Metabolic adaptations of creatine deficiency in skeletal muscle

C. Nabuurs[1], M. Romeijn[1], A. Veltien[1], H. Kan[1], D. Isbrandt[2], and A. Heerschap[1]

[1]Radiology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands, [2]Center for molecular neurobiology, Institute for signal transmission, Hamburg, Germany

Introduction: Creatine (Cr) plays an important role in maintaining ATP homeostasis in organs with highly fluctuating energy demand like muscle and brain. Defects in one of the enzymes involved in the Cr biosynthesis (i.e. arginine-glycine amidino transferase AGAT, and guanidino acetate methyl transferase GAMT) cause major adaptations in brain and muscle, if not treated with Cr supplementation *[1-2]*. A recent ^{1}H MRS study in a mouse model with AGATdeficiency (AGAT$^{-/-}$) during a Cr free diet demonstrated a total absence of Cr in brain as well as muscle. **Aim:** to investigate metabolic adaptations to Cr absence in skeletal muscle by a longitudinal *in vivo* ^{31}P MR spectroscopy study of AGAT deficient mice. Experiments were performed during Cr suppletion and subsequent removal from the diet. Ischemia was used as a challenge for metabolic readout.

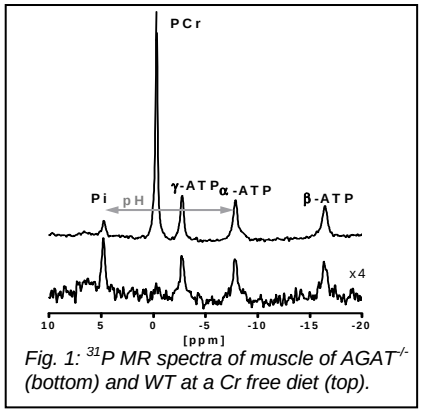

Fig. 1: ^{31}P MR spectra of muscle of AGAT$^{-/-}$ (bottom) and WT at a Cr free diet (top).

Methods: *In vivo* ^{31}P MR spectra were obtained from skeletal muscle of AGAT deficient mice (AGAT$^{-/-}$) and wildtype littermates (WT) at a 7T (MR Solutions) spectrometer (1.0-1.8% isoflurane). The Cr was administered *ad libitum* via the drinking water (5.32 g/ 500mL) with additional glucose (4.32 g/500mL). PCr and Pi changes in muscle were studied with unlocalized ^{31}P MR spectroscopy (TR = 7s, 256 ave) during >35 days of suppletion followed by 170 days of Cr restriction. Additionally, at day 2 and 21 of the Cr suppletion period and day 170 of Cr restriction, ischemia experiments (TR = 1.4s, 76 ave) were applied by occlusion of the hind limb for 20 minutes *[3,4]*. ^{31}P signals of phosphorylated Cr (PCr) and inorganic phosphate (Pi) were fitted using AMARES and normalized to ATP signals. pH was calculated from the chemical shift between Pi and α-ATP. PCr breakdown was fitted to a mono-exponential decay function. PCr decreases and Pi increases during the first 7 minutes of ischemia were used to calculate ATPase fluxes *[5]*.

Results: In addition to the absence of PCr signals, Pi levels were remarkably elevated in AGAT$^{-/-}$ muscle (fig. 1, table 1). Upon Cr suppletion, the elevated Pi in the AGAT$^{-/-}$ mice decreased only gradually. In contrast, PCr levels demonstrated instant increases beyond normal WT levels during the first two days of Cr suppletion and reached normal levels at day 11 (fig. 2). Upon Cr restriction, PCr breakdown was 1.5 ± 0.1 % day^{-1}. Elevations in Pi became most prominent when PCr levels decreased below 6 mM. Remarkably, PCr and Pi concentrations change on a different timescale during suppletion and depletion (fig.3). Upon ischemia in muscle with nearly completely depleted PCr (i.e. after 170 days of Cr restriction), initial changes in PCr were beyond detection at this timescale. The Pi levels, which were already elevated at the onset of the experiment, did not increase much further. Despite the depleted Cr, ATP levels were maintained, whereas pH decreases were significantly larger than in WT (fig. 4). The high PCr levels after 2 days of Cr suppletion enabled determination of ATPase fluxes in AGAT$^{-/-}$ muscle that had just taken up Cr. The result showed elevated fluxes when compared to WT(table 1). After 20 days of Cr suppletion, PCr, Pi and pH responses to ischemia were normalized.

