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Ongoing Dual-Angle Measurements for the Correction of Partial Saturation in ^{31}P MR Spectroscopy

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Abstract

Use of a repetition time similar to, or shorter than, metabolite T_1 's is common in NMR spectroscopy of biological samples to improve the signal-to-noise ratio. Conventionally, the partial saturation that results from this is corrected using saturation factors. However, this can lead to erroneous results in the presence of chemical exchange or non-constant T_1 's. We describe an alternative approach to correction for saturation, based on ongoing dual-angle T_1 measurements (O-DAM). Using ^{31}P MR spectroscopy of the perfused rat heart undergoing ischaemia-reperfusion, we demonstrate that signal alternations in the data acquired by the dual-angle approach are eliminated by the O-DAM correction scheme, meaning that metabolite concentration and T_1 measurements can be made throughout the course of the ischaemia-reperfusion protocol. Simulations, based on parameters pertinent to the perfused rat heart, demonstrate that accurate saturation correction is possible with this method except at times of rapid concentration change. Additionally, compared to the conventional saturation factor correction method, the O-DAM correction scheme results in improved accuracy in determining the [PCr] recovery time constant. Thus, the O-DAM procedure permits accurate monitoring of metabolite concentrations even in the setting of chemical exchange and T_1 changes, and allows more accurate analysis of bioenergetic status.

Introduction

In vivo ^{31}P NMR has been used extensively to quantify the bioenergetic status and mitochondrial synthetic capability of tissues [1,2]. In such studies, use of a repetition time (TR) similar to, or shorter than, metabolite T_1 's is common in order to improve the spectral signal-to-noise ratio [3]. However, this results in partial saturation and therefore distortion of measured metabolite amplitudes. Conventionally, correction for this is performed empirically using saturation factors calculated from the ratio of resonance amplitudes in spectra acquired with the short TR and with a long TR during an initial control period. This leads to accurate quantification provided that the degree of resonance saturation remains unchanged throughout the experiment.

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According to the Ernst formulation for saturation effects in the absence of chemical exchange [3], this correction requires only that the T_1 's of the metabolites under consideration remain unchanged. However, it has more recently been recognized that if chemical exchange is present, then this correction scheme may also fail whenever metabolite concentrations or reaction rates change, even if the T_1 's remain constant [4,5]. Although the degree of error will depend upon the specific parameters of the system, it has been shown that the errors can be substantial [5,6]. In general, metabolite concentrations, T_1 's and reaction rates are not constant for intervention experiments; in fact, it is precisely changes in these quantities in response to the intervention which are of interest. An ischaemia-reperfusion experiment in an isolated perfused rat heart is an example of this; where it is the changes in metabolite concentrations which are usually of central interest.

The issue of correcting quantification errors has been discussed in a number of previous studies, with no concrete approach to correction for saturation effects emerging besides use of a lengthy TR [4-7]; however, this limits the available SNR per unit time and so represents only a limited solution to the problem. In an attempt to address this problem, Ouwerkerk and Bottomley suggested calculating a saturation factor at the beginning and end of an intervention experiment through use of a dual-angle measurement of T_1 at these times, and then performing a linear interpolation to approximate the appropriate saturation factor to apply to acquired data throughout the intervention [8]. However, the temporal variation of saturation factors may be far from linear, especially as these measurements would be widely separated in time, and will in any case be unknown. In addition, relying upon measurements made at the end of an intervention period may be problematic due to instability of the preparation or because of endpoints such as muscular exercise to exhaustion or to a particular level of metabolite change [6].

Building on the idea of Ouwerkerk and Bottomley, we propose that ongoing dual-angle measurements (O-DAM) performed throughout an intervention may provide a quantitative correction to partial saturation through the use of a T_1 value calculated separately for each (α, β) pair [9] of the dual angle experiment. Thus, the correction for partial saturation can be based upon measurements obtained at the time of acquisition. This is in contrast to the conventional correction scheme, in which saturation factors are determined from control period measurements during which the chemical-kinetic parameters of the system may be vastly different from those applicable to the data being corrected.

In the present study, we have implemented the O-DAM correction scheme and used it to measure the variation of ^{31}P metabolites in the perfused heart during an ischaemia-reperfusion protocol. Further, we have used parameters relevant to the perfused rat heart to simulate these ischaemia-reperfusion experiments and compare the accuracy of the conventional and the O-DAM approaches for the correction of partial saturation. The ability of both techniques to accurately measure metabolite concentrations and to assess the metabolic recovery rate constant for PCr resynthesis following ischaemia has been investigated.

