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On Neglecting Chemical Exchange When Correcting *in Vivo* ^{31}P MRS Data for Partial Saturation: Commentary on: "Pitfalls in the Measurement of Metabolite Concentrations Using the One-Pulse Experiment in *in Vivo* NMR" [1]

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This article replies to Spencer *et al.* (*J. Magn. Reson.* 149, 251–257, 2001) concerning the degree to which chemical exchange affects partial saturation corrections using saturation factors. Considering the important case of *in vivo* ^{31}P NMR, we employ differential analysis to demonstrate a broad range of experimental conditions over which chemical exchange minimally affects saturation factors, and near-optimum signal-to-noise ratio is preserved. The analysis contradicts Spencer *et al.*'s broad claim that chemical exchange results in a strong dependence of saturation factors upon M_0's and T_1 and exchange parameters. For Spencer *et al.*'s example of a dynamic ^{31}P NMR experiment in which phosphocreatine varies 20-fold, we show that our strategy of measuring saturation factors at the start and end of the study reduces errors in saturation corrections to 2% for the high-energy phosphates.

In an article in this issue (*1*), Spencer *et al.* misrepresent our conclusion that chemical exchange has a negligible effect on partial saturation corrections made with saturation factors (*2*) by stating that it is based on the approximately monoexponential dependence of the saturation factor (SF) on the repetition time. Our conclusions (*2*) are in fact based on the small errors in the SFs found over a wide range of experimental conditions input to the same equations used by Spencer *et al.* (*3–5*). Moreover, Spencer *et al.*'s broad claim that "saturation factors in the presence of chemical exchange are strongly dependent upon all M_0's, T_1 and chemical exchange parameters" (*1*) is not substantiated and is not, in general, valid.

In order to illustrate their case, Spencer *et al.* limit their analysis to the "dynamic" case where creatine kinase (CK) metabolite concentrations vary during the experiment and where information about the saturation factors is incomplete (*1*), a situation which Binzoni and Cerretelli (*6*) and ourselves (*2*) had already addressed.

In this response, we first demonstrate, using differential analysis, the existence of a broad range of operating conditions over which the saturation factors are both minimally sensitive to M_0's and exchange rates, and which yield near-optimum phosphorus (^{31}P) SNR/unit time. Second, we show that the application of our interpolation strategy (*2*) to the specific dynamic experiment posed in Spencer *et al.*'s commentary (*1*) reduces errors in saturation corrections for PCr, ATP, and the ratios thereof to less than 2%, although the error in quantities involving P_i is higher ($\leq$13%). Finally, we show that a fully relaxed experiment could accomplish Spencer *et al.*'s dynamic experiment with <1% errors in all metabolite ratios, with only a 40% increase in the acquisition time required to achieve the same SNR as that provided by the optimum Ernst angle condition.

Sensitivity of SFs to k's and M_0's. To address Spencer *et al.*'s broad claim directly, the sensitivity of SF to the k's and M_0's in the three-site exchange model described in their Refs. (*2, 5*) was computed by expanding the derivative of SF in terms of its partial derivatives with respect to the various independent variables, as routinely used for determining error propagation (*7*). The root mean square fractional uncertainty in SF for species A is

$$\frac{\delta \mathrm{SF}(A)}{\mathrm{SF}(A)} = \frac{1}{\mathrm{SF}(A)}\left[\left(\frac{\partial \mathrm{SF}}{\partial M_{0\mathrm{A}}}\delta M_{0\mathrm{A}}\right)^2 + \left[\left(\frac{\partial \mathrm{SF}}{\partial M_{0\mathrm{A}}}\delta M_{0\mathrm{A}}\right]\right)^2 + \left(\frac{\partial \mathrm{SF}}{\partial M_{0\mathrm{C}}}\delta M_{0\mathrm{C}}\right)^2 + \left(\frac{\partial \mathrm{SF}}{\partial k_{\mathrm{AB}}}\delta k_{\mathrm{AB}}\right)^2 + \left[\frac{\partial \mathrm{SF}}{\partial k_{\mathrm{BC}}}\delta k_{\mathrm{BC}}\right]^2\right]^{1/2} \quad [1]$$

$$= [\Delta\mathrm{SF}(M_{0\mathrm{A}})^2 + \Delta\mathrm{SF}(M_{0\mathrm{B}})^2 + \Delta\mathrm{SF}(M_{0\mathrm{C}})^2 + \Delta\mathrm{SF}(k_{\mathrm{AB}})^2 + \Delta\mathrm{SF}(k_{\mathrm{BC}})^2]^{1/2}, \quad [2]$$

where δSF, δM_0, δk, etc., are the variations standard deviations (SDs) in the corresponding variables, and the ΔSF are fractional errors in each of the composite variables as defined by the respective terms in Eq. [1]. Similar expressions can be written for

