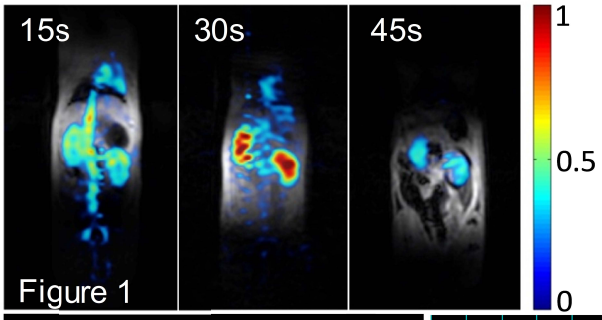
In Vivo Imaging of Hyperpolarized ^{13}C Labelled Choline and Monitoring of Metabolism

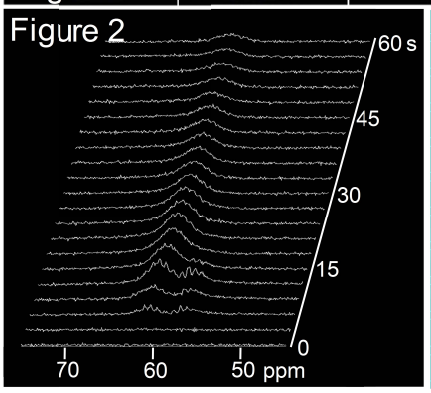
Trevor Wade[1,2], Hyla Allouche-Arnon[3,4], Lanette Friesen Waldner[1,5], Charles A McKenzie[1,5], Kundan Thind[1,5], Alexei Ouriadov[5], Albert Chen[6], J. Moshe Gomori[3], and Rachel Katz-Brull[3,4]

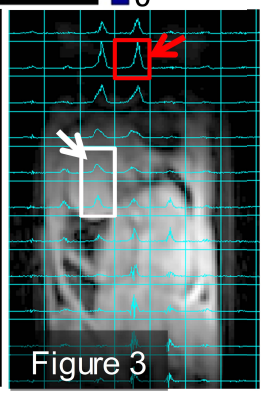
[1]Medical Biophysics, The University of Western Ontario, London, Ontario, Canada, [2]XLR Imaging, London, Ontario, Canada, [3]Department of Radiology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel, [4]BrainWatch Ltd, Tel-Aviv, Israel, [5]Robarts Research Institute, The University of Western Ontario, London, Ontario, Canada, [6]GE Healthcare

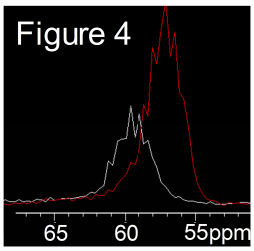
Introduction: Choline is an essential nutrient used in lipid synthesis, one carbon metabolism, and neurotransmitter metabolism. Both uptake and metabolism of choline have been shown to be altered in cancer[1]. Radioactively labelled choline analogs (^{11}C-choline and ^{18}F-choline) have been used to track and identify tumours using positron emission tomography (PET), but the use of ionizing radiation limits the applications of PET, and PET is only sensitive to uptake. Here we show the imaging results with a new hyperpolarized choline molecular agent, labelled at position one with ^{13}C and deuterated at the two methylene positions. The compound – [1,1,2,2-D3,1-^{13}C]choline chloride is termed CMP1. The ^{13}C label at position one leads to a chemical shift difference of 2.4ppm and 113.7ppm between choline its metabolic products, phosphocholine and betain respectively, while deuteration extends the T_1 relaxation time sufficiently to allow imaging and spectroscopy *in vivo*[2].

