On the Accuracy of AUC Ratio Method for Detecting Treatment Changes with Hyperpolarised ^{13}C Dynamic Spectra

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Introduction: MR Signal enhancements from Dynamic Nuclear Polarisation (DNP) allow detection of apparent enzymatic rates *in vivo* in real-time. Apparent reaction rate constants have been derived by kinetic modelling of the dynamic curves using the modified Bloch equations, which allows quantification of the data. An alternative, simpler method to quantify change is to calculate the ratio of the total areas under the curves (AUC) for a generated metabolite (e.g. lactate) and injected metabolite (e.g. pyruvate) and to compare the area ratios before and after treatment. The forward rate constant (k_{pi}) derived from kinetic modelling was previously shown to demonstrate a strong linear correlation with AUC ratios in experimental data, both *in vitro* and *in vivo* [1]. The objective of this study is to investigate the robustness of the AUC ratio method in detecting treatment induced changes in k_{pi} under a range of simulated experimental conditions.

Methods: Assuming the reaction model shown in Fig. 1, the kinetics can be described by the differential equations 1a and 1b, for the injected molecule, P and its conversion to the i^{th} metabolite, M_i with forward reaction rate k_{pi} and reverse k_{ip}. Hyperpolarised signals decay with relaxation rate r. Eq. 1a can be manipulated (independently of eq. 1b) using Laplace Transforms to give eq. 2, which demonstrates that the ratio of metabolite/pyruvate AUC is proportional to k_{pi}. Importantly, this AUC ratio is independent of both the pyruvate input curve (P_{in}) and the rate constants associated with any other metabolites (included in the term Ω in eq. 1b). This is because we assume that M_i is in exchange with the injected metabolite, but not with any other metabolites. An analogous AUC ratio could be derived from eq. 1b, but since this depends on P_{in} and Ω, this ratio does not have such a simple or useful form. The AUC ratio approach in eq. 2 is useful because changes in the AUC ratio are, in practice, dominated by changes in k_{pi} (provided $r_i > k_{ip}$), which is usually the parameter of interest. In order to investigate the robustness of the AUC ratio method to detecting treatment changes in k_{pi} we define a 'change metric' X given in eq. 3, where the superscripts refer to pre and post treatment variables. In eq. 3 the numerator is the AUC ratio of the post treatment data, and the denominator is the AUC ratio of the pre-treatment data. If r_i and k_{ip} are the same before and after treatment, then the area ratio is $X_0 = k_{pi}^{post}/k_{pi}^{pre}$. We assume that r_i does not change with treatment – this assumption is likely to be reasonable in practice, but the same is not true for the reverse apparent rate constant, k_{ip}. Therefore the purpose of this abstract is to evaluate the bias in X when k_{ip} does change with treatment. Eq. 4 relates the fractional bias in X to two dimensionless terms, Δk_{ip} and q. Δk_{ip} is the fractional change in the reverse rate constant, and smaller values will give lower bias in X. The term q depends on the particular values of r_i and k_{ip}^{pre} for the measured system, and results in lower bias when q is small, i.e. $r_i > k_{ip}^{pre}$. Data were simulated using Matlab (Mathworks) according to the modified Bloch equations [2] for the lactate-pyruvate system and physiologically relevant parameter values were chosen for the various rate constants. Simulated Gaussian random noise was added to the data with SNR of 40:1 (max. pyruvate signal: noise std) and 10,000 Monte Carlo repetitions used.

$$\frac{dM_i}{dt} = -\left(r_i + k_{ip}\right)M_i + k_{pi}P \quad (1a)$$

$$\frac{dP}{dt} = -\left(r_p + k_{pi}\right)P + k_{ip}M_i + P_{in} + \Omega \quad (1b)$$

$$\frac{\int_0^\infty M_i(t)\,dt}{\int_0^\infty P(t)\,dt} = \frac{k_{pi}}{r_i + k_{ip}} \approx \frac{\sum M_i}{\sum P} \quad (2)$$

$$X = \left(\frac{\sum M_i^{post}}{\sum P^{post}}\right) \Big/ \left(\frac{\sum M_i^{pre}}{\sum P^{pre}}\right) \quad (3)$$

$$\frac{X - X_0}{X_0} = -\frac{q\Delta k_{ip}}{1 + q\Delta k_{ip}} \quad (4)$$

$$X_0 = \frac{k_{pi}^{post}}{k_{pi}^{pre}} \quad q = \frac{k_{ip}^{pre}}{r_i + k_{ip}^{pre}} \quad \Delta k_{ip} = \frac{k_{ip}^{post} - k_{ip}^{pre}}{k_{ip}^{pre}}$$

