^{1}H MRS during creatine supplementation in GAMT deficient knockout mice elucidates an early difference in creatine uptake between muscle and brain

H. E. Kan[1], E. Meeuwissen[1], J. van Asten[1], A. Veltien[1], D. Isbrandt[2], and A. Heerschap[1]

[1]Radiology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands, [2]Center for Molecular Neurobiology Hamburg, Hamburg, Germany

Introduction. Creatine (Cr) uptake in brain of patients lacking Cr due to the absence of the enzyme guanidinoacetate methyltransferase (GAMT) is slow and Cr levels do not completely normalize after 2 years of Cr supplementation [1]. The limited permeability of the blood brain barrier (BBB) for Cr [2], competitive binding of the immediate precursor of Cr in the biosynthesis, guanidinoacetate (Gua), to the Cr transporter (CrT) [3] or saturation of the CrT due to high extracellular Cr concentrations [2] could all play a role in this slow increase. In the present study, we investigated with ^{1}H MRS the increase in Cr levels in brain and skeletal muscle during oral Cr supplementation in a mouse model of GAMT deficiency (GAMT$^{-/-}$) [4].

Methods. Localized ^{1}H MRS was performed on GAMT$^{-/-}$ mice before (n=24) and during oral Cr supplementation for 35 days [5] (n=4 per time point) and on control mice on a Cr-free diet (Con; n=4). MR spectra (7T, STEAM, TE=10 ms, TM=15ms, TR=5s) were recorded of voxels in brain (16 mm surface coil, 256 averages, 8.8µl) and skeletal muscle (Alderman-Grant coil at the magic angle, 128 averages, 16µl) (fig 1). Mice were anesthetized with 1.5% isoflurane and body temperature was maintained using a warm water blanket.
Data processing was performed with LCModel with a simulated basis set for brain MR spectra and jMRUI for skeletal muscle MR spectra. Absolute quantification was performed using the unsuppressed water signal. Linear regression was used to determine Cr uptake rates in both tissues after the first day of Cr supplementation and t-tests were used to test for differences (significant at $p<0.05$).

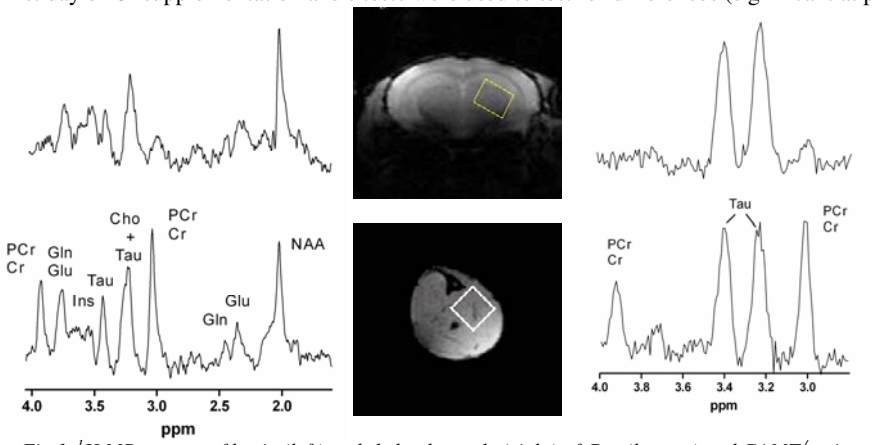

Fig 1. ^{1}H MR spectra of brain (left) and skeletal muscle (right) of Con (bottom) and GAMT$^{-/-}$ mice (top) on a Cr free diet. Voxel locations in brain (top) and skeletal muscle (bottom) are shown on gradient echo MR echo images (middle).

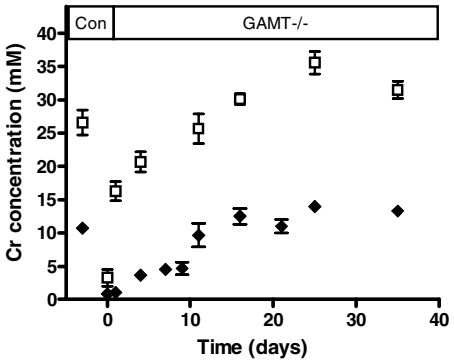

Fig 2. Cr levels in skeletal muscle (open symbols) and brain (closed symbols) in GAMT$^{-/-}$ mice during Cr supplementation. Con values of corresponding regions are shown on the left.

