Severely reduced creatine levels in skeletal muscle and brain of mice lacking arginine:glycine amidinotransferase assessed by multinuclear MRS

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Introduction. Arginine:glycine amidinotransferase (AGAT) is one of the three creatine (Cr) deficiency syndromes (CDS) that have been discovered over the last decade [1]. All CDS are characterized by significantly reduced brain Cr levels accompanied by severe neurological symptoms, highlighting the importance of intact Cr metabolism. The mouse model of the first CDS, GAMT deficiency, has been characterized extensively by biochemical means as well as by quantitative MRS and showed severe depletion of Cr in brain and skeletal muscle accompanied by an increase in the immediate precursor of Cr, guanidinoacetate [2]. Recently, a mouse model of AGAT became available ($AGAT^{-/-}$) and in the present study we show the first results of quantitative ^{31}P and ^{1}H MRS measurements of these mice. When combined with the results of (mouse models of) the other CDS, detailed biochemical characterization of AGAT deficiency is expected to further elucidate specific aspects of Cr metabolism.

Methods. All experiments were performed at a 7T, horizontal bore magnet. Mice were anesthetized with 1.5% isoflurane and body temperature was maintained by a warm water blanket during the experiments. Animals were housed according to genotype to avoid Cr intake due to coprophagia [3]. Localized ^{1}H MRS (STEAM, TE=10ms, TM=15ms, TR=5s) was performed on skeletal muscle (16µl voxel, 128 averages, n=1) and brain (18µl voxel, 256 averages, n=3) of $AGAT^{-/-}$ mice and control littermates (Con; n=3 for brain, n=4 for skeletal muscle). ^{1}H brain measurements were performed with a 16 mm surface coil, skeletal muscle measurements with an Alderman-Grant type of coil with the leg positioned under the magic angle to reduce the effect of dipolar interactions. Localized ^{31}P MRS (ISIS, TR=7s, 8 step phase cycle, 512 averages) was performed on the brain of 4 $AGAT^{-/-}$ (n=3 with 162µl, n=1 with 220.5 µl voxel,) and 4 Con mice (190µl voxel) using a quadrature coil for ^{31}P MRS combined with a ^{1}H surface coil for localization and shimming. Unlocalized ^{31}P MRS on hind leg muscle using a solenoid coil for ^{31}P and an Alderman-Grant type of coil for ^{1}H was performed on one $AGAT^{-/-}$ mouse twice: once when housed with a Con littermate, once 3 months later after solitary housing.
Data analysis of the ^{1}H brain measurements was performed using LCModel using a simulated basis set as input and the unsuppressed water signal for absolute quantification. ^{31}P MRS of hind leg and brain were analyzed using jMRUI with prior knowledge for phasing and linewidths. Tissue pH was calculated as the chemical shift difference between the resonances of inorganic phosphate (Pi) and α-ATP as no PCr signal was detectable in $AGAT^{-/-}$ mice. Differences between $AGAT^{-/-}$ and Con mice were compared with a Students t-test and considered significant at $p<0.05$ (* in the table).