Results & Discussion: Fig. 1 shows the reaction structure for the simulations. This structure is particularly relevant for *in vivo* experiments, where multiple metabolites are formed. For example, an injection of [1-^{13}C]pyruvate, will readily generate lactate, alanine and bicarbonate, which is in exchange with CO_2 [3]. Kinetic modelling of this system is complex, and we show that the AUC ratio method requires knowledge of only a small portion of this network (coloured red in Fig. 1) to quantify change from individual metabolites. Fig. 2 shows that errors in the change metric (X) are independent of change in k_{pi}, as predicted by eq. 4, and are worse with greater changes in k_{ip}. Fig. 3 shows the error in X as a function of change in k_{ip}, for different values of q. These data show that the AUC ratio method is most accurate at correctly measuring change between post and pre treatment scans for small values of q, (i.e. when r_i is large with respect to k_{ip}^{pre}), and for small changes in k_{ip}. Literature values from [2,4] result in q = 0.35 and 0.30 respectively, and from [2] k_{ip} was shown to change by 10% after treatment with etoposide, therefore from Fig. 3 we can see that using the AUC ratio method in this scenario would lead to less than 10% error in X. The AUC ratio in eq. 2 can be equivalently computed by summing the time-series of DNP ^{13}C spectra *before* taking the ratio of peak integrals of the summed metabolites. This approach has advantages when detecting and localising low-signal metabolites and would be appropriate when full kinetic modelling of the data is not required.

Conclusions: We have shown how treatment changes in the AUC ratio relate to treatment-induced changes in k_{pi} and the other rate constants. We have quantified the bias incurred in using this technique and identified various limitations, which will be a useful aid in deciding whether full kinetic modelling or the proposed AUC ratio method is most suitable. Furthermore, we have shown mathematically that, unlike the kinetic modelling approach, the AUC method is independent of hyperpolarised metabolite input function, and also independent of any other reaction pathways of the injected metabolite. This is particularly advantageous for studies where it is difficult to determine the input function and where it is not known *a priori* how many metabolites may be generated, for example when testing new hyperpolarised target molecules or novel drug treatments. We have also described how the approach may be further simplified by summing the spectra before finding the peak integrals.

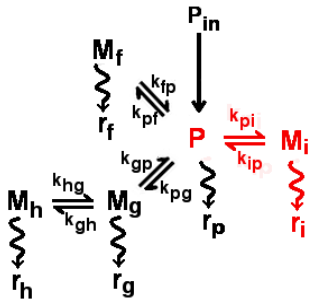

Figure 1. The reaction structure assumed for this simulation. The hyperpolarised molecule P is injected with input function P_{in}. When the reaction of interest is the conversion of P to M_i only red terms need to be considered for the AUC ratio analysis method. Kinetic modelling requires fitting of all terms depicted here. r_i is the hyperpolarised relaxation rate, k_{pi} and k_{ip} are the forward and reverse apparent rate constants for conversion between P and M_i. P may also react with other metabolites (e.g. M_f, M_g, M_h above) but we assume these do not react with M_i.

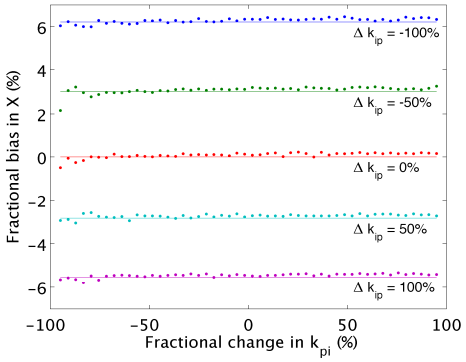

Figure 2. Errors in the change metric (X) as a function of change in k_{pi}. The different coloured plots correspond to different changes in k_{ip}, where greater changes in k_{ip} results in a larger error in X. The plot was generated using q = 0.1 and shows that errors in X are independent of changes in k_{pi}. The data were generated using the assumption that r_i is constant pre/post treatment. The dots show the mean of the Monte Carlo estimates and the lines show the prediction of eq. 4.

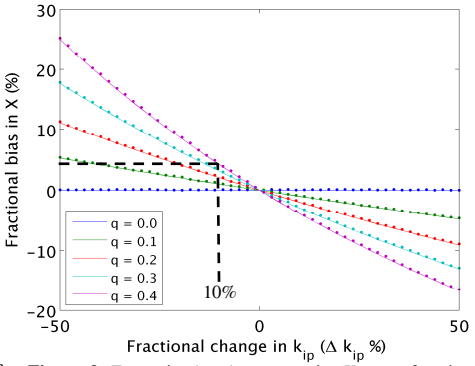

Figure 3. Errors in the change metric (X) as a function of change in k_{ip} for a range of q values and assuming no change in k_{pi}. Errors in X increase with greater change in k_{ip}. Dots show the Monte Carlo estimates and the lines show the prediction. The black dotted line represents 10% error in k_{ip} and q=0.35, which are taken from published *in vivo* data from [2], in this case the fractional bias in X incurred from using the AUC ratio method is <10%.

References. [1] Hill D. K. *et al.* Proc. Intl. Soc. Mag. Reson. Med. 18, Abstract 1738 (2012). [2] Day S.E. *et al.* Nat Med 13, 1382-1387 (2007). [3] Merritt M. E. *et al.* PNAS 104, 19773-19777 (2007). [4] Harrison C. *et al.* NMR Biomed 11, 1286-1294 (2012).**Acknowledgments.** We acknowledge the support received for the CRUK and EPSRC Cancer Imaging Centre in association with the MRC and Department of Health (England) (grants C1060/A10334 and C16412/A6269), NHS funding to the NIHR Biomedical Research Centre.