Altered Hepatic ATP Storage and Creatine Biosynthesis in Transgenic Mouse Liver Expressing Creatine Kinase

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Introduction. Cyclocreatine (CCr), a substrate of creatine (Cr) analogue, can be phosphorylated by creatine kinase (CK) to generate a synthetic phosphagen, PCCr. Since CCr has an equilibrium 30 times more toward phosphorylation than Cr[1,2], dietary CCr can be taken up and quickly accumulated, in the majority form of PCCr, in tissues expressing CK, such as skeletal muscle, heart, and brain of mammals, which results in significant reductions in creatine and its phosphagen, PCr. In contrast, oral ingestion of CCr increased ATP levels in rat brain[3] and appeared to buffer brain ATP stores[4]. On the other hand, liver, a tissue devoid of CK and PCr, biosythesizes creatine and liver ATP synthesis rate is about three times higher than in skeletal muscle and visual cortex[5]. It is therefore of interest to examine if administration of CCr to transgenic mice expressing CK in liver would alter creatine biosysthesis and hepatic ATP storage. Thus, this study applied in vivo ^{31}P MRSI and ex vitro high-resolution ^{1}H NMR to examine if marked accumulation of PCCr/CCr in liver of CK-transgenic mouse with administration of CCr may perturb creatine biosynthesis and ATP catabolism.

Methods. Animals: A group of CK-Tg mice (n=12) expressing CK_{BB} in liver were fed 0.1% CCr-fortified water for 1 day, 1-, 2-, and 6- weeks. Another group of CK-Tg mice (n=12) were fed 1% CCr-fortified water for 1-, 4- days and 1-, 2- weeks. ^{31}P MRSI data were acquired before and after the feeding to measure hepatic ATP and PCCr concentrations. MR Methods: ^{31}P MRSI data were acquired at a 9.4-T Varian Direct Drive animal MRI/MRS system (Agilent Technologies, Inc., Santa Clara, CA). Mouse in prone position was placed on a 2.5 × 2.5-cm ^{31}P receive only surface coil along with a 7-cm ID ^{1}H/^{31}P transmit and receive body coil (m2m imaging Co., Cleveland, OH). Mice were anesthetized with 1.0~1.5% isoflurane mixed with air and body temperature was maintained at 34~35 °C using warm air. A multi-slice gradient echo imaging sequence was used for scout images gated to the respiratory cycle *via* an air pressure transducer (SAII, Stony Brook, NY). ^{31}P MRSI[6] data were acquired using a 90° nonselective excitation pulse, which was followed by phase encoding (PE) gradients in all three dimensions. RF power was calibrated using K_2HPO_4 dissolved in water. 40-cm^3 FOV was encoded using a spherical sampling on a 13 × 13 × 13 grid (TR = 0.8 s, nt = 2). FIDs (512 complex points, SW = 7350 Hz) were acquired after the PE gradients. Data processing was performed using routines written in MATLAB. Concentrations of ATP and PCCr in liver were quantified using a phantom composed of 20 mmol/l KH_2PO_4 solution corrected for saturation factors calculated from measured T1 times and coil loadings[7,8]. Urine and serum samples collected prior to and post CCr feeding were prepared by mixing urine or serum with PBS buffer and D_2O with 0.04% TSP insert for chemical shift reference. Standard ^{1}H NMR spectra of urine and serum samples were measured on a Bruker DRX 600-MHz spectrometer with a 5-mm TXI probe. Metabolite integrals were normalized to TSP integral.

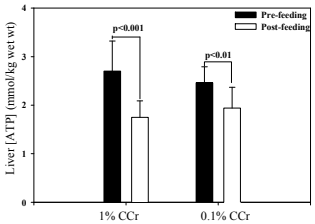

FIG. 1. Hepatic [ATP] before (solid) and after (blank) 1% (Left) and 0.1% (Right) CCr feeding.

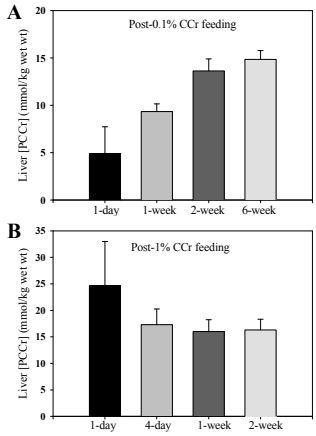

FIG. 2. Hepatic [PCCr] after 0.1% CCr (A) and 1% CCr (B) feeding.