Methods

Experimental: Implementation of O-DAM in ^{31}P NMR of perfused rat heart

All investigations conformed to Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act, 1986 (HMSO) and to institutional guidelines. Experiments were conducted on an 11.7 T (500 MHz) MR system comprising a vertical bore magnet (Magnex Scientific, Oxford, UK) and a Bruker Avance console (Bruker Medical, Ettlingen, Germany). A birdcage coil with an inner diameter of 20 mm (Rapid Biomedical, Wurzburg, Germany) was used as a transmit/receive coil for both phosphorus and protons.

Male Wistar rats ($n = 5$, $BW = 332 \pm 9$ g) were anaesthetized with an intraperitoneal injection of sodium pentobarbital (150 mg.kg^{-1} bodyweight) and their hearts rapidly excised. The hearts were cannulated via the aorta and perfused in Langendorff mode with modified Krebs-Henseleit buffer (11 mM glucose, 4.5 mM pyruvate, 0.5 mM lactate) at 37°C under a constant perfusion pressure of 80 mmHg. Left ventricular pressure was monitored throughout the experiment with a balloon inserted into the left ventricle and connected via a polyethylene tube to a bridge amplifier and PowerLab data acquisition system (ADInstruments, Oxfordshire, UK). The balloon volume was adjusted to achieve an initial end diastolic pressure of 4 mm Hg. The heart was lowered into the centre of the magnet and the position verified with scout images. After shimming, the water resonance line width was less than 50 Hz. The flip angle in the centre of the heart was calibrated via the acquisition of phosphorus spectra with varying pulse widths from a reference sample of phenylphosphoric acid (PPA) doped with gadolinium and contained within the pressure monitoring balloon.

Phosphorus spectra were acquired every minute for 60 minutes. The acquisition parameters, chosen to optimize SNR, included $TR = 0.25$ s, 8 dummy scans, 232 averages, 10 kHz bandwidth, and acquisition data size of 2048 points. Spectra were acquired alternately with nominal flip angles of $\alpha = 15^\circ$ and $\beta = 60^\circ$ as recommended by Ouwerkerk and Bottomley [10] for optimal SNR over a broad range of T_1 values. The hearts were initially perfused at a normal flow rate (approximately 25 ml/min) for 20 minutes, followed by 20 minutes at a low flow rate (0.65 ± 0.07 ml/min - ischaemia) and then 20 minutes of reperfusion at the original flow rate. At the end of this ischaemia/reperfusion protocol three further spectra were acquired with a flip angle of 45° and with the volume of PPA in the balloon being increased by 50 μl between each acquisition. These acquisitions were used to calibrate the acquired signal for the calculation of absolute concentration.

All spectra were fitted in the time-domain using the AMARES algorithm within jMRUI [11,12], with fits performed for phosphocreatine (PCr), inorganic phosphate (P_i), adenosine triphosphate (ATP), and PPA. Prior knowledge used with the AMARES algorithm included specification of the J-coupling of the ATP peaks, approximations of the peak positions, and imposition of Lorentzian lineshapes.

Correction of partial saturation by O-DAM

The observed peak amplitudes of a given metabolite, whether experimentally determined or simulated, were corrected for partial saturation using O-DAM independently for each pair of successive measurements through the following procedure;

The first measurement, with a flip angle α , yielded a signal amplitude for the relevant metabolite of $M(\alpha)$, while the second, with a flip angle of β , yielded a signal amplitude $M(\beta)$. Defining $R = M(\alpha) / M(\beta)$, an effective T_1 , referred to as $T_{1,\text{eff}}$, was calculated for each of these (α , β) measurement pairs [13]:

$$T_{1,\text{eff}} = -TR / \log_e \left[\frac{\sin \alpha - R \sin \beta}{\sin \alpha \cos \beta - R \cos \alpha \sin \beta} \right] \quad (1)$$

Each of the two data points in the (α , β) pair was then corrected for partial saturation through use of the Ernst expression for saturation factors:

$$SF(T_{1,eff}, \theta) = \frac{(1 - e^{-T_{1,eff}/TR}) \sin \theta}{(1 - \cos \theta e^{-T_{1,eff}/TR})} \quad (2)$$

Explicitly, the corrected magnetizations for the two time periods are:

$$M(\alpha)_{O-DAM} = M(\alpha) / SF(T_{1,eff}, \alpha) \quad (3)$$

and

$$M(\beta)_{O-DAM} = M(\beta) / SF(T_{1,eff}, \beta) \quad (4)$$

Note that the calculated $T_{1,eff}$ would be the actual spin-lattice relaxation time of the metabolite under consideration only if there were no changes in the chemical-kinetic parameters of the system between the α and β periods; however, here $T_{1,eff}$ is an empirical constant used to construct an approximate saturation factor correction.