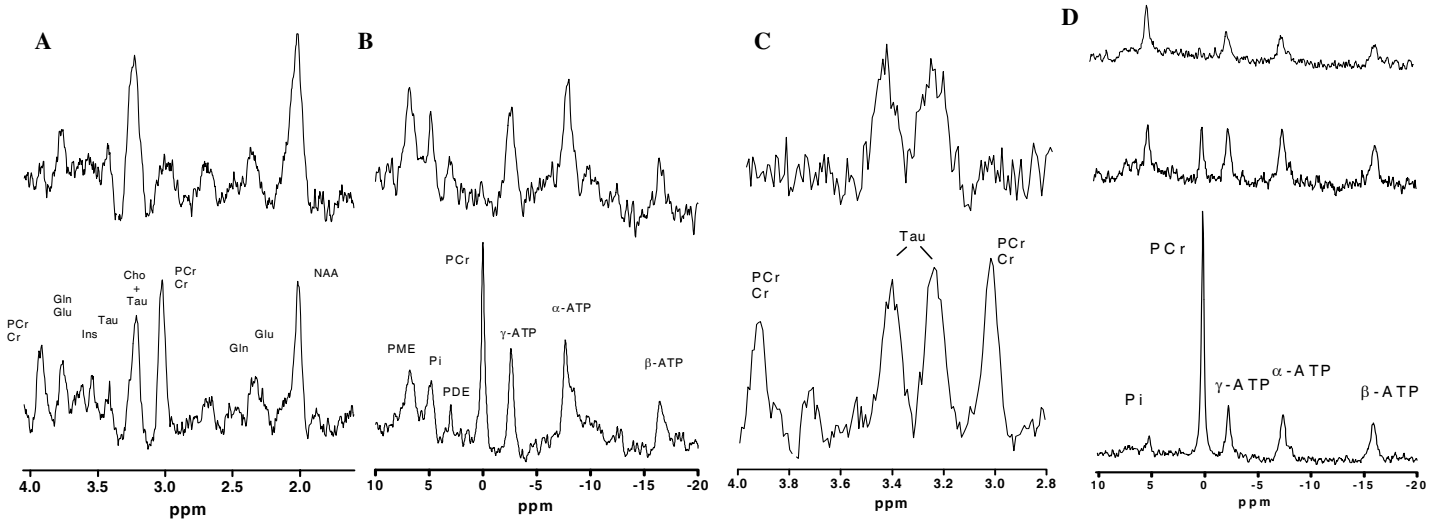

Figure 1. ^{1}H and ^{31}P MR spectra of Con and $AGAT^{-/-}$ mice. MR spectra of Con (bottom) and $AGAT^{-/-}$(top) are shown for brain ^{1}H (A) and ^{31}P (B, summed spectrum of 4 animals) and skeletal muscle ^{1}H (C) and ^{31}P (D). The middle MR spectrum in D represents the $AGAT^{-/-}$ mouse before solitary housing.

Results. All MR spectra of $AGAT^{-/-}$ tissue showed a negligible Cr content, except for the ^{31}P MR spectrum of skeletal muscle when the $AGAT^{-/-}$ mouse was housed with a Con littermate (Fig 1). Quantitative analysis of brain MR spectra showed a severe reduction in total Cr in ^{1}H MRS, undetectable PCr in ^{31}P MRS and a significant elevation of tissue pH (table 1). All other metabolites levels were similar except for a significant reduction in myo-inositol (Ins) (3.6±2.6 mM in $AGAT^{-/-}$ and 7.9±0.1 mM in Con). Skeletal muscle MR spectra showed a reduced signal to noise ratio and revealed an elevated Pi/β-ATP ratio (0.9 before and 1.8 after solitary housing) and a tissue pH of 7.27±0.04. Apart from a negligible total Cr content, the ^{1}H skeletal muscle MR spectrum showed broad resonances for the signals of taurine, indicative of increased effects of dipolar couplings possibly caused by disturbances in muscle morphology.

Conclusion and discussion. Our results show that $AGAT^{-/-}$ mice have severely depleted Cr levels accompanied by decreased Ins levels and increased tissue pH in brain. In skeletal muscle, Pi/ATP ratios show a tendency to increase with decreased PCr levels. This phenomenon has previously been observed in GAMT deficient mice [3] as well as in animals completely lacking muscle creatine kinase [4] and suggests an important role for Cr in skeletal muscle Pi homeostasis. The increased pH and reduced Ins in brain appear typical for AGAT deficiency as they were not observed in GAMT deficiency. Ins is mainly located in astrocytes and is an important cerebral osmolyte [5]. The lack of Cr in AGAT deficiency combined with the absence of an accumulating precursor like guanidinoacetate in GAMT deficiency could play a role in the decreased Ins levels in AGAT deficiency, possibly by a decrease in the number of astrocytes or by causing a osmotic disequilibrium. The reason for the increased tissue pH in brain is presently unknown.

	Con	$AGAT^{-/-}$
Cr (mM)	14.9 ± 2.7*	1.4 ± 1.2
PCr/γ-ATP	1.16 ± 0.2	-
Pi/γ-ATP	0.62 ±0.04	0.89 ± 0.4
PME/γ-ATP	0.92 ± 0.5	1.38 ± 0.3
Tissue pH	7.18 ± 0.2*	7.45 ± 0.1

Table 1. Brain metabolites in $AGAT^{-/-}$ and Con mice

In conclusion, this *in vivo* multinuclear MRS approach on AGAT deficient knockout mice further elucidates the important role of Cr in cellular metabolism, like its role in Pi homeostasis in skeletal muscle.

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