Ischemia-reperfusion injury in rat skeletal muscle assessed with T_2-weighted and dynamic contrast-enhanced MRI

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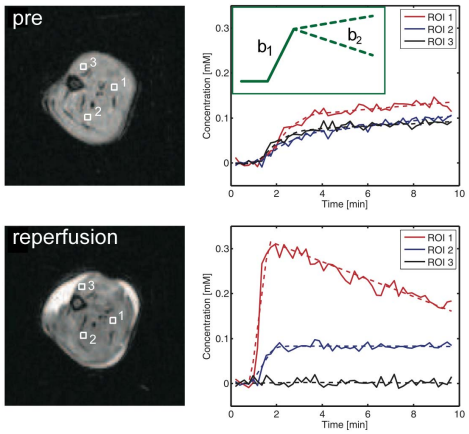
Introduction: Pressure ulcers are localized areas of soft tissue breakdown due to mechanical loading, which arise when patients are bedridden or wheelchair bound for long periods of time. Pressure ulcers represent a serious health problem to the individual and a major financial burden to the health services [1]. Susceptible individuals are subjected to pressure relief strategies to avoid long loading periods. However, these are often insufficient to prevent the formation of pressure ulcers. Therefore, it was hypothesized that also ischemia-reperfusion injury may play an important role in the etiology of pressure ulcers.

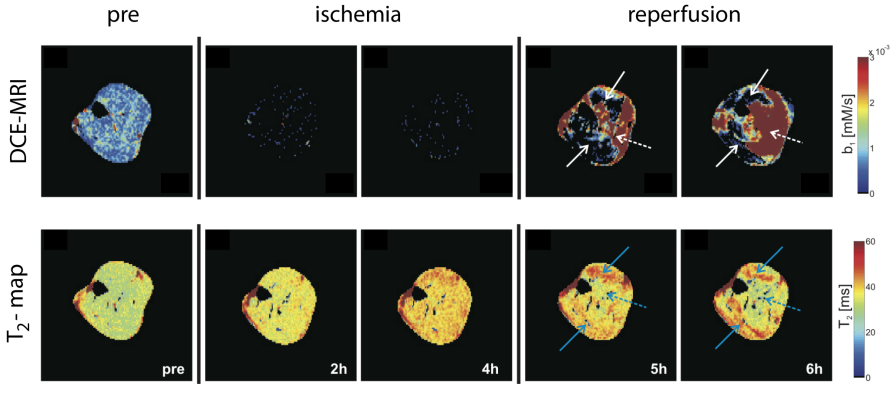
The **goal** of this study was to investigate the local interrelation between post-ischemic perfusion and changes in skeletal muscle integrity. To this aim, the hindlimbs of Brown-Norway rats were subjected to 4h ischemia followed by 2h reperfusion. Dynamic contrast-enhanced MRI (DCE-MRI) was used to investigate perfusion, and changes in skeletal muscle integrity were monitored with quantitative T_2 MRI and histology.

Materials and Methods: – *Animal model* – Four 4-5 month old, 178-183 g, female Brown-Norway rats were examined. After induction of anesthesia a catheter was implanted in the jugular vein to enable the administration of contrast agent during the DCE-MRI measurements. Rats were placed supine in a 6.3T MRI scanner with the left lower hindleg positioned in a custom-built birdcage RF coil. A silicone vessel loop was put around the thigh to enable restriction of blood flow in the complete left hindleg. DCE-MRI and T_2-w MRI measurements were performed at several time points, before, during the 4h of ischemia and during the 2h reperfusion period. – *MRI protocol* – T_1 in the leg before contrast agent administration was measured with a 3D T_1-w FLASH sequence with varying flip angle α (FOV=25×25×17.6mm^3, matrix=128×96×16, TR=10ms, TE=2.8ms, α=2°, 5°, 7°, 10°, 15°, and 20°, acquisition time~15s / flip angle). Subsequently, DCE-MRI was performed with a dynamic series of 3D FLASH acquisitions (with α=15°). After 4 baseline acquisitions, a 100μL bolus of Gd-HP-DO3A (0.2 mmol/kg) was administered, while image acquisition was continued for another 45 scans. Changes in the muscle tissue integrity were monitored by quantitative T_2 mapping, using a multi-spin-echo sequence (FOV=25×25mm^2, matrix=128×128, TR=4s, TE=10-320ms, 32 echoes, slice=1mm, 11 slices). – *Analysis* – Quantitative baseline T_1 values and the time course of signal changes during the DCE-MRI acquisition were used to obtain pixel-wise contrast agent concentration time curves C(t). To characterize the time course of contrast enhancement, a piecewise linear fit was applied to C(t) in each voxel (inset Fig 1). Initial upslope b_1 (mM/s) served as a semi-quantitative measure for muscle tissue perfusion [2]. DCE-MRI and T_2 data were coregistered in four consecutive MR slices to enable pixel- and region-wise comparisons of T_2 and DCE-MRI parameters. At the end of the examination, the animals were sacrificed and the tibialis anterior (TA) muscle of both the experimental left hindlimb and the control right hindlimb were excised. Frozen muscle was cut in transversal 5μm-thick sections and stained with hematoxylin and eosin.