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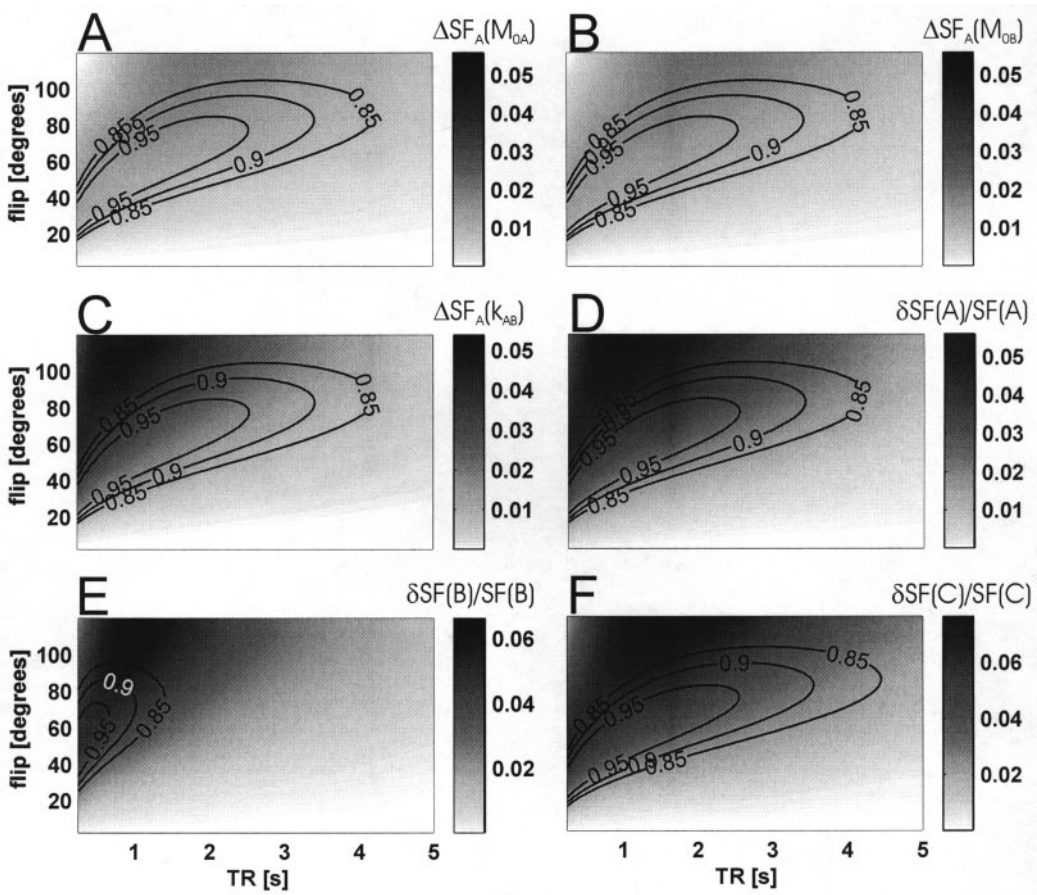

FIG. 1. Sensitivity of saturation factors to changes in equilibrium magnetization or rate constants, as measured by sensitivities to individual parameters: $\Delta SF(M_{0A})$ (A), $\Delta SF(M_{0B})$ (B), $\Delta SF(k_{AB})$ (C), and the combined sensitivities for all parameters, $\delta SF/SF$, for species A (PCr) (D), and $\delta SF/SF$ for species B (E) and C (F). A three-site linear exchange network was modeled with the following system parameters from Spencer *et al.* (1): $T_1A = 2.78$ s (PCr), $T_1B = 0.64$ s (γ-ATP), $T_1C = 2.4$ s (P_i), $M_{0A} = 6.9$, $M_{0B} = 4.3$, $M_{0C} = 1.6$, $k_{AB} = 0.7\ s^{-1}$, and $k_{CB} = 0.37\ s^{-1}$. The partial derivatives were determined by changing each parameter by 10^{-8} of the starting value, while keeping other parameters constants. The relative errors were calculated with Eqs. [1] and [2] for a 25% relative error or uncertainty in M_0's and/or k's. Pulse angle θ and T_R were each varied in 51 steps in the range $0 \le \theta \le 120^\circ$ and $0 \le T_R \le 5$ s. The contour lines on each of the plots delimit the operating conditions for T_R and θ that yield 0.85, 0.9, and 0.95 times the optimum SNR efficiency of the Ernst angle experiment.

$\delta SF(B)/SF(B)$ and $\delta SF(C)/SF(C)$. $\delta SF/SF$ is a measure of the dependence or sensitivity of $SF(T_R, \theta)$ to variations or errors in the equilibrium magnetizations and rate constants.

Figure 1 shows gray scale plots of $\delta SF/SF$ as a function of T_R and θ, calculated for PCr, ATP, and P_i using the preischemic heart muscle parameters listed in Spencer *et al.*'s commentary (*1*). The fractional variation in SF(A) due to the individual components, $\Delta SF(M_{0A})$, $\Delta SF(M_{0B})$, and $\Delta SF(k_{AB})$, are also plotted for a 25% change in the values of M_{0A}, M_{0B}, and k_{AB} (that is, $\delta M_{0'}/M_{0'} = \delta k/k = 0.25$). The variations in $\Delta SF(M_{0C})$ and $\Delta SF(k_{BC})$ were much smaller and are therefore not shown. Superimposed on the gray scale map are contours lines indicating the operating conditions for θ and T_R that yield 0.85, 0.9, and 0.95 times the optimum (Ernst angle) SNR efficiency.

The darkest areas of Fig. 1 are where the SF for PCr is maximally sensitive to changes in equilibrium magnetizations and rate constants. For PCr in this system $\delta SF/SF$ is most sensitive to changes in $k_{PCr\rightarrow\gamma\text{-}ATP}$ (see Fig. 1C). It is clear that SF(A) is most sensitive to changes in k_{AB} for high values of θ and T_R values between 0.5 and 1 s. This region of highest sensitivity to changes in SF corresponds to the operating conditions recommended by Spencer *et al.* (*5*) and lies outside the region yielding near-optimum SNR efficiency. In Figs. 1A and 1B there are two light regions where the sensitivity of SF to changes in equilibrium magnetization and rate constants appears to be minimal: (i) one very narrow region at high θ and very short T_R at the upper extreme left area of the plot; and (ii) one broad area toward low θ and long T_R covering the lower area of the plot. Region (i) with high θ and short T_R again corresponds to the experimental parameters that Spencer *et al.* recommended to keep errors in saturation corrections low (*4, 5*). But, as we discussed (*2*), these solutions lie far from the conditions for T_R and θ that yield near-optimum SNR efficiency, as indicated by the contours. Region (ii) corresponds to the broad range of operating conditions

that are more efficient in SNR per unit time and have minimal errors associated with chemical exchange as we reported (*2*). Starting anywhere in the area of 90–95% of the maximum SNR efficiency, in order to minimize δSF/SF, we may choose either to increase T_R or to reduce θ.

Calculations of δSF(B)/SF(B) and δSF(C)/SF(C) based on the parameters for the ischemic state in Spencer *et al.*'s example and on parameters in Table 2 of our paper (*2*) all showed similar patterns, as exemplified in Figs. 1E and 1F. Moreover, the magnitudes of δSF/SF for the low θ operating conditions that yield high SNR efficiency are always less than 5% in these examples, for a variation of 25% accumulating from each and all of the variables, $M_{0A'}$, M_{0B}, $M_{0C'}$, k_{AB}, and k_{BC} via Eqs. [1] and [2]. Thus, this analysis demonstrates that saturation factors in the presence of chemical exchange are *not* in general strongly dependent upon all equilibrium magnetizations and rate constants, in agreement with our paper (*2*), and contrary to Spencer *et al.*'s broad conclusion (*1*). The strong dependence of saturation factors on T_1's, be they intrinsic, observed, free, or bound, is of course trivial. As a caveat, however, we note that because we have not tested all combinations of M_0's, T_1's and k's, it is certainly conceivable that some systems may exist where substantial sacrifices in SNR efficiency may be necessary in order to avoid errors in saturation corrections due to chemical exchange effects.

