Longitudinal measurements of myocardial creatine content in normal and creatine transporter over-expressing mouse hearts using single voxel ^{1}H-MRS *in vivo*

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Introduction: Creatine (phosphorylated and unphosphorylated) plays a key role in the energy metabolism of the heart. To investigate the hypothesis that cardiac high-energy phosphate metabolism contributes to contractile dysfunction in heart failure, a transgenic mouse model that over-expresses the creatine transporter in the heart was created as a means to increase the myocardial creatine content (1). These animals show supra-normal, but variable creatine levels (65–240 nmol/mgPr compared to 58–74 nmol/mgPr in normal hearts) (1). The aim of this study was to investigate whether single voxel ^{1}H-MRS can be used to non-invasively stratify the animals according to their creatine levels and to follow to them longitudinally.

Methods: Female wild type (WT, *n*=7) and creatine transporter overexpressing (CrT, *n*=6) mice were subjected to cardiac ^{1}H-MRS *in vivo* at the age of 6 weeks, 4 months and 8 months, respectively. After inducing anesthesia in an anesthetic chamber using 4 % isoflurane in 100 % oxygen, animals were positioned supine in a purpose-built animal holder for positioning mice vertically, and maintained at 1.5-2 % isoflurane in 1 l/min oxygen flow throughout the MR experiments. Spectroscopic experiments were carried out on an 11.7 T (500 MHz) MR system comprising a vertical magnet (bore size 123 mm – Magnex Scientific, Oxon, UK), a Bruker Avance console (Bruker Medical, Ettlingen, Germany) and a shielded gradient system (548 mT/m, 160 µs rise time) (Magnex Scientific, Oxon, UK). Quadrature driven birdcage coils with inner diameters of 28 mm and 40 mm (Rapid Biomedical, Würzburg, Germany) were used to transmit and receive the NMR-signals. Cardiac triggered and respiratory gated (with steady state maintenance (2)), water suppressed and unsuppressed cardiac spectra from a 2 µl voxel positioned in the interventricular septum were acquired in diastole using a PRESS sequence (TE = 9 ms, TR ≈ 2 s, NAE=256, repeated twice). All spectra were quantitatively analyzed using the time domain fitting software jMRUI (3) and the creatine signal referenced to the unsuppressed water signal.

Results: Figure 1 and 2 show ^{1}H-MR spectra obtained from a 2 µl voxel, placed in the interventricular septum of a WT and a CrT mouse heart, respectively. The arrow indicates the supra-normal creatine level (CH_3-resonance of creatine at 3 ppm). ^{1}H-MRS was successful in 71 out of 78 cases (91%). Figure 3 shows the overall mean relative creatine content for all 13 animals individually and the error bars encode the corresponding standard deviation. The CrT (filled symbols) had significantly higher relative creatine contents than the WT (open symbols; P<0.001 at all time points). The relative creatine content was at 6 weeks: 2.7 ± 0.9/8 ± 1, at 4 months: 3.0 ± 0.6/7 ± 1, and at 8 months: 3 ± 1/6 ± 2 (Mean ± SD, WT/CrT), respectively.

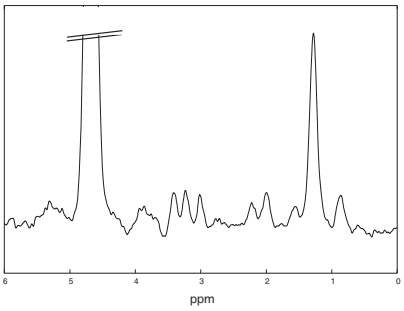

Figure 1.

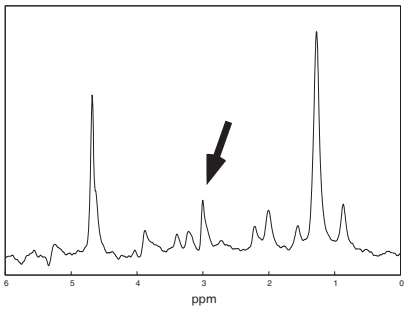

Figure 2.

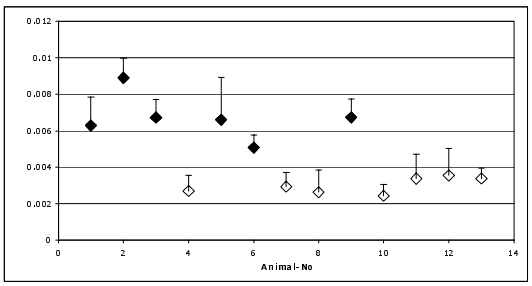

Figure 3.

Discussion: This is the first study to report on the application of cardiac ^{1}H-MRS *in vivo* to longitudinally investigate cardiac metabolism in normal and genetically modified mice. Previously, we used this technique to non-invasively confirm the absence of creatine in hearts of Guanidinoacetate N-methyltransferase (GAMT) knockout mice (4). We have now demonstrated that it is possible to use ^{1}H-MRS to stratify mouse hearts according to their relative creatine content with reasonable accuracy. This work is part of an on-going study, which will conclude with experiments on the same cohort of mice at 12 months old.

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