[1]H-MRS can be used to investigate creatine metabolism in multiple organs within a single examination in the mouse

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Introduction

Creatine is essential in energy transfer and buffering in organs with high and fluctuating energy demands such as brain, heart and skeletal muscle. Whereas a lack of creatine induces neurological, cardiac and muscular defects [1,2], creatine supplementation protects against ischemic and oxidative insults [3]. Therapies targeting creatine metabolism of these organs require a technique to monitor non-invasively creatine levels; ^{1}H-MRS is to date the only available method. Furthermore, organ-specific differences in creatine metabolism [4] necessitate the investigation / measurement of creatine concentrations in multiple organs, ideally within a single recovery MRS examination to reduce the number of animals and to increase statistical power. Therefore, the aim of this study was to evaluate the feasibility and reproducibility of a ^{1}H-MRS technique using a simple set-up, which does not require time-consuming mouse or coil repositioning.

Material and Methods

All experiments were conducted on a 9.4T/210mm bore Magnex magnet with a Varian Direct Drive console using a 33 mm quadrature birdcage coil (Rapid Biomedical). The same PRESS sequence was used to scan the heart, the thalamus and the tibialis anterior (TE/TR=8/2000 ms). Mice were anaesthetized with Isoflurane and cardiac and respiratory parameters recorded throughout the experiment.

1. Reproducibility experiments
   - Cardiac MRS: Three water-suppressed (256 averages) and three unsuppressed (16 averages) spectra were acquired interleaved during diastole from a 2 μl septal voxel using a cardiac/respiratory-gated PRESS sequence in 33 wild-type mice and 53 transgenic mice with elevated cardiac levels, as previously described [5].
   - Brain MRS: Three water-suppressed (256 averages) and three unsuppressed (16 averages) spectra were acquired from a 8 μl voxel localised in the thalamus of the same wild-type mouse on five different days. No gating was used.
   - Skeletal muscle MRS: Three water-suppressed (256 averages) and three unsuppressed (16 averages) spectra were acquired from a 4.5 μl voxel localised in the tibialis anterior of the same wild-type mouse on five different days. No gating was used.
2. Follow-up of creatine concentration in multiple organs

A wild-type mouse was scanned on two following days. Three scans were acquired from the cardiac septum, one from the thalamus and one from the tibialis anterior. Between each organ, the mouse was repositioned in the scanner by simply pushing the whole cradle inside the volume coil, repositioning the voxel and reshimming using an automatic method [6].

3. Data analysis

Creatine and water peak amplitudes from all spectra were quantified using AMARES [7]. The water peak of the non-suppressed spectra was used an internal reference.

Results

1. Reproducibility experiments

Representative spectra from all three organs are shown in Figure 1. The average intra-day Coefficient of Variation (CV) of the creatine cardiac measurements was of 16.9%. The average intra-day CV of the creatine thalamic measurements was of 6.6% and the inter-day of 4.1%. In the tibialis anterior, the intra-day and inter-day CV were of 5.8% and 2.8% respectively.

These results confirm the reproducibility and repeatability of the method. However, due to a higher intra-day variability of the method in the heart, three cardiac spectra were acquired in the multi-organ examination, whereas single spectra were acquired from the brain and the skeletal muscle.

2. Follow-up of creatine concentration in multiple organs

The whole acquisition of spectra from the heart, the brain and the skeletal muscle was performed within a single anaesthesia and lasted roughly 2.5 hours, including preparation time. During this period the physiological parameters were stable (HR=418±13, RR=28±12). The variations in the creatine measurements between the two consecutive days were of 9% for the heart, 2.5% for the brain and 6.7% for the skeletal muscle.

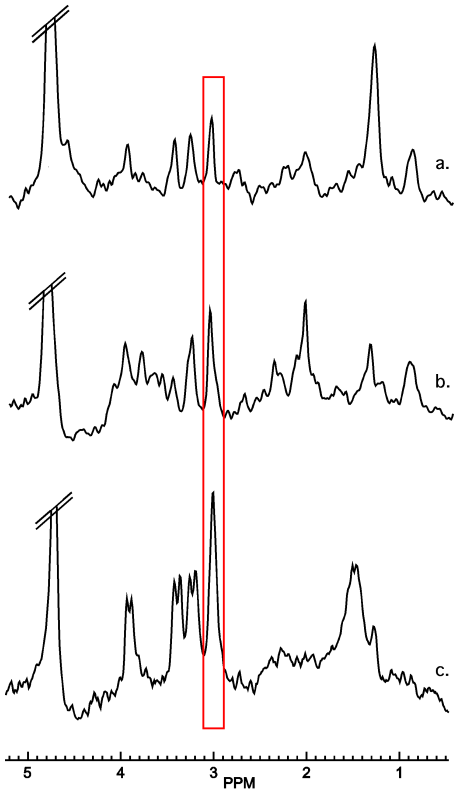

Figure 1: Representative spectra from the myocardium (a), the thalamus (b) and the anterior tibialis (c). The creatine peak is highlighted in red.

Conclusions / Discussion

We have shown that creatine metabolism can be assessed in three different organs using a simple set-up without the need for RF coil repositioning as would be required for surface coils. Very few studies in rodents have investigated the reproducibility of MRS. However, our inter-day CV is similar to the one from a study by Öz et al. [8] in the mouse brain with a CV for creatine measurement less than 5%.

Therefore, this technique allows us to accurately follow creatine levels *in vivo* in multiple organs. An extended study in mice with different pathophysiological conditions is ongoing.

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Acknowledgements: This work was funded by the British Heart Fundation (BS/06/001 and FS/07/065)