Correction of Experimental Data

Amplitudes measured experimentally with flip angles of $\alpha=15^\circ$ and $\beta=60^\circ$ were corrected according to the above procedure. These corrected amplitudes were then used to calculate absolute metabolite concentrations using the signal measured from the PPA and the heart weight as measured after completion of the MR data collection. The calculation incorporated an assumed intracellular volume ratio of 0.47 to enable conversion of a given heart mass into a corresponding intracellular volume [14].

Overall simulation approach: Evaluation of O-DAM corrections for partial saturation

Simulations were performed to evaluate the accuracy of metabolite concentrations determined using O-DAM correction of saturated resonances. A prescribed set of input parameters $M_0(\text{PCr})$, $M_0(\gamma\text{-ATP})$, $M_0(\text{Pi})$, $T_1(\text{PCr})$, $T_1(\gamma\text{-ATP})$, $T_1(\text{Pi})$, $k_{\text{PCr} \rightarrow \gamma\text{-ATP}}$, and $k_{\text{Pi} \rightarrow \gamma\text{-ATP}}$ was defined for the period during which a flip angle of α was used, and are referred to as the α parameters. Similarly, a set of β parameters were also prescribed. For a stable system, the α parameters and the β parameters would be identical. This is the case in which dual angle acquisition provides a method for measuring T_1 [13], but it is not the case of interest for intervention studies. For the ischemic heart or in the setting of any intervention or instability, the α and β parameters will generally differ from one another in an unknown way.

With the α parameters specified, the observed magnetization during the α period was calculated according to the model for steady-state resonance amplitudes incorporating the effects of chemical exchange [15]. The observed magnetization during the β period was likewise calculated. These two observed magnetization values were then used to obtain corrected magnetization values for the α and the β period as described above.

With this simulation approach, the input metabolite values are precisely known, permitting the calculation of the error in the derived corrected magnetizations. For a given metabolite S_i , with known input magnetization $M_0(S_i)$ as defined by the input parameters to the simulation, and with corrected magnetization $M_{O-DAM}(S_i)$, the percent error is defined as:

$$\%error M(S_i) = \frac{M_{O-DAM}(S_i) - M_0(S_i)}{M_0(S_i)} \quad (5)$$

This error calculation is performed separately for the α and the β periods, and separately for each metabolite.

Simulation of O-DAM-based correction in the setting of reciprocal changes in [PCr] and [Pi]

Any of the chemical parameters describing the system may change during an intervention [16–18]. Therefore, there is an exceedingly wide range of non-steady-state conditions which may be explored. As an initial test of the accuracy of the O-DAM correction scheme, we simulated a simple experiment where there were reciprocal changes in the concentrations of two of the phosphorus containing metabolites, namely PCr and Pi. This means that as PCr decreased, the level of Pi increased commensurately, such that the sum PCr + Pi remained constant.

According to these considerations, we can describe the change between the α period and the β period by a single parameter, defining the fractional decrease in PCr which occurs between the α period and the β period. To be explicit, with f denoting this fractional decline, we have

$$M_0(PCr, \beta) = M_0(PCr, \alpha)(1 - f) \quad (6)$$

and

$$M_0(Pi, \beta) = M_0(Pi, \alpha) + f M_0(PCr, \alpha) \quad (7)$$

Although any intervention experiment will generally encompass several pairs of α and β periods, we can focus on a single α and β period pair to fully describe the errors due to partial saturation throughout the experiment because results from a given α and β pair are analyzed and corrected for saturation independently of the results from any other acquisitions.

We calculated the percent error in the α period and the β period for an assumed fractional decline, f , in PCr occurring between these two time periods. We expect that a smaller f will result in smaller errors, so that the analysis will in effect allow us to place bounds on f which will limit errors to within a specified bound. It is important to note, however, that even for $f = 0$ there will be a non-zero error; this is because the analysis of the dual-angle method [13] does not incorporate exchange into the formalism. For these simulations, we assumed α period magnetizations proportional to $[PCr] = 6.9 \mu\text{mol/g ww}$, $[\gamma\text{-ATP}] = 4.3 \mu\text{mol/g ww}$, and $[Pi] = 1.6 \mu\text{mol/g ww}$ based on our experimental results (below). For the β period, we maintained $M_0(\gamma\text{-ATP}, \beta) = M_0(\gamma\text{-ATP}, \alpha)$ and constrained $M_0(PCr)$ and $M_0(Pi)$ to vary according to equations (6) and (7). Note that here and throughout, the ATP resonance of interest is $\gamma\text{-ATP}$, which undergoes chemical exchange in the context of the usual 3-site exchange model. Use of α and β is restricted to describing the flip angles for the first and second periods of the O-DAM data collection, rather than referring to the ^{31}P nuclei within ATP. For both the α period and the β period, we took $T_1(PCr) = 2.78 \text{ s}$, $T_1(\gamma\text{-ATP}) = 0.64 \text{ s}$, and $T_1(Pi) = 2.4 \text{ s}$, [19]. We also used $k_{PCr \rightarrow \gamma\text{-ATP}} = 0.7 \text{ s}^{-1}$ and $k_{Pi \rightarrow \gamma\text{-ATP}} = 0.37 \text{ s}^{-1}$ [16] with all other rates set to zero, consistent with a linear three-site exchange model [19–20].