This analysis also confirms our main conclusion that for many realistic parameters of the CK reaction studied by ^{31}P MRS *in vivo*, it is possible to choose operating parameters that both avoid large errors in saturation corrections due to chemical exchange effects and still yield near-optimal SNR efficiency (*2*). In essence, the main difference between our analysis of the problem of saturation corrections in the presence of chemical exchange and the solutions suggested by Spencer and colleagues (*3–5*) is that we identify experimental conditions that both minimize errors due to chemical exchange effects *and* retain appreciable SNR efficiency on the other side of the δSF/SF maximum in Fig. 1.

Spencer *et al.* criticize the Monte Carlo method used by Binzoni and Cerretelli (*6*) to gauge the sensitivity of SF to exchange rates, pointing out that under certain conditions the sensitivity of SF to exchange rates may be more significant than suggested by the SD of SF when determined with k randomly varied over a large range. It is indeed unrealistic to vary k over such a large range, because the result will be unduly weighted by the value of SF near its asymptotic value for large k (approaching infinity). For similar reasons the change of 29% calculated by Spencer *et al.* in Fig. 1 of their commentary is unrealistic because $k_{PCR\rightarrow\gamma\text{-ATP}}$ will never change from zero to infinity. In their own example $k_{PCR\rightarrow\gamma\text{-ATP}}$ is still 0.2 [s^{-1}] in the ischemic state and it is inappropriate to assume that this will be zero as long as there is any remaining enzyme and substrate. From Fig. 1 in their commentary we can see that the SF for PCr changes by only about 8% between the values for $k_{PCR\rightarrow\gamma\text{-ATP}}$ of 0.7 [s^{-1}] in the normoxic state and 0.2 [s^{-1}] in ischemia state. The changes in SF for P_i due to these variations in $k_{PCR\rightarrow\gamma\text{-ATP}}$ are less than 2%, and we calculated the corresponding change in the SF for ATP to be 3%.

Dynamic experiments. Earlier papers by Spencer and colleagues focused on analyzing differences between observed T_1 and intrinsic T_1's and errors in SFs caused by chemical exchange (*3–5*) in static experiments where M_0's and k's were kept constant. To support their commentary Spencer *et al.* (*1*) now present an example of a dynamic experiment analogous to those analyzed by Binzoni and Cerretelli (*6*), and by us (*2*). We offered a practical solution for minimizing the errors in saturation corrections caused by the effect of chemical exchange by measuring SFs at the start and end of the experiments, and using linearly interpolated values in between (*2*). The specific example given by Spencer *et al.* (*1*) reiterates the point that large errors might arise when such solutions are not implemented in dynamic studies, and when ^{31}P MRS saturation correction factors derived from a preischemic measurement alone are used to correct metabolite levels that are significantly altered by ischemia. Thus, the errors shown in Table 1 of their commentary (*1*) result from the use of a single measurement of saturation factors performed at the beginning of a dynamic experiment wherein P_i increases 11-fold, and PCr and ATP concentrations are reduced 20-fold. Before judging that the errors of about 20% in PCr or PCr/β-ATP due to this strategy are unacceptable, these errors need first to be placed in the context of the typical *in vivo* experiment where a starting SNR is often much less than 100 for PCr, at best. As PCr and ATP are depleted by a factor of 20 in this experiment, a 20% error in the final PCr/ATP ratio would actually only be comparable to the underlying noise and therefore meaningful, if the nearly completely depleted PCr can be measured with SNR > 5 at this point.

TABLE 1

The Maximum Relative Error in SFs for Metabolites and Metabolite Ratios at Any Point in a Simulated Dynamic Experiment in Which the Metabolite Levels Vary Linearly between the Begin and End Points Defined by Spencer *et al.* in Table 1 of their Commentary (*1*)

	Saturation factors used	
	Start only	Interpolated
Max % error PCr	20.4	1.3
Max % error γ-ATP	25.7	0.4
Max % error P_i	12.4	13.2
Max % error PCr/P_i	14.0	12.3
Max % error PCr/γ-ATP	7.2	1.4
Max % error PCr/β-ATP	20.4	1.3
Max % error P_i/γ-ATP	15.4	11.9
Max % error P_i/β-ATP	15.4	11.6

Note. The flip angle was 60° and repetition time was 1 s. The SFs used either are determined only at the start (start only) or are interpolated from saturation factors measured only at the start and at the end of the experiment (100 intermediate points calculated).

