Creatine deficiency, uptake and breakdown studied in brain and muscle of Arginine:Glycine Amidinotransferase deficient mice

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Introduction: The importance of creatine (Cr) for normal brain function is demonstrated by the severe symptoms of mental and muscular abnormalities in patients with Cr deficiency syndromes (CDS) *[1]*. Oral Cr administration is used succesfully to replenish Cr levels in brain of patients with inborn errors of Cr biosynthesis enzymes (arginine-glycine amidino transferase AGAT, and guanidino acetate methyl transferase GAMT) *[2,3]*. Cr uptake in patients with AGAT deficiency was suggested to be faster, which was ascribed to toxic effects of guanidino acetate (Gua) accumulation in GAMT deficient patients and the competition between Cr and Gua uptake. As a result, lower Cr doses are prescribed for AGAT$^{-/-}$ than for GAMT$^{-/-}$ patients *[2,3]*. This difference could only be investigated in a few patients. The recent generation of a mouse model for AGAT$^{-/-}$ enables further investigation on the effects of AGAT$^{-/-}$ on brain and muscle metabolites and the response to Cr treatment without interference of accumulated Gua levels. Here we present a longitudinal metabolic study using *in vivo* ^{1}H and ^{31}P MR Spectroscopy (MRS).

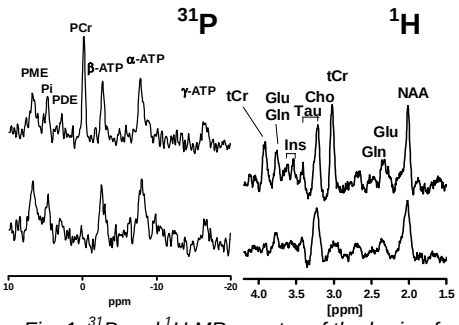

Fig. 1: ^{31}P and ^{1}H MR spectra of the brain of AGAT$^{-/-}$ (bottom) and WT(top)mice.

Methods: MR spectra were obtained from muscle and brain of AGAT$^{-/-}$ and wild type (WT) mice on a 7T spectrometer (MR Solutions). For comparison all procedures were similar to previous studies on GAMT$^{-/-}$ mice using 1.0-1.8% isoflurane *[4,5]*. ISIS localized ^{31}P MR spectra were acquired from brain (n=4,160-220 µL voxel, TR = 6750 ms, 512 ave). ^{31}P spectra of hind limb muscle were measured without localisation (TR=7s, 128 ave). Localized ^{1}H MR spectra were obtained in brain (STEAM, 8.8 µL voxel, TE = 15 ms, TM = 10 ms, TR = 5 sec, 256 ave) and muscle (12.3µL voxel). Cr uptake in brain and muscle were studied with identical ^{1}H MRS measurements during >35 days of suppletion and 120 days of breakdown. The Cr was administered *ad libitum* via the drinking water (5.32 g/ 500mL) with additional glucose (4.32 g/500mL). LCModel was used to obtain metabolite concentrations from the ^{1}H MR spectra using water signals as an internal reference. ^{31}P signals of PCr, Pi and PME were analyzed with AMARES and normalized to ATP signals. Breakdown rates of Cr were determined by fitting the depleting tCr levels to a mono exponential curve.

Results: The ^{1}H MR and ^{31}P MR spectra demonstrate an almost complete absence of total Cr and PCr in AGAT$^{-/-}$ brain (fig 1-2) and muscle (spectra not shown here). In muscle, significantly high Pi levels were found (table 1), whereas in brain phospho mono esters (PME) were elevated. AGAT$^{-/-}$ mice showed a gradual Cr uptake in brain during 45 days of Cr suppletion, reaching normal levels at ~20 days, which is comparable to Cr uptake in brain of GAMT$^{-/-}$ mice (fig. 2). In contrast, skeletal muscle shows a very fast uptake response, that was not seen in GAMT$^{-/-}$ mice *[5]*. Note the high tCr concentrations at the first two days of Cr suppletion in AGAT$^{-/-}$ muscle(fig. 2). Breakdown of tCr was similar in both tissues (fig. 3: brain: 2.1 ± 0.4 % day^{-1}; muscle: 1.0 ± 0.2 % day^{-1}). Interestingly, taurine levels in muscle show additional changes upon Cr suppletion and depletion.