Methods: *Animals:* All experiments were performed on Male Sprague-Dawley rats, weighing 400+/-20 g (n=6), under a protocol approved by the University of Western Ontario's Animal Use Subcommittee. Animals were anesthetised with isoflurane, and were given atropine intravenously at a dose of 1 mg/Kg to avoid adverse cholinergic effects. Hyperpolarized media were injected via a tail vein catheter. *Hyperpolarization:* 27.3 mg (0.189 mmol) CMP1 was mixed with 7.1 mg of an aqueous solution of OX063 (61 mM) and ProHance (2.9 mM). The DNP sample was hyperpolarized in a dissolution DNP polarizer (Hypersense, Oxford Instruments, Abingdon, UK) at 1.4 K, 94.1 GHz, and 50 mW[3].A volume of 2.5 ml of this hyperpolarized medium was typically injected into the rats for a final dose of 12.6-to-50.0 mg/kg. The bolus injection to the tail vein started typically 15-20s following the start of dissolution. *Imaging and Spectroscopy*: MRI and MRS were performed in a 3T clinical MRI scanner (Discovery MR750,GE Healthcare, Waukesha, WI, USA), equipped with a transmit/receive birdcage ^{13}C RF coil, (Morris Instruments, Ottawa, ON, Canada), and a custom built ^{1}H surface coil within the ^{13}C coil. Coronal projection^{13}C imaging was performed using a variable flip angle [4] gradient echo sequence (matrix: 64x64, FOV: 20cm, acquisition time: 1s, BW ± 2.0kHz). CMP1 was injected as a 12s bolus for each experiment. Images were acquired 15-45 s after the start of the bolus injection. Time resolved ^{13}C spectra were acquired using a pulse-acquire pulse sequence with a slice-selective excitation pulse from a 6 cm slab containing the torso and abdomen of the rat, matching the region demonstrated in the hyperpolarized ^{13}C images. Consecutive spectra of 10^{o} flip angle were acquired with readout filter of 10,000 Hz / 4096 points and a repetition time of 3s. 2D,^{13}C chemical shift imaging (FID-CSI, GE Healthcare) was acquired in the same region described above for imaging and spectroscopy, covering the entire torso and abdomen in the coronal orientation. A 12 x 12 matrix was used with in-plane resolution of 1.0 cm. The tip angle was 10^{o} and the CSI acquisition started 5 s after the beginning of the bolus injection, total scan time was 19 s (10,000 Hz / 1024 points readout, TR: 130 ms).









Results: Imaging of hyperpolarized CMP1 (Figure 1) demonstrated a time resolved distribution of hyperpolarized CMP1. The distribution of signal depended on the time between the start of the bolus injection and the image acquisition. At the 15s point, signal appeared to be largely vascular. By 30s post start of injection, signal from the heart and vasculature was greatly reduced, while signal from the kidneys was even larger than that at 15s. This suggests an efficient choline transport mechanism in the kidney leading to rapid uptake. At 45s, the strongest signal was still from the kidneys but was 57% lower. Dynamic spectroscopy of the abdomen (Figure 2) provided information on the time-course of the metabolism of CMP1. During the bolus injection, two distinct signals were visible. These signals were assigned to choline (CMP1) at 55.7ppm and [1,1,2,2-D_4,1-^{13}C]phosphocholine at 58.9 ppm, in agreement with previous ex vivo studies. At the end of the bolus, the signal from phosphocholine was already higher than CMP1, and by 18s from the start of injection, phosphocholine was the predominant signal, suggesting complete conversion of CMP1 to phosphocholine. By fitting the signal decay from this point on it was possible to estimate the T_1 of phosphocholine *in vivo* to be 21.7s. The regional metabolism was monitored using a 2D CSI sequence (Figure 3) with the acquisition timed to cover the period when uptake and metabolism occurred. The signal from a voxel in the heart is compared to the signal from the kidneys (Figure 4) and indicates that the hyperpolarized agent has been rapidly metabolised by the kidneys into phosphocholine, with a chemical shift of 2.4 ppm. These findings suggest that the uptake and metabolism if CMP1 in the kidneys is extremely fast.

Discussion and Conclusion: We have successfully imaged choline distribution in-vivo for the first time using a noninvasive technique without the need for ionizing radiation. The rapid uptake in kidneys is in agreement with previous studies using an ^{11}C-choline PET agent in rabbits [5], but while PET is only sensitive to uptake we were able to show here that within 18s of the start of the injection, the ^{13}C image in the normal rat body is dominated by phosphocholine metabolised from the injected choline. We have successfully demonstrated that imaging of choline uptake and metabolism is possible within the timescales available to hyperpolarized ^{13}C MRI.

References: [1]M. Beheshti et. al. *Radiology* 254:925-933 (2010) [2] R. Katz-Brull et. al. *Cancer Res*. 67:1966-1970 (2002) [3] J. H. Ardenkajaer-Larsen et. al. *Proc. Natl. Acad. Sci.* USA 100:10158-10163 (2003) [4] G. E. Santyr et. al. *Magn. Reson. Med.* 59:1304-1310 (2008). T. Hara et. al. *J. Nuc. Med.* 38:842-847 (1997)

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