Investigation of CEST effects in hexoses and pentoses of the glycolytic pathway

Francisco Torrealdea[1], Marilena Rega[1], Mark Lythgoe[2], David Thomas[1], Simon Walker Samuel[2], and Xavier Golay[1]
[1]*UCL Institute of Neurology, University College London, London, London, United Kingdom,* [2]*UCL Center of Advanced Biomedical Imaging, University College London, United Kingdom*

Introduction. The use of techniques that exploit the Magnetization Transfer effects, such as CEST and APT, allow imaging of small concentration of metabolites *in vivo*. Recently, GlucoseCEST has been proposed to detect glucose uptake in subcutaneous models of tumour xenografts [1,2], in a way similar to that of ^{18}F-Fluorodeoxyglucose (FDG) commonly used in nuclear medicine to assess malignancy or response to therapy [3], yet at a cheaper cost and without the need for radio-labelled isotopes. So far however, it is not clear if the measured signal in GlucoseCEST comes from glucose only, in which case it would be mostly extracellular [2], or if other sugars transformed by enzymatic reaction along the Embden-Meyerhof pathway would be detectable using similar processes, thereby providing further evidence of the intracellular provenance of the GlucoseCEST in these experiments. In addition, the presence of signals from these sugars would open the possibility of exploring the kinetics of the cancer metabolism non-invasively under the MRI. In this study we investigate the first four molecules present in the glycolytic pathway at concentrations close to the expected values in vivo and assess their CEST signal intensity at constant pH and depending on the temperature.

Theory. CEST utilises the exchange of pre-saturated protons in glucose hydroxyl groups and bulk water to reduce the measured signal from water. The measured asymmetric magnetisation transfer ratio can be described analytically by: $MTR_{asym}(\Delta\omega) = \frac{k\alpha x_{sg}}{R_{1w}+kx_{sg}}\{1 - e^{-(R_{1w}+kx_{sg})t_{sat}}\}$ (Eq. 1), for the OH groups in sugars [4], where x_{sg} = N[*sg*]/2[H_2O] is the fraction of exchangeable sugar protons, k is the OH proton exchange rate, R_{1w} is the longitudinal relaxation rate of water, α is the saturation factor (assumed to be 1) and t_{sat} is the saturation time. Thus, the change in the area under the MTR_{asym} curve is proportional to the concentration of a particular sugar multiplied by the number of OH groups present. As such, if the exchange rate was constant for all hexoses and pentoses of the glycolytic pathway, one would expect a ratio of the CEST effects to be in the same proportion than the number of exchangeable hydroxyl groups {5,4,4,3}.

Methods and Materials. The two first hexoses (glucose and glucose-6-phosphate) and first two pentoses (fructose-6-phosphate and fructose-1,6-biphosphate) were chosen in this theoretical study, as they contained the largest number of exchangeable OH groups (resp. 5, 4, 4, 3) and as they are part of the preparatory phase of the Embden-Meyerhof pathway (and cost therefore ADP rather than producing it), generally present with slower enzymatic reactions *in vivo*. All phantom solutions were prepared using distilled water in 1% PBS giving pH=7.4. A 10mM concentration of solute was used to mimic *in vivo* conditions. The phantom was scanned at four different temperatures, ranging from 22C° to 41C°. A lapse of 30 minutes was given in between each step to guarantee a constant temperature of the phantoms. The sequence used was a modified EPI (resolution of 128x128, FOV=40x40x2mm, TR=1s and TE=15ms) with 5s pre-saturation train of Gaussian RF pulses (N=100, Length=80ms, FA= 4000°, 95% duty cycle) applied off-frequency over a 20ppm range centred at the water frequency. Each set of frequencies was acquired twice starting from negative to positive offsets and then reversed to compensate for any signal drifts. The phantoms were scanned in a Varian 9.4T Agilent VNMRJ system using a 40mm millipede receive/transmit coil. Calculation of the Chemical Exchange Saturation Transfer effect was done by fitting of a Lorentzian to the Z-spectra on a pixel-by-pixel basis, incorporating both direct saturation (±0.5ppm) and regions outside the asymmetrical CEST and NOE effects (>±8ppm). This procedure allows correction of B0 effects and is more precise than the direct left-right subtraction of the MTR spectra, especially *in vivo* where exchange-mediated NOE might mitigate and counterbalance CEST effects. Calculation of the MTR asymmetry was done by integration of the difference between the measured Z-spectra and the fitted Lorentzian between 1 and 3 ppm.

