Expanding on Characterization of the "Forgotten Muscle“ during Knee Osteoarthritis

Cadence Gatterer[1], José Cruz Ayala[1], Vinod Dasa[2], Luis Marrero[2,3]

Louisiana State University Health Sciences Center School of Medicine[1], Department of Orthopaedics[2], and Morphology and Imaging[3]

Introduction

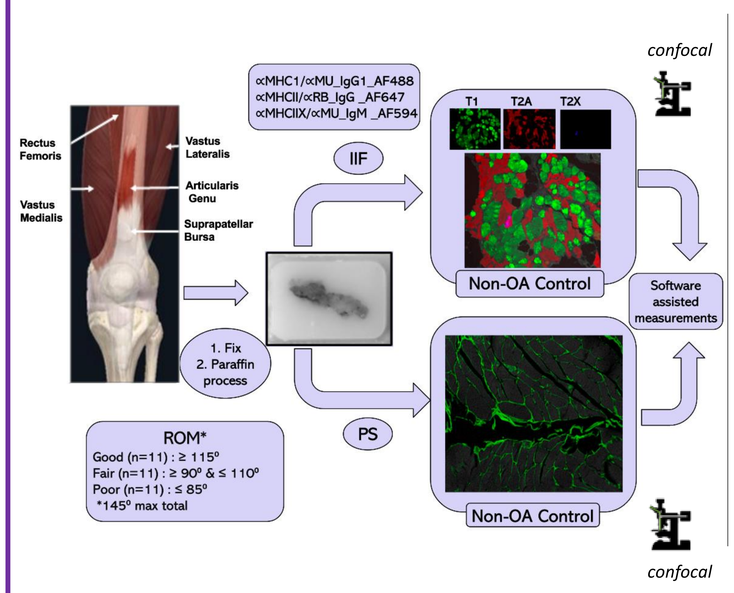
Skeletal muscles are composed of type-I (T1), high endurance, slow-twitch myofibers, and type-II (T2), high strength, fast-twitch myofibers. The former derive energy from oxidative phosphorylation, and are rich in mitochondria. On the other hand, T2 fibers mostly rely on glycolysis for energy metabolism, and fatigue easily. T2 fibers are further classified into T2a and T2x subtypes. Myofibers can be identified by specific expression of different myosin heavy chains (MHC). MHC1, 2, and 7 specifically and respectively expressed by T1, T2a, and T2x subtypes. Additionally, two different MHC can be processed and co-expressed by myofibers in a transitional state between subtypes, and thus termed hybrids, such as T1/2a, and T2a/x.

The *Quadriceps femoris* (QF) complex in younger individuals is normally composed of an approximately even ratio of T1 and T2a fibers. The presence of T2x and T2a/x hybrids in the healthy QF is rare. Changes to T1:T2 distribution is dynamic, depending on activity type and levels. For example, the ratio of T1 over T2 will shift to favor higher T1 counts in older individuals due to aging-related, preferential de-innervation of T2 fibers. Moreover, because T2x fibers are required for sudden high strength contractions, and are the most fatigable, higher incidences of T2a/x hybrids are associated with inactivity and sedentary behavior.

The normal integrity of the QF is compromised by functional limitations caused by knee osteoarthritis (kOA). Loss of OA knee motility is clinically assessed by measuring active range of motion (ROM). In other words, higher ROM deficits relate to higher knee disuse, and therefore, based on studies on the OA *Vastus lateralis* (VL), a higher risk of atrophy, fibrosis, and deleterious switching between myofiber subtypes to T2a and T2a/x hybrids. The *Articularis genu* (AG) is a small intra-articular muscle in close proximity to the synovium and continuous to the *Vastus intermedius* (VI). The AG coordinates movement of the suprapatellar fat pad and tightens the synovial membrane to prevent impingement of the synovial folds.

The AG can be sampled during total knee arthroplasty (TKA) for OA and from non-OA organ donors and preserved in our biobank. Consistent with normal QFs, healthy AGs also contain a nearly equivalent T1:T2 composition and less than 0.5% T2x or T2a/x hybrids. Nearly a year ago, we generated evidence that similar to the OA VL, banked OA AGs from our TKA patients undergo myofiber atrophy and progressive switching of T1 to T2 fibers in association with deficits in ROM. Our data are compared to similar studies in the VL during OA (Noehren et al. 2018). Notably, our lab recently published evidence that the severity of synovial fibrosis (SFb) during OA also correlates to ROM metrics. To that end, we predict a higher incidence of T2a/x hybrids in the AG of OA knees that is dependent on increasing ROM deficits. Concurrent with this myofiber phenotype and also in a ROM deficit dependent fashion, we anticipate measuring higher fibrosis of the AG (AGFb) in association with high SFb in neighboring synovium.