Results: Fig 1 shows a representative example of the temporal concentration of the contrast agent in three ROIs in the leg. In the initial pre-ischemic phase (top) DCE-MRI contrast agent time curves are rather similar in different areas indicating homogeneous tissue perfusion. However, in the reperfusion phase (bottom) large spatial differences were observed, characterized by regions with rapid influx of contrast agent at high concentrations (ROI 1), regions with almost normalized influx (ROI 2), as well as regions with no-reflow (ROI 3). This pattern is also illustrated in Fig 2, which contains coregistered maps of b_1 and T_2 of one animal pre, during ischemia, and in the reperfusion phase. DCE-MRI b_1 values were homogeneous in the pre-ischemic phase and essentially zero during ischemia, indicating that blood flow was fully obstructed, as intended. In the reperfusion phase a heterogeneous distribution was observed with areas of high b_1 (ranging from 14 to 76% of the area of the hindlimb) as well as regions with no-reflow (ranging from 5 to 77%). For T_2, a gradual increase in the complete leg was observed during the 4h ischemic period (from 34 to 41ms). During the reperfusion phase, a heterogeneous distribution of T_2 was present. Areas with high b_1 were associated with a decrease in T_2 (to 38ms) towards pre-ischemic levels, whereas no-reflow areas exhibited a further increase in T_2 (to 42ms). At the end of the experiments, histological analysis revealed heterogeneous morphological changes throughout the muscle, with large regions of hypertrophic fibers and increased interstitial space, which confirmed the T_2-based muscle damage assessments.



^ Figure 1: (left) Anatomical cross-sectional images of the rat hindleg pre-ischemia and in the reperfusion phase. (right) DCE-MRI contrast agent time curves (solid lines) for the ROIs shown on the left and piece-wise linear fittings (dashed lines) according to the model shown in the inset.



^ Figure 2: Coregistered maps of DCE-MRI b_1 and T_2 of one animal pre, during ischemia, and in the reperfusion phase. In the reperfusion phase a heterogeneous distribution was observed with areas of high b_1 (dashed arrows) as well as regions with no-reflow (solid arrows). Areas with high b_1 were associated with a decrease in T_2 towards pre-ischemic levels (blue dashed arrows), whereas no-reflow areas exhibited a further increase in T_2 (blue solid arrows).

Conclusions: DCE-MRI revealed the presence of no-reflow areas in the hindlimb subjected to ischemia and reperfusion. These areas were associated with a post-ischemic increase in T_2 and additional muscle damage. For skeletal muscle tissue that is subjected to prolonged periods of mechanical loading associated with ischemia, these results show that reperfusion after prolonged ischemia may not be complete, thereby continuing the ischemic condition and aggravating tissue damage. Clearly, the influence of reperfusion on muscle damage development is complex and heterogeneous, which must be considered when designing appropriate reposition and pressure relief strategies.

References: [1] Severens JL et al., Adv Skin Wound Care (2002) 15, 72–77; [2] Luo Y et al., J Magn Reson Imaging (2002) 16, 277–283.