Skeletal Muscle State Estimation by T_2 and Rotating Frame Relaxations in Ischemic Hind Limb Mouse Model

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Introduction Increasing trend towards personalized medicine, also in gene therapy of ischemic skeletal muscle, has created a demand for non-invasive assessment of muscle viability. Recently, MRI T_2 and water diffusion anisotropy was applied to follow up regeneration of muscle after hind limb ischemia[1]. Rotating frame relaxations ($T_{1\rho}$ and Relaxation Along a Fictitious Field (RAFF) T_{RAFFn}[2,3]) have shown to be potential quantitative MRI markers for disease progression in several applications, including brain and myocardial ischemia[4,5]. In this study, T_1, T_2 and the rotating frame relaxation times (T_{RAFF2}, $T_{RAFF4,}$ and $T_{1\rho}$) were applied to estimate skeletal muscle viability in mouse ischemic skeletal muscle with and without AdhVEGF-D mediated gene therapy.

Materials and Methods The femoral artery and vein were ligated in right hind limb of eight female $LDLR^{-/-}ApoB^{100/100}$ mice. After the operation, an injection of either AdhVEGF-D or AdLac-Z control was made. The mice were anesthetized with 1.5 % isoflurane in ($70\%N_2$:$30\%O_2$) and imaged 1, 4, and 7 days after the ligation at 7T Bruker Pharmascan using volume transmitter and surface receiver coil. The MRI scans consisted of T_{RAFF2} and T_{RAFF4} (RAFF2 or 4-pulses[2] pulse train length of 0 - 36 ms, $\gamma B_1/(2\pi)$ = 1250Hz for RAFF2 and 648 for RAFF4), $T_{1\rho}$ ([3]spin-lock time=0-45.4ms, $\gamma B_1/(2\pi)$=1250 Hz), T_2 (adiabatic Hahn double echo preparation with TE=8-22ms), T_1 (saturation recovery with TR=200-5000ms) and B_1 (altering hard pulse lengths between 0.2 and 1.6ms) measurements. Fast spin echo sequence (TR=4s, effective TE=8ms, ETL=8, FOV= 20x10 mm^2, matrix size 256x128, and slice thickness of 1 mm) with fat suppression was used as readout imaging sequence. ROIs excluding skin, subcutaneous fat, and bone were hand drawn for further analysis based on T_2 weighted images. After imaging at the last time point, the mice were sacrificed for histology. Hematoxylin Eosin staining was prepared and areas for regenerative, necrotic and normal muscle were differentiated. The relaxation times in ischemic leg were also divided into three sections, where the normal muscle was determined based on the control leg relaxation times and the rest of the range was divided half and half to regeneration and necrotic tissue.

Results and Discussion

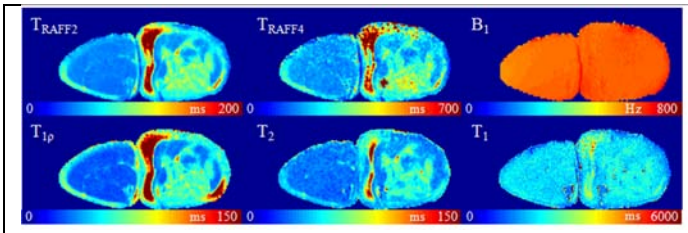
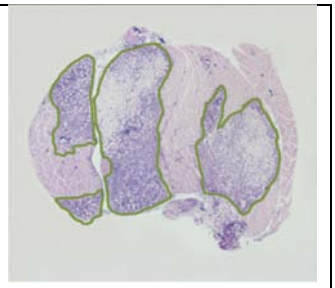


Fig. 1 (left): Relaxation time and B_1 maps for a single mouse with VEGF-D gene therapy at day 7. The ischemic leg is on the right and the control leg on the left.

Fig. 2 (right): Histological section with hematoxylin-eosin staining. Early regeneration areas marked with green. The rest of the tissue was indicated as normal.



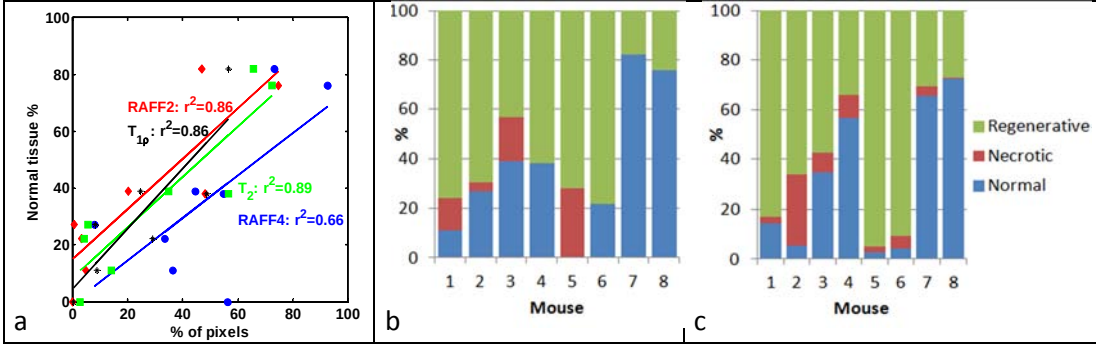


Fig. 3: Correlation between the percentage of normal tissue and percentage of the pixels representing normal relaxation time values in the ischemic leg (a), differentiated tissues as the percentage of the whole muscle cross section using histology (b) and T_2 (c). Mice numbers 1-4 are treated with VEGF-D and 5-8 with Lac-Z.

The mean relaxation time over the ischemic leg was significantly higher ($p<0.01$) compared to the control leg at day 7 with all methods used (**Fig. 1**). The relative number of pixels with short relaxation times (T_{RAFF2}, $T_{RAFF4,}$ $T_{1\rho}$, and T_2) in the ischemic leg on day 7 correlated with the percentage of normal area determined from HE staining (**Fig. 2**). The highest correlations between normal areas determined using histology and relaxation time was obtained with T_2, $T_{1\rho}$ and T_{RAFF2} (**Fig. 3**). Corresponding correlations in necrotic areas were low, which may be due to small fraction of necrotic areas in the muscles. For analysis, we assumed that the control leg represents normal relaxation times, highest 1/3 necrotic and the rest regenerating area and tuning of the relaxation time thresholds may lead to better correlations. The present data suggest that MRI relaxation time constants, especially T_{RAFF2}, $T_{1\rho}$, and T_2, could be used to evaluate ischemic muscle state non-invasively and provide potential imaging marker for therapy outcome. **References** 1. Heemskerk AM et al. Radiology 2007, 2. Liimatainen T et al. MRM 2010, 3. Liimatainen T et al. ISMRM 2012, 4. Gröhn et al. MRM 1999, 5. Musthafa HNS et al. MRM 2012. **Acknowledgements** Academy of Finland, and Sigrid Juselius Foundation.