# Feasibility of rapid and direct detection of DNP hyperpolarised betaine synthesis

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**Target Audience:** This abstract is targeted to those interested in hyperpolarised $^{13}$C MRI/S and/or metabolism monitoring.

**Introduction:** Choline is an essential nutrient found at the metabolic crossroads of lipid synthesis, one carbon metabolism, and neurotransmitter metabolism [1]. It has been shown that deuteration of the choline molecule leads to a prolongation of $T_1$ [2]. This should allow monitoring of choline oxidase activity using hyperpolarised $^{13}$C MRI/S; however, Gadda [3] has shown that deuteration of choline leads to a decrease in choline oxidase enzyme activity.

**Purpose:** To detect metabolism of hyperpolarised [1,1,2,2-$D_4$,2-$^{13}$C]choline to betaine aldehyde and betaine using the enzyme, choline oxidase, and to determine if there is an isotopic effect on the enzyme kinetics.

**Methods:** *Thermal equilibrium:* Twelve to 14units of choline oxidase from *Alcaligenes* (E.C.1.1.3.17) (Sigma Aldrich) were dissolved in 100mM potassium phosphate buffer and placed in a 5mm NMR tube. Oxygen was bubbled into the solution for *ca.* 3min. Choline chloride (3mM) (Sigma Aldrich) or [1,1,2,2-$D_4$]choline (3mM) (Sigma Aldrich) were added to the reaction mixture and gently mixed before being placed in an 11.8T spectrometer (Varian) for $^1$H-NMR spectroscopy (water suppression, 4 transients, TR=10s). Additional $^1$H spectra of the enzymatic solutions containing choline or [1,1,2,2-$D_4$]choline, were acquired 8 and 13 days after the reaction onset, respectively. *Hyperpolarised state:* Six to 24units of choline oxidase was dissolved in 100mM tris buffer and placed in a 5 mm NMR tube. Oxygen was bubbled into the solution until the medium was saturated with oxygen. A DNP solution including [1,1,2,2-$D_4$, 2-$^{13}$C]choline (CMP2) (donated by BrainWatch Ltd., BW-42, BrainWatch Ltd., Tel-Aviv, Israel) with trityl radical OX063 (62 mM) (GE Healthcare, London, UK) and ProHance (Bracco Diagnostics Inc) was prepared and polarised as previously described [4]. The polarised sample was dissolved in 4mL Tris buffer. This solution was added to the enzyme mixture (final CMP2 concentration of 3mM), gently mixed, and placed at 37$^0$C in a 14.1T spectrometer (Bruker). 128 consecutive $^{13}$C spectra were acquired (repetition time=1s, 10° flip angle).

**Results and Discussion:** Figures 1 and 2 show representative spectra acquired at thermal equilibrium of the enzymatic oxidation of deuterated choline. The metabolic products were assigned to betaine aldehyde hydrate (BAH) and betaine (Bet) using standards (BAH prepared in house). Analysis of products' signals using native or deuterated choline resulted in a significant decrease in the enzymatic reaction yield using deuterated choline (data not shown). While the enzyme activity is affected, deuteration is required to prolong the $T_1$ sufficiently to enable hyperpolarised studies. The time course of oxidation of CMP2 is shown in Fig 3. Build-up of the metabolic products, [1,2,2-$D_3$,2-$^{13}$C]BAH and [2,2-$D_2$,2-$^{13}$C]Bet is clearly detected.

**Conclusion:** Detection of metabolism of hyperpolarised [1,1,2,2-$D_4$,2-$^{13}$C]choline to betaine aldehyde hydrate and betaine using the enzyme, choline oxidase, is feasible. While deuteration of choline decreased the enzymatic reaction yield, deuteration is required to prolong the $T_1$ sufficiently to enable hyperpolarised studies.

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**References:** [1] Blusztajn JK. Science 1998;281(5378):794. [2] Allouche-Arnon H, *et al.* Contrast Media & Molecular Imaging, 2011;6(6):499. [2] Gadda G, Biochimica Et Biophysica Acta-Proteins and Proteomics, 2003;1650(1-2):4. [3] Fan F, et al. Biochemistry, 2006;45(6):1979. [4] Allouche-Arnon H, *et al.* Contrast Media & Molecular Imaging, 2011;6(3):139.

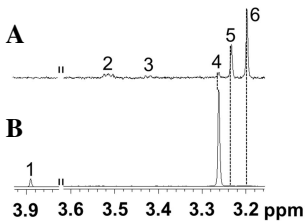


**Fig 1:** $^1$H spectra showing enzymatic oxidation of choline (peaks labeled 2 and 6) to BAH (peaks 3 and 5) and Bet (peaks 1 and 4). A) Spectrum at 11min shows BAH and Bet formation. B) Spectrum at 13days demonstrates full conversion of Cho to Bet. Spectra are not to scale since spectrum A was recorded with a higher gain.

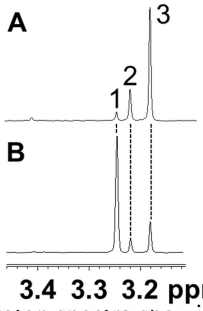


**Fig 2:** Proton spectra showing enzymatic oxidation of [1,1,2,2-$D_4$]choline (labeled 3) to [1,2,2,-$D_3$]BAH (2) and, [2,2-$D_2$]betaine (1). A) Spectrum recorded at 12 min shows BAH and Bet formation. B) Spectrum at 8 days shows enzymatic reaction progression.

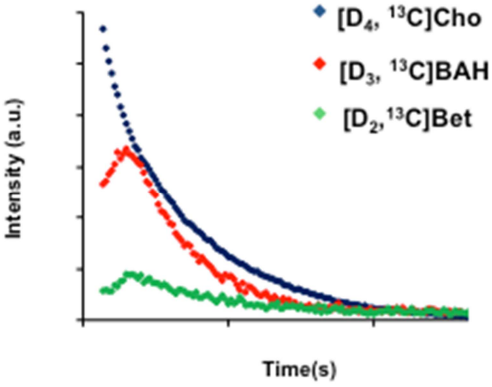


**Fig 3:** Time course of a typical CMP2 oxidation reaction as observed by hyperpolarised $^{13}$C NMRS. The CMP2 signal intensity was reduced 5 fold.