in T2 values seen in the PTOA model may potentially reflect inflammation effects associated with muscle remodeling. The decrease in MMF may be a consequence of the combined effect of a decrease in myofibrillar proteins and an increase in collagen that reflect the loss of fiber integrity and fibrosis respectively. Our study also showed that increase in fibrosis increase is accompanied by adipose infiltration leading to overall % increase in non-contractile tissue that will potentially affect quadriceps strength of the injured leg. Muscle injury has been shown to result in a loss of fiber integrity that is reflected as a decrease and increase in FA and MD respectively. The changes in FA and MD seen in the quadriceps of the injured leg in this study are consistent with loss of fiber integrity.

SIGNIFICANCE/CLINICAL RELEVANCE: The clinical significance is that this study confirms that muscle remodeling occurs in the post-traumatic OA murine model and that this remodeling can be successfully monitored by multiparametric MRI. Our approach offers a non-invasive way for comprehensive characterization of muscle which in turn will enable one to target muscle dysfunction early to develop strategies to slow or prevent the progression of post-traumatic OA[5].

References:

1. Cameron KL, Hsiao MS, Owens BD, Burks R, Svoboda SJ. Incidence of physician-diagnosed osteoarthritis among active duty United States military service members. *Arthritis Rheum*. 2011;63(10):2974-2982. doi:10.1002/art.30498. 2.Cho Y, Jeong S, Kim H, et al. Disease-modifying therapeutic strategies in osteoarthritis: current status and future directions. *Exp Mol Med*. 2021;53(11):1689-1696. doi:10.1038/s12276-021-00710-y. 3.Alnahdi AH, Zeni JA, Snyder-Mackler L. Muscle Impairments in Patients With Knee Osteoarthritis. *Sports Health*. 2012;4(4):284-292. doi:10.1177/1941738112445726. 4.Shang X, Hao X, Hou W, et al. Exercise-induced modulation of myokine irisin on muscle-bone unit in the rat model of post-traumatic osteoarthritis. *J Orthop Surg Res*. 2024;19(1). doi:10.1186/s13018