Results. CCr feeding significantly lowered hepatic ATP concentrations estimated by ^{31}P MRSI (Fig.1). Hepatic [ATP] decreased from 2.70 ± 0.62 (SD) at baseline to 1.75 ± 0.34 mmol/kg wet wt ($p<0.001$) after 1%-CCr water feeding. [ATP] decreased from 2.46 ± 0.33 to 1.94 ± 0.43 mmol/kg wet wt ($p<0.01$) after 0.1%-CCr water feeding. With 0.1%-CCr water feeding, hepatic [PCCr] increased gradually over time (Fig 2A) and did not alter serum and urine creatine levels (Table 1). With 1% CCr hepatic PCCr content reached maximum after only one day feeding (Fig.2B) and resulted in reduced serum creatine content (Table 1, $p = 0.008$) and elevated urine creatine content (Table 1, $p = 0.023$). Serum acetate levels increased after both 0.1%-CCr and 1% CCr feeding (Table 1). Urine CCr content after 1%-CCr feeding is much higher than that after 0.1%-CCr feeding (Table 1, $p < 0.001$). Urine creatinine levels didn't change with administration of CCr.

Discussion and Conclusion. Liver, one of the primary tissues responsible for creatine biosynthesis, does not phosphorylate creatine to a significant extent. This physical separation of synthesis and utilization provides the advantage that creatine biosynthesis can be more readily regulated in liver than in other tissues expressing CK[2]. However, in CK-Tg mouse liver expressing CK, this advantage may be perturbed with administration of CCr. With ingestion of CCr, significant amounts of PCCr/Cr accumulate in the CK-Tg mouse liver and may serve as an additional feedback co-repressor of creatine biosynthesis. Reduced serum creatine levels observed with 1% CCr feeding are consistent with this assumption. However, increased urinary excretion of creatine with administration of 1% CCr may also be caused by reduced uptake and retention of creatine within muscle and nerve, which presumably significantly accumulate PCCr/CCr. The lowered hepatic ATP concentration with CCr feeding is probably caused by less conversion from PCCr to ATP since PCCr is a poor substrate for CK. Also, ATP production from glycolysis didn't appear to decrease since serum lactate level didn't decline with CCr feedings. Nevertheless, oxidative phosphorylation may have produced less ATP since its precursor acetyl CoA may have been favorably converted to acetate, as suggested by increased serum acetate levels. To conclude, hepatic ATP concentration estimated by ^{31}P MRSI decreased in CK-Tg mice fed CCr and biosynthesis of creatine may be impaired, with significant amounts of PCCr/CCr stored in CK-Tg mouse liver.

Table 1. Relative metabolite integrals in ^{1}H NMR spectra of serum and urine samples: Pre- vs. Post- CCr feeding

	serum acetate	serum creatine	serum lactate	urine creatine	urine creatinine	urine CCr
Pre- 0.1%	0.019±0.007	0.029±0.015	0.47±0.21			
Post- 0.1%	0.086±0.062*	0.027±0.006	0.56±0.12	0.42±0.16	0.61±0.29	1.46±0.68
Pre- 1%	0.025±0.020	0.034±0.011	0.54±0.18	0.44±0.15	0.55±0.18	
Post- 1%	0.075±0.049*	0.025±0.008*	0.66±0.28	1.01±0.44*	0.51±0.19	6.55±0.94‡

*$p<0.05$ Pre- vs. Post-, ‡$p<0.05$ Post-CCr feeding: 0.1% vs. 1%.

References. [1]Annesley TM et al. Biochem Biophys Res Commun 1977; 74:185-190, [2]Walker JB. Adv Enzymol Relat Areas Mol Biol 1979; 50:177-242. [3]Matthews RT et al. J Neurosci 1998; 18:156-163. [4]Woznicki DT et al. J Neurochem 1980; 34:1247-1253. [5]Schmid AI et al. NMR Biomed 2008; 21 :437-443. [6]Landis CS et al. Hepatology 2006; 44:1250-1258. [7]Meyerhoff DJ et al. NMR Biomed 1990; 3:17-22. [8]Chmelík M et al. Magn Reson Med 2008; 60:796-802.