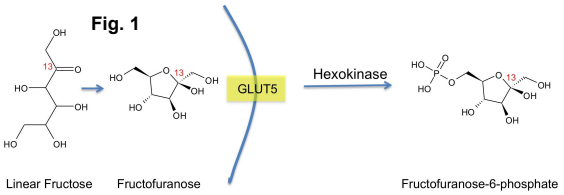
Hyperpolarized [2-^{13}C] Fructose: A hemiketal substrate for in vivo metabolic imaging

K. R. Keshari[1], D. M. Wilson[2], A. P. Chen[3], R. Bok[2], P. E. Larson[2], S. Hu[2], M. Van Criekinge[2], J. M. Macdonald[4], D. B. Vigneron[2], and J. Kurhanewicz[2]
[1]University of California, San Francisco, San Francisco, Ca, United States, [2]University of California, San Francisco, [3]GE Healthcare, [4]University of North Carolina, Chapel Hill

INTRODUCTION: Hyperpolarized ^{13}C labeled molecular probes have shown the potential to investigate important metabolic pathways[1] and other physiologic parameters, such as pH[2], in *in vivo* pre-clinical models of cancer. Carboxylate carbons have been the primary targets for development of hyperpolarized ^{13}C labeled molecular probes due to their relatively long T_1 relaxation times. However, there are a number of potentially important hyperpolarized probes that do not contain a carbonyl but contain quaternary carbons that also have relatively long T_1's. Fructose, occurring as an isomeric mixture of five and six membered rings, has as its most stable isomer β-fructopyranose with a quaternary carbon in the C_2 position. The one-step metabolism of fructose via hexokinase to the phosphorylated fructose-6-phosphate is analogous to the first step of glycolysis, in which glucose is phosphorylated to glucose-6-phosphate (**Figure 1**). Therefore the goal of this study was to investigate a new non-carbonyl hyperpolarized ^{13}C probe, [2-^{13}C]-fructose for the study of metabolism *in vivo*.

Fig. 1



METHODS: *Hyperpolarized [2-^{13}C]-Fructose:* A 4.0M solution of [2-^{13}C]-fructose (Isotec) in water containing 15mM OX063 trityl radical (Oxford Instruments) was hyperpolarized on a Hypersense® (Oxford Instruments) as previously described[3] and dissolved in 1X phosphate buffered saline, with a pH of 7.6. *Ex vivo:* NMR studies were performed on an 11.7T Varian INOVA spectrometer (125MHz ^{13}C, Varian Instruments) using a 10mm broadband direct detect probe and temperature controlled at 37°C. Hyperpolarized [2-^{13}C]-fructose spectra were acquired using a 5º pulse and acquire sequence with proton decoupling during acquisition (NT=100, TR= 3s). Corresponding thermal spectra were acquired using the same sequence with a 90º pulse (NT=16, TR=300s). T_1's were determined from a mono-exponential fit to the time series of hyperpolarized spectra. Solution state polarizations were calculated by correcting the enhancement for the T_1 relaxation during the transfer time (12s) and the thermal polarization at 11.7T (9.6 ppm). *Hexokinase Studies:* Hyperpolarized [2-^{13}C]-fructose was reacted with 400U of hexokinase (Sigma Aldrich) in the presence of 15mM ATP, 50mM TRIS and 13mM $MgCl_2$ to observe the conversion of fructose to fructose-6-phosphate, and identify the hyperpolarized ^{13}C resonances. *In Vivo:* T_1 studies were performed using a 3T GE Signa™ scanner (GE Healthcare) equipped with the MNS (multinuclear spectroscopy) hardware package similar to studies at 11.7T, and temperature maintained using a heating pad calibrated to 37ºC. A dual-tuned ^{1}H-^{13}C coil with a quadrature ^{13}C channel and linear ^{1}H channel was used. For animal studies, T_2-weighted fast spin echo images were acquired prior to MRSI studies to determine the region of interest. MRSI studies utilized a compressed sensing double spin echo 3D MRSI acquisition scheme as previously published[4]. 500 µl of of 80 mM [2-^{13}C]-fructose (0.0013 mmols/kg) were injected similar to previously described methods for [1-^{13}C] pyruvate in a transgenic model of prostate cancer (TRAMP)[1]. Maps of ^{13}C fructose and its metabolites were generated from the peak heights in each voxel and overlaid on the corresponding T_2-weighted image.

