Effects of Resistance Training and \(\beta\)-hydroxy-\(\beta\)-methylbutyrate (HMB) on Muscle Fiber CSA and Lean Body Mass in Aged Rats: A DTI and DEXA Study

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Introduction:
Aging mammalian skeletal muscles exhibit sarcopenia, which includes loss of muscle mass and strength. Estimates indicate that approximately 45% of older Americans are sarcopenic [1]. The healthcare cost of sarcopenic patients in the United States of America was estimated at $18.5 billion in 2000 [2]. Diffusion tensor imaging has shown high accuracy and sensitivity to study muscle architecture [3, 4] and microstructure [5, 6]. In this study, the effects of \(\beta\)-hydroxy-\(\beta\)-methylbutyrate (HMB), a dietary supplement known to promote muscle strength and lean body mass (LBM) [7, 8], were investigated in a pre-clinical model of aged rats during resistance training (RT).

Methods:

Experimental setup: Sixteen 19-month-old Sprague-Dawley female rats were divided randomly into three groups: BL (Baseline), HMB (0.46 g/kg b.w./d) and Non-HMB. HMB and Non-HMB groups underwent intense resistance training (weighted ladder climbing) every third day for 10 weeks. 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% paraformadehyde. The fixed muscle tissues were washed with phosphate buffered saline (1xPBS) at least one day prior imaging, and immersed in fresh 1xPBS for scanning.

Imaging protocol: DTI images (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 \(\mu\)m, with a slice thickness of 500 \(\mu\)m. The DTI acquisition parameters were as following: TE = 20.5 ms, TR = 2.75 s, \(\Delta = 12.7\) ms and \(\delta = 2.1\) ms. Also, a high resolution (40 \(\mu\)m) 3D gradient-recalled echo (GRE) image was acquired (Fig.1) (TE/TR= 10/150 ms) for anatomical and volumetric measurements. In vivo and prior to sacrifice, pre- and post-RT LBM was assessed by dual energy X-ray absorptiometry (DEXA).

Data Analysis: After acquisition, the images were processed by MedINRIA software to calculate the diffusion tensor, providing the parameters fraction anisotropy (FA), apparent diffusion coefficient (ADC) and eigenvalues \((\lambda_1, \lambda_2, \lambda_3)\). A region of interest (ROI) was chosen in the widest region of the soleus muscle for processing as shown in Fig. 2. Tukey’s HSD test was used to determine if there were any statistical differences between groups. The statistics was performed using SPSS 17.

Results and Discussion:
Diffusion tensor imaging of the soleus muscle showed no change in the principal eigenvalue \((\lambda_1)\) while \(\lambda_2\) and \(\lambda_3\) increased (+17% and +20%, respectively) significantly (p<0.05) in both groups after RT, indicating a significant increase in cross sectional area (CSA) of the muscle fiber (data is shown in Fig. 3 below). The increased CSA was also evident in the decreased FA (-30%) and increased ADC (+13%). The findings suggest that the RT muscles are either swollen or contain hypertrophic myofibers due to resistance training. As assessed by DEXA, RT also improved LBM (+21%, p<0.05) in both HMB and Non-HMB groups. However, no between-group differences (HMB v. Non-HMB) were identified for all variables tested.

Conclusions:
Results indicated that HMB failed to facilitate the changes in LBM and soleus CSA during high intensity RT. However, an RT-induced increase in the CSA of the soleus muscle was identified in addition to associated changes in FA and ADC when compared to the baseline group, which indicates that at least RT played a role in muscle maintenance with age. Further corroborative studies are underway to determine the effect of age on muscle fibers and impact of HMB on sedentary conditions. Future analysis also will evaluate the effects of HMB and RT on the gastrocnemius muscle.

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References: