Diffusion Tensor Imaging to Track Changes in Skeletal Muscle Architecture of Sarcopenic Rats

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Introduction:
Aging is usually accompanied with a loss in muscle strength and mass, referred to clinically as sarcopenia. While the wasting effects of sacropenia on skeletal muscle have been studied in both rodents [1,2,3] and humans [4,5], only one study has employed diffusion tensor imaging (DTI) techniques to study these aging effects in elderly humans compared to younger volunteers [6]. DTI has shown high accuracy and sensitivity in the investigation of muscle architecture [7, 8] and microstructure [9, 10]. However, it has not yet been utilized to study sarcopenia in rodent models, which can be monitored over the longitudinal course of disease progression to indentify therapeutic windows and potential treatments that might reverse or delay muscle wasting. In this study, the effects of age on myofiber dimensions (MD) and characteristics of the rat soleus muscle are investigated using DTI.

Methods:
Experimental setup: Eighteen Fisher 344 male rats were divided into four groups based on their age at the time of sacrifice: 44 (n=4), 60 (n=4), 86 (n=5) and 102 (n=5) weeks. Animals were housed in a 12:12-h light-dark cycle and fed *ad libitum*. Animals were perfusion fixed using 4% paraformadehyde (PFA) and a trans-cardial procedure, after which the gastrocnemius and soleus muscles were harvested and directly immersed in 4% PFA. The fixed muscle tissues were washed with phosphate buffered saline (1xPBS) at least one day prior to imaging, and immersed in 1xPBS for MRI.
Imaging protocol: DTI datasets of the muscles in 7-noncollinear gradient directions were acquired using a widebore 11.75-T vertical magnet with a Bruker Avance console and Micro2.5 gradients. Using a 15-mm birdcage coil, spin echo (SE) DTI scans were acquired with b values of 0, 500 and 1000 s/mm^2 at in-plane resolution of 50×50 μm^2, and a slice thickness of 500 μm. The DTI acquisition parameters were as following: TE = 20.5 ms, TR = 2.75 s, Δ = 12.7 ms and δ = 2.1 ms. Also, a high resolution (40-μm^3) 3D gradient-recalled echo (GRE) image was acquired (TE/TR = 10/150 ms) for anatomical and volumetric measurements.
Data Analysis: After acquisition, the images were processed with MedINRIA (http://www-sop.inria.fr/asclepios/software/MedINRIA/) to calculate diffusion tensor parameters such as: fraction anisotropy (FA), apparent diffusion coefficient (ADC) and eigenvalues (λ_1, λ_2 and λ_3). The region of interest (ROI) was chosen in the widest region of the soleus muscle for processing as shown in Fig. 1. Tukey's HSD test was used to determine if there were any statistical differences between groups. The statistical analysis was performed using SPSS17 software.

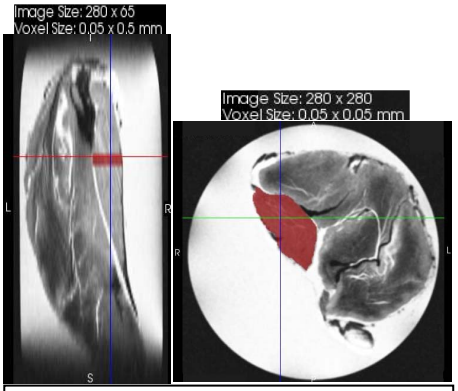

Figure 1: Sagittal (left) and axial (right) B0 images of soleus muscle (Old sample) with the processed ROI drawn in red

Results and Discussion:
DTI analysis (summarized in Table 1) showed that the second and third eigenvalues decreased with age (as indicated by the up to 14% change in λ_3) until age 86 weeks, after which time there is no further loss in either parameter. Clearly, the most significant drops in the second and third eigenvalues are seen at earlier time points. The same trend was observed in the ADC (-10% between 44 and 86 weeks), suggesting that water diffusion experiences more overall restrictions with age until a plateau is reached. The opposite trend is evident for FA values, which increased with age (up to 32%) until reaching a plateau. This data suggest that the muscle becomes more anisotropic and restricted with age. Because the change in anisotropy is attributable to alterations in the second and third eigenvectors as opposed to the principal eigenvalue (λ_1), this restriction may be related to the age-related muscle wasting of sacropenia and its more direct impact on cross sectional area (CSA) and myofiber diameter instead of fiber length.

Conclusions:
Results showed the ability and sensitivity of DTI to track the changes in muscle fiber CSA with sarcopenia. It has been shown in this study that the architecture of soleus muscle changes with age, particularly the apparent CSA and diffusivity. Future work includes analyzing sarcopenic gastrocnemius muscle, and studying the impact of different interventions such as exercise and/or dietary supplements on sarcopenic skeletal muscles.

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Table 1: DTI parameters measured at different ages of sarcopenic rats.

Age [wks]	FA	ADC [μm^2/ms]	λ_1 [μm^2/ms]	λ_2 [μm^2/ms]	λ_3 [μm^2/ms]
44	0.118±0.009	1.12±0.03	1.25± 0.02	1.13±0.04	0.99±0.03
60	0.137±0.005	1.06±0.01	1.19±0.01	1.06±0.01	0.91±0.002^{x}
86	0.175±0.018 ^{xy}	1.01±0.05^{x}	1.19±0.05	0.99±0.05$^{x\,y}$	0.85±0.05^{x}
102	0.171±0.015 ^{xy}	1.04±0. 04^{x}	1.21±0.04	1.02±0.04^{x}	0.87±0.04^{x}

^{x} indicates a statistical significant difference when compared to the 44-week group, while ^{y} indicates a statistical significant difference when compared to the 60-week group.