Fig. 2

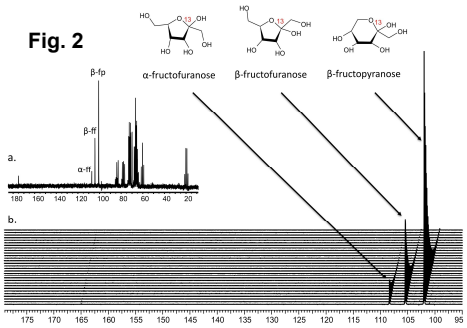


Fig. 3

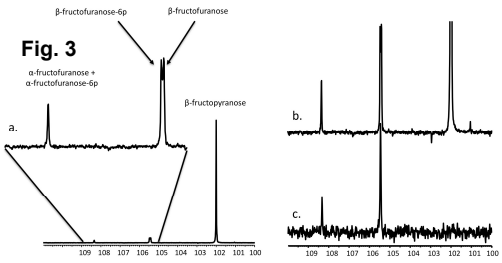
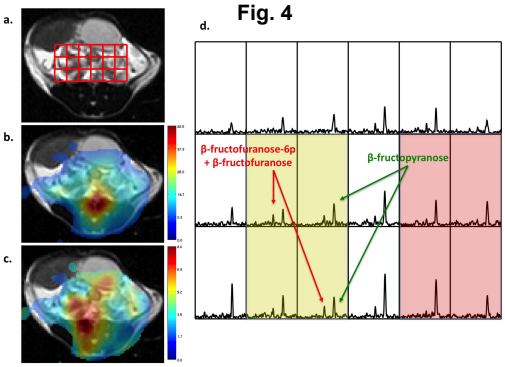


Fig. 4



RESULTS AND DISCUSSION: The natural abundance (**a**) and hyperpolarized spectra (**b**) of fructose are shown in **Figure 2** demonstrating the isomeric distribution of the two ring forms (pyranose and furanose). Corresponding T_1's for the C_2 labelled fructose carbons are given in **Table 1** for the 3 most abundant cyclic isomers (β-fructofuranose, β-fructopyranose, α-fructofuranose) at both 11.7T and 3T. There was no significant difference in the C_2 T_1 between the cyclic isomers of fructose, most likely due to the fast chemical exchange of the isomeric forms[5]. Percent polarizations (**Table 1**) show similar values for the isomers of fructose with an average solution state polarization of 12% at 37ºC. The reaction of hyperpolarized fructose with hexokinase yields the fructofuranose-6-phosphate within 5 seconds (**Figure 3**). An expansion of the downfield region of the spectrum (**Figure 3a**) shows the split in the 105.5ppm resonance, which is a combination of both β-fructofuranose and the β-fructofuranose-6-phosphate. **Figure 3** also compares the first scan of the hyperpolarized acquisition (**Figure 3b**) to the thermal spectrum acquired over 85 minutes post DNP (**Figure 3c**). It is apparent that the enzyme has now fully converted the fructose to fructose-6-phosphate and there is no longer a resonance corresponding to β-fructopyranose. **Figure 4** demonstrates the in vivo distribution of β-fructopyranose and the composite β-fructofuranose and β-fructofuranose-6-phosphate peak in a TRAMP mouse. There was tumor only in the left side of the murine prostate, providing a direct comparison of hyperpolarized fructose uptake/ delivery and metabolism between benign (red) and malignant (yellow) prostate tissues. The MRSI data demonstrated that the resonance corresponding to the composite β-fructofuranose and β-fructofuranose-6-phosphate was higher in the regions of tumor as compared to the benign prostate tissues (**Figure 4d**), which is accentuated in the overlay maps of the total fructose (**Figure 4b**) and composite 105.5ppm resonance (**Figure 4c**).

Table 1

Isomer	T_1 sec (11.7T)	T_1 sec (3T)	%pol
β-fructopyranose	16.3 ± 0.5	14.5 ± 0.3	12.0 ± 2.2
β-fructofuranose	15.8 ± 0.5	13.4 ± 0.5	11.6 ± 2.5
α-fructofuranose	15.8 ± 0.5	13.4 ± 0.4	11.8 ± 2.0

CONCLUSIONS: In this study, [2-^{13}C]-fructose was hyperpolarized using the DNP method and shown to have sufficiently long T_1's and polarizations for hyperpolarized ^{13}C NMR spectroscopic and MRSI studies. The hemiketal C_2 of fructose demonstrates the first non-carbonyl to be hyperpolarized for use as a metabolic probe and suggests the potential of using other hyperpolarized probes involving quaternary carbons even those in a ring structure. Hyperpolarized [2-^{13}C]-fructose has the potnetial to measure changes in carbohydrate metabolism that occur with the evolution and progression of cancer as well as a number of other human diseases such as non-alcoholic fatty liver disease.

[1] Albers et al. Cancer Res. 68(20):8607-15 **[2]** Gallagher et al. Nature 453(7197):940-3 **[3]** Ardenkjaer-Larsen et al. PNAS 100(18):10158-63 **[4]** Hu et al. J Magn. Reson. 192(2):258-64 **[5]** Goux. JACS 107(14):4320-4327 **ACKNOWLEDGEMENTS:** NIH R21 EB007588 and R21 GM075941-01