Methods



IIF: indirect immunofluorescence; PS: Picrosirius technique for collagen fibers

Results

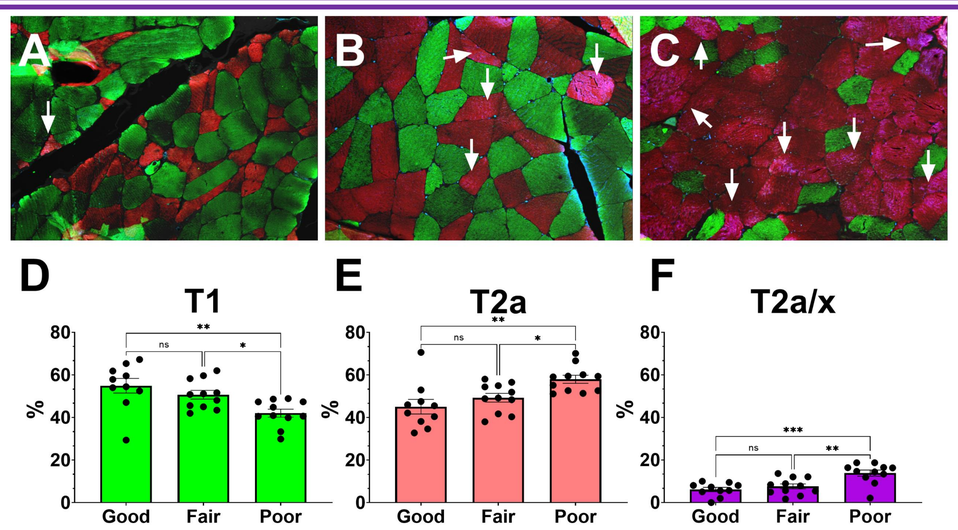


Figure 1. Representative 200x confocal photomicrographs of MHC7 forT1 (green), MHC2 for T2a (red), and MHC1 for T2a/x hybrid (magenta) myofiber distribution in OA AGs by indirect immunofluorescence grouped by (A) good, (B) fair, and (C) poor ROM groups (n=11 each). Percentage of each myofiber subtype was quantified as a percentage of (D) T1, (E) T2, and (F) T2a/x hybrids over total myofibers and groups compared by one-way ANOVA with Tukey's multiple comparison test and alpha set at 0.05. *p<0.05; **p<0.01, and ***p<0.001. Arrows indicate T2a/x hybrids.

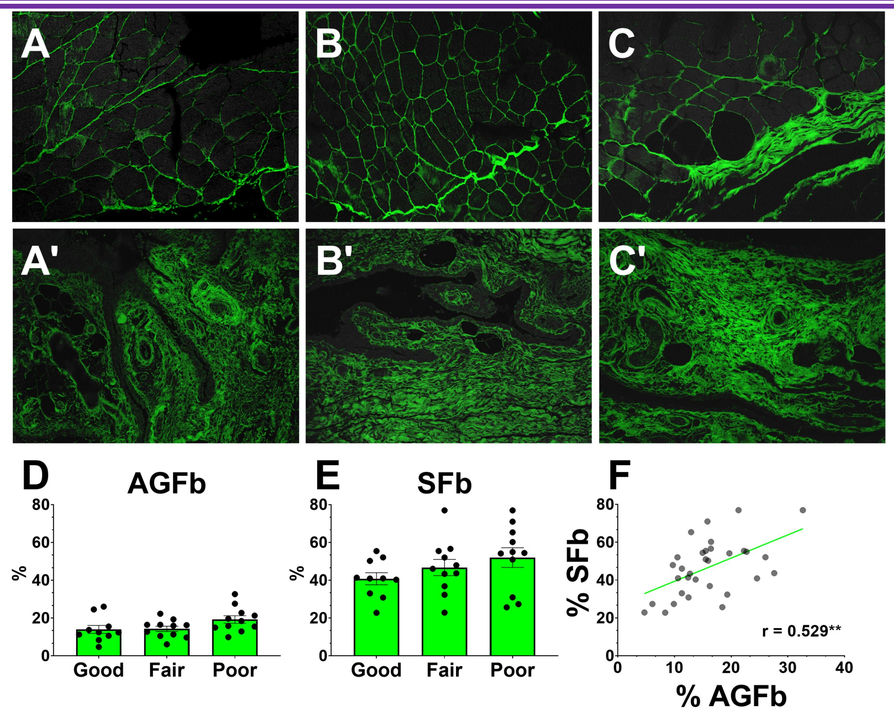


Figure 2. Representative 200x confocal photomicrographs of Picrosirius (PS)-stained sets of AGs grouped by patients presenting with (A) good, (B) fair, and (C) poor pre-TKA ROM, each with corresponding (A'-C') synovium underneath. Fibrous collagen deposits in the AG endomysium and synovial subintima were measured using software assisted thresholding and percentages over total tissue area of (D) AG and (E) synovium compared between ROM groups using one-way ANOVA. (F) We measured a significant Pearson's correlation (r) between fibrosis in the AG with that in the synovium. **p<0.01.