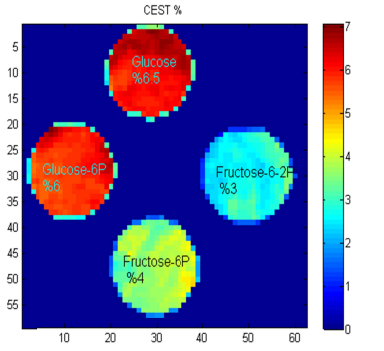

Figure 1 Reconstructed CEST image of all four sugars, counter clockwise: glucose; glucose-6-phosphate; fructose-6-phosphate; fructose-1,6-biphosphate

Results and discussion: Figure 1 shows an image where CEST MTR_{asym} signals were integrated between 1 and 3 ppm. The two first hexoses (glucose and glucose-6-phosphate) and first two pentoses (fructose-6-phosphate and fructose-1,6-biphosphate) are shown in 4 phantoms acquired at the same time, starting with glucose on top counter-clockwise. As can be seen from the scale in this image, the amplitude of the CEST effect is clearly different for all four sugars. Figure 2 shows each of the four spectra, averaged over a large ROI placed in the centre of each of the phantoms. Finally, Figure 3 demonstrate the dependency of each of these signals in function of temperature.

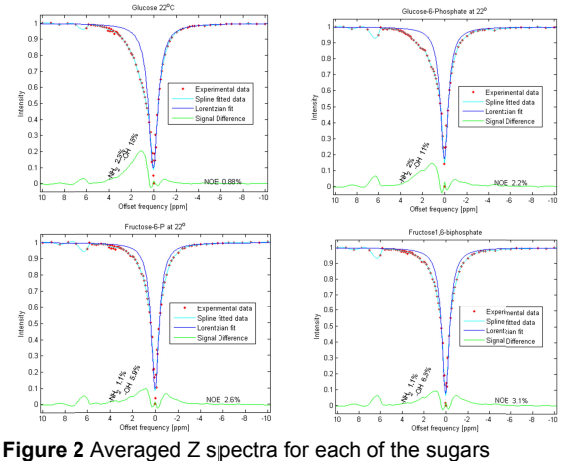

Figure 2 Averaged Z spectra for each of the sugars

Interestingly, one can see that the ratio of the CEST effects are not {5,4,4,3} or {1.0, 0.8, 0.8, 0.6}, but effectively {1.0, 0.92, 0.62, 0.46}, showing therefore the two hexoses having a very similar CEST effect, while the pentoses CEST effects are much more reduced. One can only conclude that the exchange rates of each of these sugars are different, which might limit the detection *in vivo* of these pentoses, therefore reducing the time during which a signal can be detected intracellularly using glucoseCEST [1]. On the other hand, it is reasonable to assume that, since both glucose and glucose-6-phosphate show a CEST effect of similar amplitude, one can detect intracellular glucose using this method.

Another interesting observation is that contrary to what had been seen in glycogen [4], the CEST signal seems to decrease with temperature. This effect, although counterintuitive, as the exchange rate scales linearly with temperature, may be explained by the fact that each of these sugars have a much smaller number of exchangeable protons than glycogen, and are also much smaller and more mobile, leading to a higher probablility of encounter with water molecules and a subsequent larger intrinsic k value. Now, for CEST to be working ideally, k should be in the intermediate exchange rate, and both too large and too small values lead to reduced CEST effects [5]. So here, one can estimate that k is slightly too high at body temperature, and will become better suited for CEST when the temperature drops.

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References: [1] Walker-Samuel *et al.*, Proc. ISMRM 2011, Montreal, #962; [2] Chan et al, Proc. ISMRM 2011, Montreal, #551; [3] Tanimoto, et al. Nuclear medicine communications, 2010, 31(6):604-9; [4] van Zijl et al., PNAS, 2007 Mar 13;104(11):4359-64; [5] Goffeney et al., JACS. 2001;123(35):8628-9.

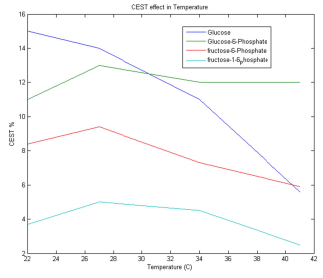

Figure 3 Temperature dependency of the CEST effect in each of the sugars.