Validation of Rat Muscle Volume Measurement by MRI as a Frailty Biomarker

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Introduction

Mammalian aging is characterized by a decline in skeletal muscle mass and strength, termed sarcopenia. In animal studies, the assessment of muscle size is usually by directly weighing dissected muscles. This has the major limitation of requiring euthanasia of the animals thus necessitating cross-sectional study designs at much greater costs (more animals and reagents). Moreover, the muscle weight endpoint obtained cannot be directly compared to clinical studies where muscle size is often estimated by imaging. To bridge these gaps, in the present study we validated MRI for the noninvasive detection of muscle volume changes in rats by demonstrating comparable estimates of muscle atrophy by MRI and by muscle weight after unilateral sciatic neurectomy (USN) in old rats.

Methods

The animal study was approved by our Instititional IACUC. Twenty male ~22-month old Sprague Dawley rats (521 ± 51g) were imaged to establish the baseline. Animals were then randomized into four groups (N = 5) for the following procedures. Rats in group 1 (Control) were euthanized after baseline imaging. Rats in groups 2, 3 and 4 were subjected to USN of the right leg under isoflurane anesthesia as described previously [1], and the right leg re-imaged at 2, 6, and 12 days after the USN, respectively. All animals were euthanized after the final round of imaging and all leg muscles with the tendon of insertion traversing the ankle joint (namely, the tibialis anterior, extensor digitorum longus, soleus, plantaris, gastrocnemius, flexor hallicis longus, flexor digitorum longus, tibialis caudalis and peroneal muscles) were dissected from each of the right and left legs and weighed. The difference in the pooled muscles weight between the right and left legs was used to calculate the degree (%) of USN-induced muscle atrophy for each time point.

MRI was performed on a 7T Bruker Biospec scanner equipped with a 12 cm ID gradient insert (up to 20 G/cm). Animals were anesthetized using isoflurane (3% induction, 1-1.5% maintenance) and placed on their left side with the right leg secured to a thin slab of Plexiglas. The Plexiglas slab with the attached leg was positioned inside the 38mm quadrature Litzcage coil (Doty Scientific) and inside the magnet. Axial Fat Suppressed FLASH anatomical images were acquired in the axial aspect with the following imaging parameters: TE= 3.776 ms, TR=25 ms, FA=30°, NA=1, MTX =256×256×128, Resolution = 110×110×400 μm. To segment muscle on MR images, first an intensity classifier was used to generate a muscle mask. However, due to the similarities in the intensities of skin, inflamed tissue and muscle, all three tissues were included in the muscle mask. Muscle was separated from other tissue types by using a clustering approach in AFNI [2]. The formation of unconventional shapes caused by connection of clusters by thin necks was minimized by eroding voxels that weren't connected to other voxels and dilating post clustering. In a few cases, some further manual editing was required. Figure 1 shows the representative results of the process. To ensure consistent quantification across rats and time-points the distal tibia-fibula bifurcation was used as a fiducial marker. Muscle volume over a 4mm section, proximal to this point was determined. Analysis was limited to this section to prevent interference from thigh muscles which insert at variable points below the knee, but usually above the selected section. Muscle volumes for all subjects and all time points were calculated. For each rat, the difference between the muscle volume at baseline and at subsequent imaging time points was used to calculate the degree (%) of USN-induced muscle atrophy.

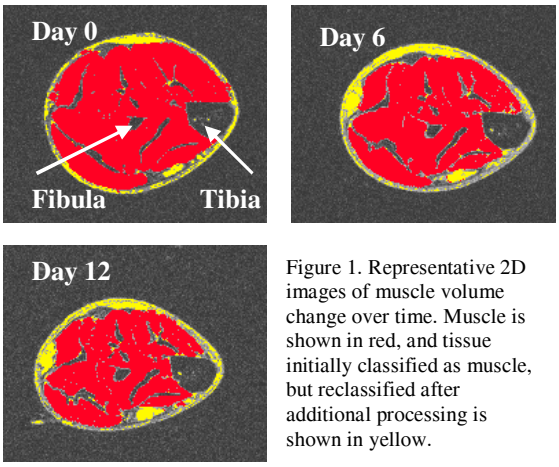

Figure 1. Representative 2D images of muscle volume change over time. Muscle is shown in red, and tissue initially classified as muscle, but reclassified after additional processing is shown in yellow.

Results

USN is known to produce a rapid decline in muscle weight [1]. There was no statistically significant change in MRI measured muscle volume two days after USN. However, at 6 and 12 days after USN the change in muscle volume became significant. Figure 2 shows mean percent atrophy calculated based on MR volume and muscle weight. There was also a significant correlation between the percent atrophy estimated by MRI versus muscle weight (R^2 =0.66).

Conclusion

The measurement of muscle volume by noninvasive MRI method provides a valuable biomarker for tracking longitudinal changes in muscle size in preclinical studies of frailty/sarcopenia. It is characterized by sensitivity and accuracy similar to the muscle weight endpoint without the need to sacrifice the animal at any given time point. The already good correlation between MRI and dissection estimates of muscle size may further be improved by incorporating MRI density estimates and dissected muscle volume into the analysis.

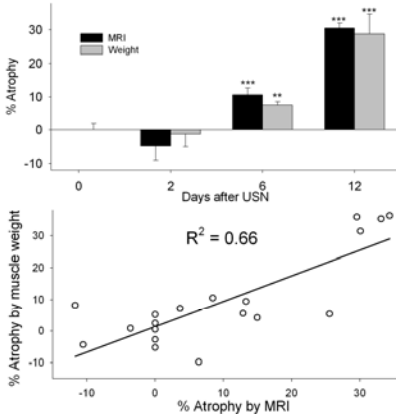

Figure 2. Dynamics (A) and correlation (B) of muscle atrophy measured by MRI and dissection. **, *** - significant difference from day 0 (P<0.01, 0.001 respectively)

References:
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[2] Cox RW(1996) *Computers and Biomedical research* 29,162-173