Conclusions

- Similar to the OA VL, the OA AG undergoes a transition to T2 fibers with a high occurrence of disuse-related T2a/x hybrid myofibers.
- Also similar to the OA VL, the OA AG undergoes the hallmark fibrosis of the endomysium. In addition, AGFb presentation increases relative to functional limitations and moderately correlates to fibrosis of the neighboring synovium. This suggests that the AG is not impervious to the chronic effect of pro-fibrotic factors that also affect the OA synovium. The type and concentration of fibrogenic analytes can be measured in synovial fluid relative to severity of SFb and therefore can be also used to assess AGFb status.
- Taken together, evaluation of AG myofiber dynamics and fibrosis severity would potentially facilitate the development of a serum and/or synovial fluid-based diagnostic tool that would allow for tailoring individualized post-TKA rehabilitation strategies.

Limitations

- Limited patient sample size may potentially affect the significance of differences between ROM groups, particularly in fibrosis metrics.
- Lack of corresponding samples of VL impairs validation of the AG as a surrogate for QF status during OA.
- Lack of additional approaches to measure MHC content and proteins related to myofiber switching to corroborate immunohistochemical results.
- Limited control tissue from organ donors with healthy knees.
- Lack of gene expression analyses related to myofiber dynamics of OA-related fiber transitions, atrophy, and fibrogenesis.

References

1. Moreillon, M., *et al*. Hybrid fiber alterations in exercising seniors suggest contribution to fast-to-slow muscle fiber shift. *J Cachexia Sarcopenia Muscle*. 10, 687-695 (2019).
2. Bougea, A., *et al*. An Age-Related Morphometric Profile of Skeletal Muscle in Healthy Untrained Women. *J Clin Med*. 5, (2016).
3. Aagaard, P., Magnusson, P.S., Larsson, B., Kjaer, M. & Krustrup, P. Mechanical muscle function, morphology, and fiber type in lifelong trained elderly. *Med Sci Sports Exerc*. 39, 1989-1996 (2007).
4. Noehren, B., *et al*. Alterations in quadriceps muscle cellular and molecular properties in adults with moderate knee osteoarthritis. *Osteoarthritis and Cartilage*. 26, 1359-1368 (2018).
5. Tanner, C.J., *et al*. Muscle fiber type is associated with obesity and weight loss. *American Journal of Physiology-Endocrinology and Metabolism*. 282, E1191-E1196 (2002).
6. Fink, B., *et al*. Morphologic changes in the vastus medialis muscle in patients with osteoarthritis of the knee. *Arthritis Rheum*. 56, 3626-3633 (2007).
7. Grob, K., Gilbey, H., Manestar, M., Ackland, T. & Kuster, M.S. The Anatomy of the Articularis Genus Muscle and Its Relation to the Extensor Apparatus of the Knee. *JB & JS open access*. 2, e0034-e0034 (2017).
8. Kobayashi, H., *et al*. Morphological Analysis of Articularis Genus and Involvement in Muscle Synchronicity with Vastus Intermedius. *International Journal of Morphology*. 36, 859-863 (2018).
9. Saito, A., *et al*. Functional status of the articularis genus muscle in individuals with knee osteoarthritis. *Journal of musculoskeletal & neuronal interactions*. 16, 348-354 (2016).
10. Willemse, H., Theodoratos, A., Smith, P.N. & Dulhunty, A.F. Unexpected dependence of RyR1 splice variant expression in human lower limb muscles on fiber-type composition. *Pflugers Arch*. 468, 269-278 (2016).
11. Hodgeson S, O'Brien S, Simkin J, Plakotaris E, McCarthy C, Dasa V, Marrero L. Differences in synovial fibrosis relative to range of motion in knee osteoarthritis patients. J Orthop Res. 2021 Apr 29. doi: 10.1002/jor.25061. Epub ahead of print. PMID: 33913554.

Acknowledgements

We would like to thank all study participants and members of the Marrero Laboratory and the Morphology and Imaging Core for technical assistance and thoughtful discussions. Collection, preservation, and storage of samples in the LSU Integrated Musculoskeletal Biobank (LIMB) and used in study was supported in part by a grant (U54 GM104940) from the NIGMS of the NIH, which funds the Louisiana Clinical and Translational Science Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

This research project was supported by Award Number: DBI-2051440 through the National Science Foundation (NSF), Research Experiences for Undergraduates (REU) Program