In vivo ^{1}H MRS and ^{31}P MRSI in Transgenic Mouse Liver Expressing Creatine Kinase

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Introduction. In vivo ^{31}P MRS[1]/MRSI[2] has been employed to measure phosphocreatine (PCr) in hepatocytes of creatine-fed CK-Tg mice expressing creatine kinase BB (CK_{BB}) in liver. However, hepatic creatine has not yet been measured using in vivo ^{1}H MRS. The scarcity of Cr[3] in rodent liver and respiratory motion of rodent abdomen both make in vivo ^{1}H MRS detection of hepatic Cr challenging. The purpose of this study is thus to find a combined in vivo ^{1}H and ^{31}P MRS technique to measure creatine and its phosphagen in liver of CK-Tg mice when creatine is supplemented in the diet. Cyclocreatine (CCr), a Cr analogue, was also fed to CK-Tg mice to boost hepatic accumulation of total CCr (tCCr) for the detection of tCCr and phosphocyclocreatine (PCCr) using ^{1}H MRS and ^{31}P MRSI.

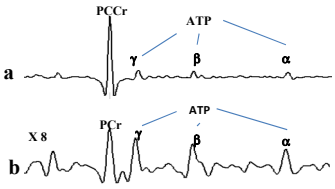

FIG. 1. ^{31}P-NMR spectra of CK-Tg mouse liver fed CCr (**a**) or Cr (**b**).

Methods. Animals: CK-Tg mice (32.5 ± 7.4 g, n = 21) expressing CK_{BB} in liver were studied. Some mice were fed water fortified with either 0.1% or 1% CCr and regular chow for 1 day, 4 days, 1 week or 2 weeks. The rest were fed water fortified with 1.3% Cr and 10% Cr-coated chow followed by daily i.p. injection of 300 µl 1.3% Cr water. 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 the 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 surface temperature, monitored using a surface temperature probe, 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[2] data were acquired using a 90° nonselective hard 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. A 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. Hepatic PCCr concentration was quantified using a phantom composed of 20 mM KH_2PO_4 corrected for saturation factors and coil loadings. ***^{1}H MRS*** data were acquired right after ^{31}P MRSI acquisition following mouse repositioning within a 35-mm ID quadrature ^{1}H volume coil (m2m Imaging Co., Cleveland, OH). MR data acquisition was gated to the respiratory cycle. Water-suppressed ^{1}H MRS data were acquired *via* LASER sequence on a 3 × 3 × 4 mm^3 voxel in the middle liver lobe (TR = 0.8~1.1 s, TE = 36 ms, nt =1024, complex points = 1024, SW = 4006 Hz). Water was suppressed by WET sequence. Shimming routinely resulted in unsuppressed water signal line widths (FWHM) of 20 ~ 40 Hz or 0.1 ~ 0.2 ppm. To provide an internal reference, a non-suppressed water spectra (TR = 10 s, TE = 36 ms, nt = 1 or 4) were also acquired from the same metabolic voxel. T1 of hepatic water and methylene proton of lipid were measured to be 1.76 ± 0.15 s and 0.76 ± 0.05 s, respectively. T1 value of tCr methyl proton from rat brain[4] at 9.4 T was used as T1 of tCCr to correct for TR differences due to gated acquisition. tCCr signal intensity was represented relative to water signal from the same voxel after correction of saturation factors.

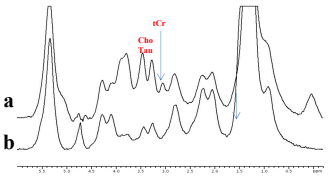

FIG. 2. ^{1}H-NMR spectra of CK-Tg mouse liver before (**b**) and after (**a**) Cr supplementation.

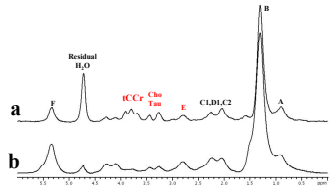

FIG. 3. ^{1}H-NMR spectra of CK-Tg mouse liver before (**b**) and after (**a**) CCr supplementation. A~F represent protons from hepatic lipid.