Table 1: ATPase fluxes upon ischema at different PCr levels (*# p<0.05 compated to WT values)*

group	Timepoint [days]		$[PCr]_0$ [mM]	$[Pi]_0$ [mM]	ATPase flux PCr decrease [mM/min]	ATPase flux Pi increase [mM/min]
Wt	0	N=5	25.2 ± 1.0	4.1 ± 0.3	0.11 ± 0.03	0.07 ± 0.03
AGAT$^{-/-}$	170 Cr restr.	N=3	3.2 ± 0.6	12.2 ± 1.7$^{\#}$	n.d.	n.d.
AGAT$^{-/-}$	2 Cr suppl.	N=3	37.4 ± 4.4	13.9 ± 1.9$^{\#}$	0.25 ± 0.09	0.18 ± 0.05$^{\#}$
AGAT$^{-/-}$	21 Cr suppl.	N=4	22.2 ± 2.1	5.6 ± 0.2$^{\#}$	0.14 ± 0.03	0.08 ± 0.03

Discussion: The results show that upon Cr modifications, both fast and slow processes are taking place in skeletal muscle. The initiation of Cr suppletion to Cr deficient mice immediately results in a fast Cr replenishment and phosphorylation. This causes a nett elevated total phosphate level in the muscle cells during the first two days of Cr suppletion because Pi is still elevated then. The slow processes that are observed are (1) the degradation of PCr (2) the changes in Pi levels upon Cr suppletion and Cr restriction and (3) adaptations in ATPase fluxes. Firstly, the depletion of PCr, nicely follows the non-enzymatic degradation of Cr 1-2% per day *[6]*. Secondly, the slow responses in Pi levels to Cr modifications indicate that the ATP↔ADP+Pi equilibrium gradually adapts to a new equilibrium. Upon decreasing PCr, muscle tissue tries to maintain Pi at normal levels, but as soon as PCr levels decrease below about 6 mM, the ATP↔Pi equilibrium shifts towards the right. As this process is dependent on changes in mitochondrial expression it occurs at a different timescale than the fast changes in PCr. Thirdly, although ATPase fluxes cannot be determined directly in nearly complete absence of PCr, the results of the ischemia experiments at the second day of Cr suppletion suggest upregulated ATPase rates.The results of this *in vivo* ^{31}P MRS study applied to a mouse model for AGAT$^{-/-}$ point to fast metabolic uptake responses but slow transcriptional adaptations of ATPases upon modifications in Cr level.

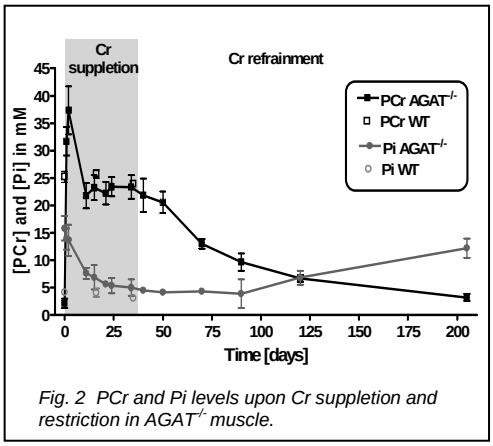

Fig. 2 PCr and Pi levels upon Cr suppletion and restriction in AGAT$^{-/-}$ muscle.

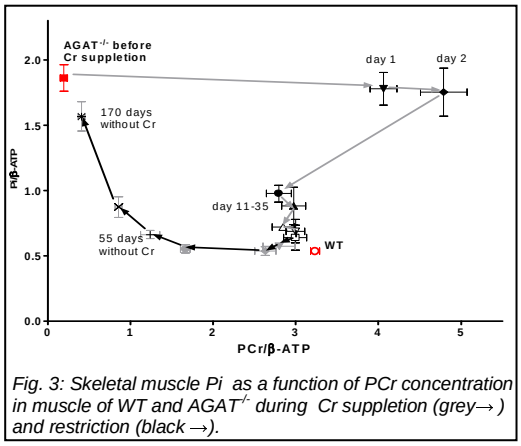

Fig. 3: Skeletal muscle Pi as a function of PCr concentration in muscle of WT and AGAT$^{-/-}$ during Cr suppletion (grey→) and restriction (black →).

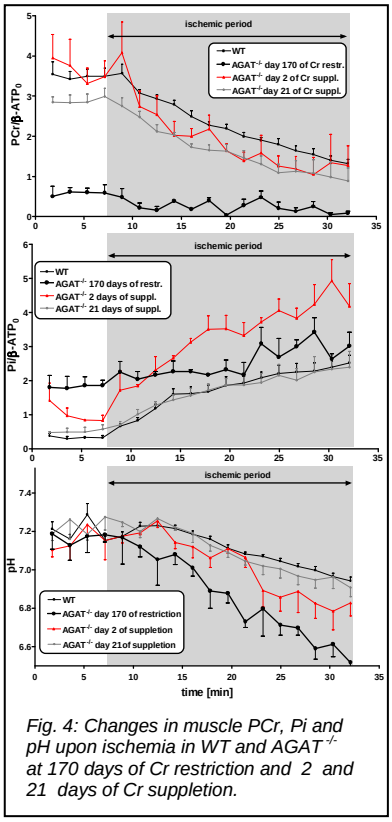

Fig. 4: Changes in muscle PCr, Pi and pH upon ischemia in WT and AGAT$^{-/-}$ at 170 days of Cr restriction and 2 and 21 days of Cr suppletion.

References: *[1] Stockler Metab. 1997, [2] Biancini et al AJNR 28, [3] Kan et al. J. Physiol 2004, [4] Kan et al. JAP 2007[5] Blei et al. J Physiol 1993 [6] Kan et al. MRM 2006*