Results. Before Cr supplementation, Cr levels in skeletal muscle and brain of GAMT$^{-/-}$ animals were severely reduced compared to Con (fig 1) and N-acetylaspartate (NAA) levels were slightly increased (table 1). During Cr supplementation, Cr levels initially increased rapidly in skeletal muscle. Thereafter, Cr levels increased linearly in skeletal muscle until day 25 and in brain until day 16 (0.8 ± 0.06 mM/day and 0.8 ± 0.07 mM/per day respectively). After 35 days of Cr supplementation, Cr levels in both tissues exceeded those Con animals (fig 2, table 1) and NAA levels in brain normalized to control levels.

Conclusion and discussion. Our results show that Cr levels are higher after one day of Cr supplementation in skeletal muscle compared to brain of GAMT$^{-/-}$ mice but that Cr uptake rates are similar between these tissues after the first day of Cr supplementation. This similarity in Cr uptake rates in skeletal muscle and brain after the first day indicates that the combined effect of both the specific activity and the number of CrTs is similar between the tissues. Apparently, during this period, the BBB does not provide an additional limitation for Cr uptake compared to skeletal muscle in these mice. At low intracellular Cr concentrations in skeletal muscle, however, uptake kinetics of the CrT are increased [6] and this could underlie the initial rapid increase in Cr levels in skeletal muscle. After the first day, Cr levels increased linearly in both tissues and increased faster compared to normal mice during Cr supplementation [5], suggesting that *in vivo* high Gua levels [4] do not dramatically limit the uptake of Cr into these tissues.

	Con	GAMT$^{-/-}$	GAMT$^{-/-,Cr}$
Cr - brain	10.7 ± 0.5	0.8 ± 0.6$^{*\#}$	13.7 ± 0.8*
NAA	8.6 ± 1.5	11.8 ± 1.4$^{*\#}$	9.5 ± 1.2
Glutamate	10.4 ± 1.2	9.7 ± 1.8	10.0 ± 2.2
Glutamine	3.0 ± 1.3	3.6 ± 1.0	3.2 ± 0.5
Taurine	7.8 ± 1.6	6.2 ± 1.4	5.6 ± 1.8
Myo -inositol	5.1 ± 0.8	5.7 ± 1.1	6.8 ± 1.5
Cr – skeletal muscle	27.7 ± 3.9	3.4 ± 3.9*	34.9 ± 3.7*

Table 1.Concentrations are in mM and GAMT$^{-/-,Cr}$ = GAMT deficient mice after 25 and 35 days of Cr supplementation. *=significantly different from Con $^{\#}$ = significantly different from GAMT$^{-/-,Cr}$ at $p<0.05$

In conclusion, the slow increase in brain Cr levels in GAMT deficiency compared to skeletal muscle is probably caused by a difference in Cr uptake kinetics that is only present at low intracellular Cr concentrations in skeletal muscle. The linear and similar increase in Cr levels in both tissues after the first day shows that a specific limitation of the CrT on the BBB or high plasma Gua levels are unlikely to play a role in the slow uptake of Cr in brain. Our results can provide the basis for further experiments to optimize Cr supplementation strategies in GAMT deficient patients, for instance the application of intermittent Cr supplementation protocols to prevent saturation of the CrT.

References. [1] Stöckler, S. et al. *Pediatr Res*, 1994. [2] Schulze, A. et al. *Mol Genet Metab*, 2001. [3] Ohtsuki, S. et al. *J Cereb Blood Flow Metab*, 2002. [4]. Schmidt, A. et al. *Hum Mol Genet*, 2004. [5] Ipsiroglu, O.S. et al. *Life Sci.*, 2001. [6] Brault, JJ. et al. *J Appl Physiol*, 2003.