Results. ***^{31}P MRSI***: As shown in Fig. 1, both Cr and CCr feeding resulted in strong signals from their phosphagens, i.e., PCr and PCCr, respectively. PCCr accumulated much more than PCr in CK-Tg liver since CCr has an equilibrium 30 times more toward phosphorylation than Cr[5]. ***^{1}H MRS***: When CK-Tg mouse was fed 1.3%-Cr water for more than 1 week, no tCr was detected with in vivo ^{1}H MRS. Addition of 10%-Cr coated chow didn't result in detectable tCr signal either. Then daily i.p. injection of 300-µl 1.3%-Cr water was added, which resulted in detectable tCr methyl proton at 3.0 ppm as shown in Fig. 2a. On the other hand, feeding CK-Tg mouse with 1%-CCr water even for 1 day resulted in detectable tCCr signals resonated between 3.6 ppm and 4.0 ppm. Shown in Fig. 3 are ^{1}H-NMR spectra of CK-Tg mouse liver before (Fig. 3b) and after 1-wk 1%-CCr water feeding (Fig.3a). The relative tCCr signal intensity *via* ^{1}H MRS was correlated with hepatic PCCr concentration estimated by ^{31}P MRSI (R=0.631, p=0.012), as shown in Fig. 4.

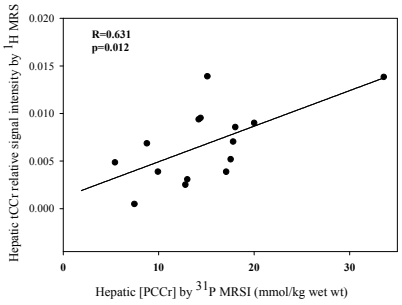

FIG. 4. Correlation between hepatic [PCCr] measured by ^{31}P MRSI with hepatic tCCr signal intensity from ^{1}H MRS.

Discussion and Conclusion. Creatine has been routinely measured in brain and muscle *via* in vivo ^{1}H MRS in both human and rodents. However, its detection by in vivo ^{1}H MRS has not been reported in liver. Besides its scarcity in liver compared to muscle, i.e., 0.4 mmol/kg wet wt vs. 28.2 mmol/kg wet wt[3], respectively, respiratory motion of rodent abdomen also makes hepatic ^{1}H MRS Cr detection challenging. In addition, the creatine methyl proton only resonates 0.2 ppm upfield from the choline methyl proton and 0.2 ppm downfield from the diallylic methylene proton of hepatic lipid (Fig. 3a E). In CK-Tg mouse without Cr supplementation, shimming around 20~40 Hz in a 3 × 3 × 4 voxle in liver results in resolved choline and taurine (Fig.2b and Fig.3b) and efficient water suppression (Fig. 2 and Fig. 3). When Cr accumulated enough in CK-Tg mouse liver, tCr methyl proton is detectable and resolved from choline methyl proton and diallylic methylene proton of hepatic lipid, as shown in Fig. 2a. We believe this is the first reported detection of tCr in liver *via* in vivo ^{1}H MRS. However, its low signal-to-noise ratio may impede its quantification in response to physiologic or dietary change. Thus, CCr, a substrate of Cr analogue, was fed to CK-Tg mouse since a significant amount of CCr/PCCr can quickly accumulate in CK-Tg mouse liver expressing CK. Hepatic tCCr relative signal intensity by in vivo ^{1}H MRS correlates well with hepatic [PCCr] determined by in vivo ^{31}P MRSI. However, H6, H5, H4-1, H3 and H2 of hepatic glycogen also resonate in the region between 3.7 and 4.0 ppm, which may complex tCCr quantification *via* in vivo ^{1}H MRS. In summary, tCr and tCCr and their corresponding phosphagens can be measured in CK-Tg mouse liver expressing CK_{BB} *via* in vivo ^{1}H MRS and ^{31}P MRSI, respectively. These combined techniques may provide a noninvasive method to estimate CK activity in rodents with CK expressing hepatocytes. **References.** [1]Koretsky AP et al. Proc Natl Acad Sci U S A 1990; 87:3112-3116, [2]Landis CS et al. Hepatology 2006; 44:1250-1258. [3]Henderson GD et al. Comp Biochem Physiol 1983; 76B:295-298. [4]Cudalbu C et al. MRM 2009; 62:862-867. [5]Annesley TM et al. Biochem Biophys Res Commun 1977; 74:185-190.