INTRODUCTION: Hyperpolarized $^{13}$C labeled molecular probes have shown the potential to investigate important metabolic pathways and other physiologic parameters, such as pH. In vivo pre-clinical models of cancer and cardiovascular disease 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 carbens 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 $\beta$-fructopyranose with a quaternary carbon in the C2 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. 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.

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 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=500s). $T_1$'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 MgCl2 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. 500 µl of 80 mM [2-$^{13}$C]-fructose (0.0013 mmols/kg) was injected similar to previously described methods for [1-$^{13}$C] pyruvate in a transgenic model of prostate cancer (TRAMP). 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.

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 C2 labelled fructose carbons are given in Table 1 for the 3 most abundant cyclic isomers ($\beta$-fructofuranose, $\beta$-fructopyranose, $\alpha$-fructofuranose) at both 11.7T and 3T. There was no significant difference in the C2 $T_1$ between the cyclic isomers of fructose, most likely due to the fast chemical exchange of the isomeric forms. Percent polarizations (Table 1) show similar values for the isomers of fructose with an average solution state polarization of 12% at 37°C. The reaction of hyperpolarized fructose with hexokinase yields the fructose-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 $\beta$-fructofuranose and the $\beta$-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 $\beta$-fructopyranose. Figure 4 demonstrates the in vivo distribution of $\beta$-fructopyranose and the composite $\beta$-fructofuranose and $\beta$-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 $\beta$-fructofuranose and $\beta$-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).

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 C2 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 potential 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.