Creatine uptake kinetics in brain and skeletal muscle of GAMT deficient knockout mice

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Introduction. Guanidinoacetate methyltransferase (GAMT) deficiency is a rare disease characterized by reduced creatine (Cr) and elevated guanidinoacetate concentrations in body fluids. ^{1}H MRS has been instrumental in the diagnosis of these patients, showing characteristically low Cr signals in brain. Treatment of patients focused on elevating Cr concentrations in skeletal muscle and brain, however, the time course of Cr increase in these tissues has only been investigated in case studies [e.g. 1,4]. To enable in depth study of the disorder, GAMT deficient knockout mice (GAMT-/-) were generated [3]. The purpose of the present study was to investigate in detail by ^{1}H MRS the increase of Cr in brain and skeletal muscle of GAMT-/- mice receiving Cr supplementation.

Methods. ^{1}H MRS measurements of both skeletal muscle (SM) and brain (B) were performed at 7.0T (STEAM TE=10ms, TM=15ms, TR=5s, B=256 averages and SM=128 averages). For B, a 16-mm surface coil was used and MR spectra were recorded of a voxel in the midbrain (thalamus/hippocampus) (8.8µl). For SM, MR spectra were recorded of a voxel (16µl) placed in the calf muscles using an Alderman-Grant type of coil, oriented at the magic angle to reduce the spectral effect of dipolar interactions. Mice were anaesthetized with 1.5% Isoflurane and body temperature was maintained using a warm water blanket.

GAMT-/- mice were supplemented for 35 days with 2g/kg Cr monohydrate dissolved in the drinking water. After recording a MR spectrum before supplementation, GAMT-/- mice (n=20) were divided in 4 groups and measured at different time points during the supplementation period. This resulted in n=3 or 4 per time point for B and n=2 (except day 1) for SM. Spectra of the same locations were recorded for control animals (B: n=4 and SM: n=3) on a Cr-free diet.

Data processing for both B and SM was performed using the unsuppressed water signal for eddy current correction, phasing and normalization. MR spectra of B were processed using LCModel, MR spectra of SM were processed using jMRUI. For SM, the linewidth of the Cr signal was constrained and T2 correction was applied for absolute quantification. Linear regression was used to compare the increase in Cr between SM and B during different periods of Cr supplementation and t-tests were used to test for differences between groups (significant at $p<0.05$.)

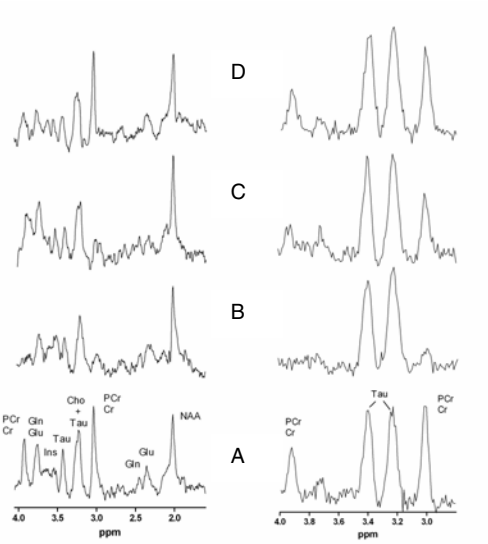

Figure 1. Brain (left) and skeletal muscle (right) ^{1}H MR spectra of a control mouse (A) and a GAMT-/- mouse before (B) and after 4 (C) and 16 (D) days of Cr supplementation. The increase in Cr signal intensity at 3.0 and 3.9 ppm is clearly visible.

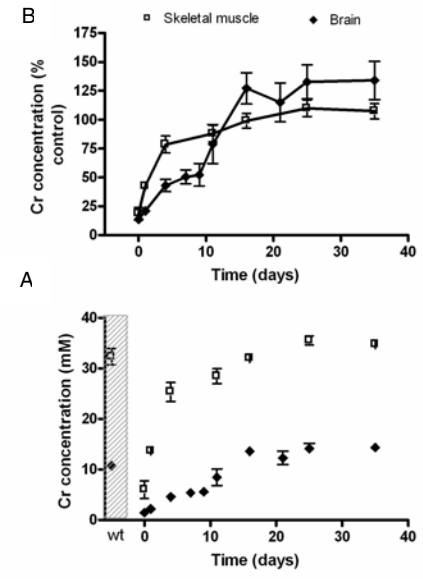

Figure 2. Absolute (A and relative (compared to control) (B) increase in Cr concentration in skeletal muscle and brain of GAMT-/- mice. wt = Cr concentrations in control animals.

Results. Cr supplementation resulted in an increase in Cr concentration in GAMT-/- mouse brain and skeletal muscle (Fig 1). During the first week, the increase of Cr was more rapid in SM compared to B, both absolutely (Fig 2A) (4.6±0.8 mM/day and 0.6±0.1mM/day respectively) as relatively (compared to control values) (Fig 2B) (14.3±2.4 %/day in SM and 5.4±0.9%/day in B). Thereafter (until day 25), the absolute increase in Cr concentration was similar for both tissues. The relative increase, compared to control values, at the end of the supplementation period was higher for B compared to SM.

Conclusion and discussion. These results show that GAMT-/- mice take up Cr in skeletal muscle and brain in the course of days and that the increase is faster in skeletal muscle compared to brain. This faster increase in Cr concentration in skeletal muscle was already suggested in a case study on a GAMT-/- patient [1] where skeletal muscle Cr was studied before and once during Cr therapy. The present detailed analysis of the time course of Cr increase in both tissues shows that the faster increase in skeletal muscle is only apparent at the beginning of the supplementation period and that the increase in skeletal muscle is biphasic.

The observed differences in uptake kinetics between skeletal muscle and brain could well be influenced by the limited permeability of the blood brain barrier for Cr [2] and the regulation and expression of the Cr transporter in both tissues.

References

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