

# Skeletal muscle degeneration and regeneration following femoral artery ligation in the mouse, as monitored using diffusion tensor imaging

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## Introduction:

Ischemic injury of skeletal muscle is frequently encountered in association with trauma, peripheral diseases, compartment syndrome and surgery. Animal models are commonly used to investigate the processes underlying this type of muscle injury. These models include the induction of prolonged ischemia by ligation of the femoral artery, causing muscle tissue to degenerate and regenerate over time (1 to 3 wks). The initial phase (degeneration) of muscle repair is characterized by necrosis of damaged tissue and activation of an inflammatory response, followed by processes leading to new myofiber formation (regeneration).

MRI is a powerful tool in muscle disease diagnosis. In general a  $T_2$  enhancement is found with either a homogeneous or heterogeneous regional appearance, but the findings are usually not specific for a given disease. Diffusion tensor imaging (DTI) might offer additional diagnostic value as the technique can be used for characterization of the microscopic geometrical properties of tissue. Recently, the utility of DTI has been shown as a marker for ischemic muscle damage (1). The goal of this study was to evaluate quantitative diffusion MRI for monitoring tissue regeneration following femoral artery ligation in mouse skeletal muscle.

## Materials and Methods:

**Animal model:** Ischemia was induced by ligation of the femoral artery above the origin of the natural collaterals. Care was taken to leave the femoral vein and nerve untouched. A similar dermal incision was made in the contralateral limb as a sham operation. C57Bl/6 mice were measured directly before ligation (n=5) and directly after (n=15), 3 days after (n=12), 10 days after (n=7) and 21 days (n=4) after ligation. The mice were anesthetized with isoflurane (1.0-1.5% in air). The body temperature was maintained at 38°C and the animal's respiration was continuously monitored. After the MR experiments the mice were perfusion fixed with formaldehyde for histological analysis; directly before ligation (n=1) and directly (~2.5 h) after (n=3), 3 days after (n=4), 10 days after (n=3) and 21 days (n=4) after ligation.

**MRI:** MR was performed at 6.3 Tesla. A diffusion-weighted spin echo sequence with fat suppression was used. The diffusion gradients were applied along 6 noncolinear directions and one reference image was recorded without diffusion weighting. Scan parameters were: FOV=20x20 mm<sup>2</sup>, matrix size=128x64, slice thickness = 1.5 mm, TE=30 ms, NSA=2, TR=1.5 s,  $\Delta$ =13 ms,  $\delta$ =8 ms and b-value=0 or 572 s/mm<sup>2</sup>. A multi-echo spin-echo sequence with fat suppression was used to obtain  $T_2$  maps, with: 6 echoes, FOV=20x20 mm<sup>2</sup>, matrix size=128x64, slice thickness = 1.5 mm, NSA=2, TE=13.3 – 79.8 ms, TR=4 s.

**Data analysis:** From the DTI datasets the eigenvalues ( $\lambda_1$ ,  $\lambda_2$  and  $\lambda_3$ ) and eigenvectors,  $ADC = \text{Trace}(\mathbf{D})/3$  and fractional anisotropy (FA) were calculated. The mean effects on parameter values over time were determined from the mean of the modulus of frequency distributions per animal. Furthermore, the DTI and  $T_2$  data were clustered to visualize the regional changes of the MR indices in a single image. K-means clustering was performed for the slice approximately mid-belly for the tibialis anterior (based on  $\lambda_3$ , FA and  $T_2$  values).

## Results and Discussion:

The ADC and  $T_2$  values changed dynamically over time for the ligated limb and showed regional differences between the MR indices and between time points (Fig 1a and 1b). After three days, the ADC was increased over almost the entire limb. The  $T_2$  was primarily enhanced in the outer rim and was less enhanced within the muscles. At 10 days,  $T_2$  enhancement was also observed within muscles. Only small spots, central in the muscles with ADC increase were still present. It appeared that within muscles an increase in ADC preceded  $T_2$  increase and  $T_2$  enhancement progressed from superficial to deep. The non-ligated limb hardly showed any changes.

Fig 2 depicts the overall mean changes in ADC and  $T_2$  values as determined by histogram frequency analysis. Directly after ligation no difference in ADC was measured, whereas an increased  $T_2$  was present. At day 3 both the ADC and  $T_2$  were increased. After 10 days most pixels showed a decreased ADC and an enhanced  $T_2$ . After 21 days most MR indices were normalized.

