# $T_2$ and $T_{1\rho}$ detect early regenerative changes in ischemic skeletal muscle

Hanne Hakkarainen[1], Galina Wirth[1], Petra Korpisalo-Pirinen[1], Seppo Ylä-Herttuala[1], and Timo Liimatainen[1,2]
[1]*University of Eastern Finland, A.I. Virtanen Institute for Molecular Sciences, Kuopio, Finland,* [2]*Imaging Center, Kuopio University Hospital, Kuopio, Finland*

**Purpose** Although MRI $T_2$ and water diffusion anisotropy were applied to follow up muscle regeneration after hind limb ischemia[1], the MR relaxation parameters have received only little attention in the diagnosis and follow up of limb ischemia. Rotating frame relaxation $T_{1\rho}$ has shown to be a potential quantitative MRI marker for disease progression in several applications, including brain and myocardial ischemia[2,3]. In this study, $T_2$, $T_1$, and $T_{1\rho}$ were applied for detecting signs of early regeneration in a mouse ischemic skeletal muscle.

**Materials and Methods** 19 female LDLR$^{-/-}$ApoB$^{100/100}$ mice underwent a ligation of both, the common femoral artery and vein of the right hind limb resulting in acute ischemia. The mice were imaged 1, 4, 7, 14, and 29 days after the ligation at 7T Bruker PharmaScan using volume transmitter and surface receiver coil. The MRI scans consisted of $T_2$ (adiabatic Hahn double echo preparation with TE=8-22ms), $T_{1\rho}$ ([4]spin-lock time=0-45.4ms, $\gamma B1/(2\pi)$=1250 Hz), $T_1$ (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, effTE=8ms, ETL=8, FOV= 20x10 mm$^2$, matrix size 256x128, and slice thickness 1 mm) with fat suppression was used as readout imaging sequence. ROIs were hand drawn for further analysis based on $T_2$ weighted images to match with histological sections. At day 29, the mice were sacrificed for histology and sections from the posterior calf muscle were stained with Hematoxylin Eosin. From the stained sections, the areas of normal, necrotic, very early, early, mediatory and late regeneration tissues were differentiated. The relaxation times in the ischemic leg were also divided into sections representing these tissue morphology types based on the relaxation time maps and the correlations with histology results were calculated.

**Results and Discussion**

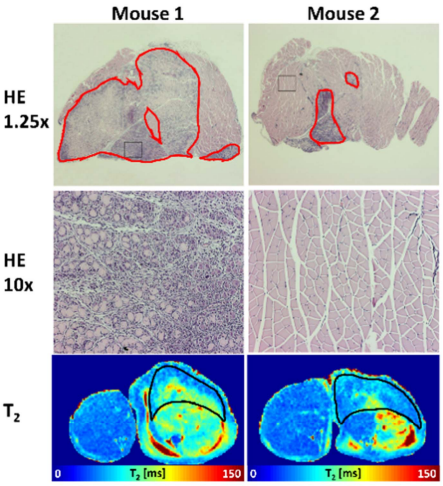


**Figure 2** Hematoxylin Eosin stained sections of the calf muscles of two mice at day 7 with 1.25x and 10x magnifications. The red lines delineate the areas containing early signs of regeneration (62% on mouse 1 and 15% on mouse 2). Bottom row shows the $T_2$ maps from the same mice. Black line delineates the area of histology section.

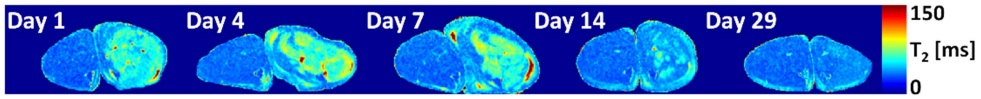


**Figure 1** Example of $T_2$ map time series of one mouse.

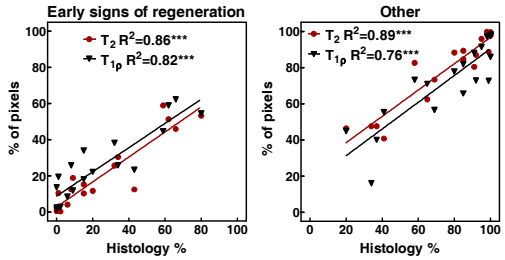


**Figure 3** Correlations between histologically derived and $T_2$, and histologically derived and $T_{1\rho}$ relaxation time based percentages of areas showing early signs of regeneration and areas of other tissue morphologies in the calf muscle. ***P<0.001.

The ischemia model showed variable ischemic changes at different time points, with early signs of regeneration (characterized by atrophic myofibers surrounded by activated satellite cells and basophilic regenerating myofibers with centrally located nuclei) and necrosis (represented by necrotic fibers with pale, flocculated sarcoplasm and loss of nuclei) at days 1-7, and mostly late phase of regeneration (defined by eosinophilic regenerating myofibers with centrally oriented nuclei) and fully recovered tissue (angular shaped muscle fibers with peripherally oriented nuclei) at days 14-29. The relaxation times increased until day 7 in the ischemic leg and decreased to the level of intact leg at day 29 (**Figure 1**). The percentages of early regeneration areas defined with $T_2$ and $T_{1\rho}$ correlated significantly ($R^2$=0.86 and $R^2$ = 0.82, respectively) with the histologically derived percentages (**Figures 2 and 3**) while correlation was lower with $T_1$ ($R^2$ = 0.57). Similarly, the percentages of the other tissue morphologies (mediatory and late regeneration, necrosis, and normal tissue) defined using $T_2$ and $T_{1\rho}$ correlated significantly with the histology ($R^2$=0.89 and $R^2$=0.76, respectively). In the early regeneration phase satellite cells are activated and are proliferating, while muscle cells are swollen. Cell swelling increases the tissue water content and leading to increased $T_2$ and $T_{1\rho}$, providing an explanation for measured increase of $T_2$ and $T_{1\rho}$.

**Conclusion** $T_2$ and $T_{1\rho}$ are able to separate activation of muscle regeneration in the ischemic tissue which could be used for localization of therapies into the areas which would benefit from therapy.

**References** 1. Heemskerk AM et al. Radiology 2007, 2. Gröhn et al. MRM 1999, 3. Musthafa HNS et al. MRM 2012.

**Acknowledgements** Academy of Finland, Sigrid Juselius Foundation, and Instrumentarium foundation.