TABLE 2

Correction Factors for PCr/P_i, PCr/γ-ATP, and P_i/γ-ATP Ratios at Various T_R Values and Two Flip Angles, and the Factor by Which the Experiment Time Has to Be Increased to Obtain the Same SNR Realized with the Optimum T_R

			Metabolite ratio correction			Time penalty factor		
θ	T_r [s]	State	PCr/P_i	PCr/γ-ATP	P_i/γ-ATP	PCr	γ-ATP	P_i
90°	1.0	Preischemic	1.05	0.65	0.63	1.36	1.04	1.38
		Ischemic	0.92	0.76	0.83	1.57	1.10	1.44
	2.0	Preschemic	1.05	0.85	0.81	1.01	1.29	1.02
		Ischemic	0.94	0.87	0.93	1.10	1.00	1.05
	5.5	Preischemic	1.01	0.99	0.98	1.44	2.49	1.35
		Ischemic	0.98	0.97	0.99	1.11	1.27	1.17
60°	1.0	Preischemic	1.04	0.80	0.77	1.01	1.21	1.01
		Ischemic	0.94	0.86	0.91	1.06	1.00	1.03

Note. A T_R of 5.5 s for $\theta = 90°$ yields saturation correction factors for the metabolite ratios that are only 1–2% from unity.

Even so, adoption of the strategy that we propose, that of measuring the saturation at the start and the end of the dynamic experiment, would reduce the errors in this example to about 2% for the PCr and γ-ATP if they are measurable, as indicated in our Table 1 here. However, note that the errors in quantities involving P_i are higher at 12–13% when the intepolation method is used. This is due to the inaccuracy of the linear interpolation used and the large difference in intrinsic T_1's, and moreover because the P_i/ATP ratio changes by a factor of 220 over the course of this experiment. It is not justifiable to conclude from this example that such errors will occur in all dynamic experiments. The quantification of P_i *in vivo* may be dubious in any case, given questions of NMR visibility (*8, 9*), or overlap with blood 2,3-diphosphoglycerate in heart studies (*9*). Note also that the use of β-ATP ratios instead of the γ-ATP ratios does *not* reduce errors in the PCr/ATP ratios in this case.

Fully relaxed experiments. Spencer *et al.* raise valid concerns about the practicality of measuring saturation factors at the end of a dynamic experiment. We showed that with the dual angle method (*10*), multiple measurements of the saturation factors or the observed T_1's are possible with near-optimum SNR. As noted in our discussion, the option of performing an additional SF measurement based on the fully relaxed magnetization at $T_R = 5T_1$ with 90° pulses is worth considering, even though it takes 2.5 times longer to obtain the same SNR as in the optimized 90° pulse experiment with $T_R = 1.26T_1$ (*2*).

However, if metabolite ratios instead of metabolite levels are the quantities to be measured as in the example given by Spencer *et al.* (*1*), the effects of chemical exchange can actually be used to significantly reduce the time penalty for performing all experiments under fully relaxed conditions (*11*). In this case we define a fully relaxed experiment as one that yields metabolite ratios that differ by 1% or less from the true ratios. Our Table 2 shows that the minimum T_R required to yield fully relaxed values of each of the three ratios PCr/P_i, PCr/γ-ATP, and P_i/γ-ATP is only 5.5 s for the 90° pulse experiment. This experiment achieves the same SNR as that which satisfies the optimum Ernst angle condition for PCr with 90° pulses with only a 44% increase in acquisition time! Thus, with only a modest increase in acquisition time beyond that yielding optimum SNR, or conversely a 20% SNR penalty for the same acquisition time, fully relaxed ratios can be measured directly in dynamic studies, obviating the need for saturation corrections altogether.

Finally, we do not agree with Spencer *et al.*'s assessment that accurate metabolite quantification *in vivo* requires a significant departure from current practice (*1*), nor the significant SNR penalties prescribed earlier (*5*). Our analysis, based on the same equations as Spencer *et al.* and not challenged by them, shows that the errors in quantification are negligible over a broad range of operating conditions and exchange rates for the nondynamic experiment, and, in the case of dynamic studies, can be accommodated either by start and end saturation measurements or by using the above strategy for measuring fully relaxed metabolite ratios. These ranges of conditions encompass those that provide optimum and near-optimum SNR as is current practice today, while the use of a fully relaxed protocol *in vivo* is not uncommon.

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