Clustering of the MRI data was used to emphasize the regional differences between the responses of DTI indices and  $T_2$  (Fig 1c) and to enable a comparison with histology (Fig 3). Over the different time points the clusters changed in location and in size (Fig 1c), but had similar MR values. The cluster with highest ADC values (red) was surrounded by a cluster with highest  $T_2$  values (blue). At 10 days also a cluster with lower ADC, higher FA and normal  $T_2$  values was present (orange). The correspondence between a clustered image and the corresponding histological slice is remarkable (compare Fig 3a and 3b), taking the deformations into account. An area with high ADC appears to correspond with an area with large, swollen cells (Fig 3c), whereas an area with high  $T_2$  appears to correspond with an area with lots of infiltrates and collagen (Fig 3d).

The histological findings are in agreement with the observation that muscle regeneration follows a centripetal gradient (2), which results in the formation of different zones within a regenerating muscle, each zone being in a different phase of degeneration or regeneration. This centripetal gradient is observed also on the DTI-derived maps and the  $T_2$  map, and these maps highlighted different spatial areas. The regional difference between DTI and  $T_2$  suggests that these indices report on different processes.

## Conclusion:

ADC and  $T_2$  dynamically change in response to ischemia-induced muscle injury and regional changes in these indices correspond with histological findings. Therefore, the data strongly suggest that the combination of DTI and  $T_2$  enables the differentiation of the degenerative and regenerative phases in tissue regeneration. Also, diffusion MRI can be used to longitudinally study effects of therapeutic interventions that are aimed to reduce muscle degeneration or promote tissue repair.

- References:** 1) Heemskerk AM et al., Proc. 13th Scientific Meeting ISMRM, Miami 2005  
2) Carlson BM and Gutmann E, Anat. Rec. 183(1):47-62, 1975

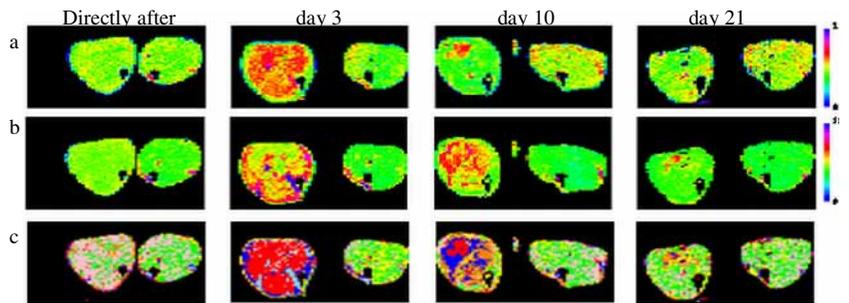


Fig 1) Parametric images for one animal at different time point. a: ADC; b:  $T_2$ ; c: k-means clustered image based on  $\lambda_3$ , FA and  $T_2$ .  $T_2$  (ms), ADC ( $\times 10^{-3}$  mm<sup>2</sup>/s). The ligated limb is situated left.

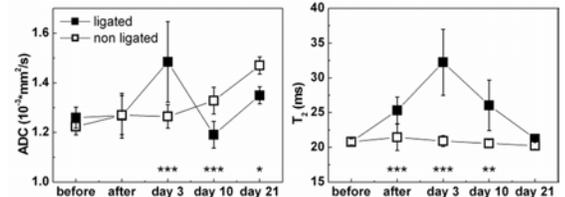


Fig 2) Changes in MR indices. The values are the mean values of the modulus of the histogram frequency distributions. Error bars:  $\pm$  SDs. \*, \*\*, \*\*\*  $p < 0.05$ , 0.01, 0.005 of ligated versus non-ligated limb.

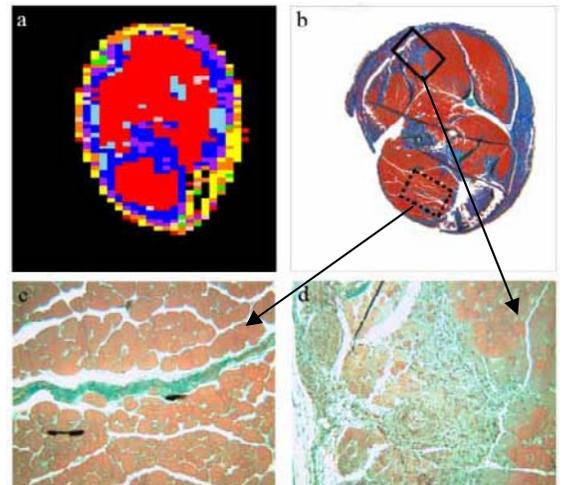


Fig 3) Comparison of clustered MR data (a) and histology (b) with: swollen cells (c) and area with inflammatory cells (d).