Table 1: Brain and muscle metabolite concentrations in mM and pH in AGAT$^{-/-}$ and WT. # statistically different from WT at p<0.05

	Brain		**Muscle**	
	AGAT$^{-/-}$(n=4)	**WT** (n=5)	**AGAT$^{-/-}$**(n=8)	**WT** (n=6)
[tCr]	0.98 ± 0.39 #	11.06 ± 1.10	4.1 ± 1.5 #	28.9 ± 2.9*
[PCr]	0.2 ± 0.1#	3.5 ± 1.0	2.1 ± 0.9 #	25.2 ± 1.0
[Pi]	2.2 ± 0.6	1.5 ± 0.5	15.8 ± 2.24 #	4.2 ± 0.3
[PME]	3.1 ± 0.6#	2.0 ± 0.3	n.d.	n.d.
pH	7.13 ± 0.11	7.19 ± 0.11	7.29 ± 0.04 #	7.20 ± 0.05

Discussion: This *in vivo* MRS study demonstrates that AGAT$^{-/-}$ brain and muscle tissue do not have an alternative high energy phosphor compound such as phosphorylated Gua in GAMT$^{-/-}$ *[2,4]* to compensate for the lack of the creatine kinase system. However, the elevated Pi levels in AGAT$^{-/-}$ muscle indicate a change in the equilibrium of the phosphate homeostasis. In brain, Cr uptake is very slow when compared to the immediate replenishment of Cr in muscle. This suggests that the blood brain barrier delays transport of Cr into the brain. Moreover, the rate of Cr uptake in brain of the AGAT$^{-/-}$ mice was similar to that observed in GAMT$^{-/-}$ mice *[5]*, though this is in conflict with previous outcome on patients. Thus, the Gua accumulation in GAMT-/- mice did not have an inhibiting effect on Cr uptake in brain. In contrast to brain, muscle of AGAT$^{-/-}$ mice demonstrated a remarkably faster Cr uptake than muscle of GAMT$^{-/-}$ mice. Thus the inhibiting role of accumulated Gua in the treatment of CDS becomes more manifest in muscle, when compared to brain tissue. The changes in taurine levels in response to alterations in tCr levels indicate competition between taurine and Cr in osmolitic homeostase. This mouse model for CDS enabled the determination of Cr breakdown rates in muscle and brain accurately over a 0 to 100 range, which are in good agreement with the outcome of labeling studies which only focus at a 100-115% range *[6]*. In combination with non-invasive MRS techniques this mouse model, that can be switched to and from Cr depleted conditions, provides us with a unique opportunity to study Cr uptake and metabolic effects in CDS without interfering postnatal developmental adaptations or toxic Gua accumulations.

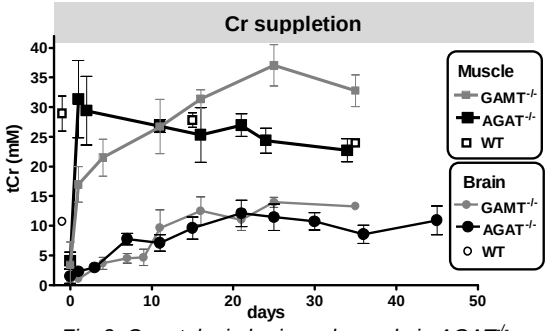

Fig. 2: Cr uptake in brain and muscle in AGAT$^{-/-}$ and GAMT$^{-/-}$ mice. GAMT$^{-/-}$ data presented with permission of HE Kan [7]

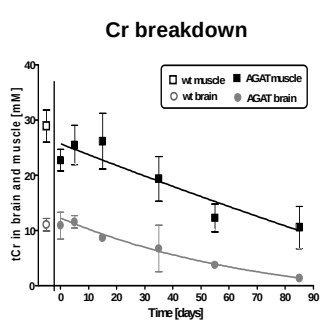

Fig. 3: Creatine breakdown in AGAT$^{-/-}$ muscle and brain during a Cr free diet

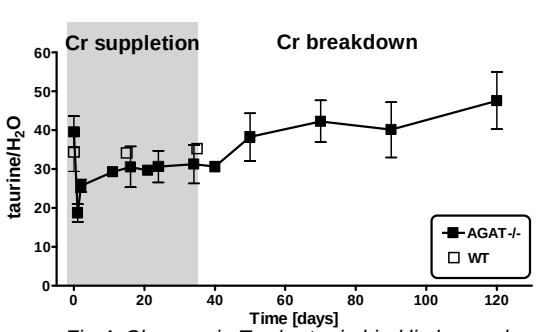

Fig.4: Changes in Tau/water in hind limb muscle of AGAT$^{-/-}$ mice during and after Cr suppletion.

Ref: *[1] Stockler Metab. 1997, [2] Schulze & Battini, Subcell biochem,2007, [3] Biancini et al AJNR 2007, [4] Kan et al. J. Physiol 2004, [5] Kan et al. JAP 2007,[6] Kan et al.MRM 2006*