Simulation of O-DAM-based correction during cardiac ischaemia

The O-DAM based partial saturation correction method as described above was also applied to time-course data corresponding to the isolated perfused rat heart ischaemia-reperfusion experiments that we conducted. We used the same pre-ischaemic metabolite concentrations as above, and post-ischaemic concentrations were taken as $[PCr] = 0.345 \mu\text{mol/g ww}$, $[\gamma - \text{ATP}] = 0.215 \mu\text{mol/g ww}$, and $[Pi] = 19.2 \mu\text{mol/g ww}$. The time course of $[PCr]$ was modelled as an exponential decrease during the 20 minute ischemic period, and as an exponential increase during the 20 minute reperfusion, while that of $[Pi]$ was modelled as an exponential increase and decrease during these time periods, respectively. Note that in these simulations, the concentrations of PCr and Pi are not linked as they were for the study of reciprocal changes described in the preceding section. A linear decrease in $[ATP]$ was assumed during ischaemia, with a linear recovery during reflow. We used T_1 , $k_{PCr \rightarrow \gamma\text{-ATP}}$, and $k_{Pi \rightarrow \gamma\text{-ATP}}$ values as above for the control period. During ischaemia, T_1 's were taken to approach end-ischaemia values of $T_1(PCr) = 2.22 \text{ s}$, $T_1(\gamma\text{-ATP}) = 0.50 \text{ s}$, and $T_1(Pi) = 3.6 \text{ s}$ [5] in a linear fashion and reaction rates were taken to linearly approach $k_{PCr \rightarrow \gamma\text{-ATP}} = 0.2 \text{ s}^{-1}$ and $k_{Pi \rightarrow \gamma\text{-ATP}} = 0.1 \text{ s}^{-1}$ [6]. A linear recovery to control values was assumed to occur during reflow for T_1 's and k 's.

The time course simulations consist of successive (α, β) periods. We used $\alpha = 15^\circ$ and $\beta = 60^\circ$ for O-DAM. For each (α, β) pair, simulated saturated magnetizations were calculated [6], and corrected for each pair of (α, β) measurements using O-DAM as described above. Percent errors over the time course of the simulated experiment were determined for each metabolite using equation (10).

For comparison with the O-DAM correction, we also simulated the conventional correction to partial saturation. We assumed an experimental flip angle of 60° throughout for this. A saturation factor was calculated from the ratio of observed magnetizations acquired with short TR, that is with the TR used for data acquisition throughout the experiment, and long TR during an initial control period. This SF was then used to correct for saturation effects throughout the intervention [7,10]. Explicitly, the SF measured (or calculated, in the case of our simulations) during the control period is:

$$\begin{aligned} SF(TR, \theta) &= M(TR, \theta) / M(TR = \infty, \theta) \\ &= M(TR, \theta) / M_0 \sin \theta \end{aligned} \quad (8)$$

Again, the effects of chemical exchange are included in the calculation of $M(TR, \theta)$ [6]. The corrected value for a measurement of the magnetization performed at time t , $M(t)$, is:

$$M(t)_{\text{conv}} = M(t) / SF(TR, \theta) \quad (9)$$

The error is calculated in the same fashion as for the O-DAM method:

$$\%error M(t)_{\text{conv}} = \frac{M(t)_{\text{conv}} - M_0(t) \sin \theta}{M_0(t) \sin \theta} \quad (10)$$

where $M_0(t)$ is the actual, known, input magnetization at time t . Again, this conventional correction is accurate only when chemical exchange is absent and T_1 remains constant.

Percent errors over the time course of the simulated experiment were determined for each metabolite using equations (9) and (10).

Simulation of O-DAM-based determination of [PCr] recovery time constant

The time constant of [PCr] recovery after ischaemic or hypoxic stress, τ_{PCr} , is regarded as an index of mitochondrial phosphorylation capacity [1,21]. τ_{PCr} is determined from a fit of the [PCr] recovery curve to

$$[PCr]_{\text{recovery}}(t) = C_o + C_1 \exp(-t/\tau_{PCr}) \quad (11)$$

Data were simulated using as input a recovery time constant of $\tau_{PCr} = 2.78 \text{ min}^{-1}$, determined from our experimental measurements. We calculated τ_{PCr} in two separate ways, first using the O-DAM correction for partial saturation of data acquired with alternating flip angles of $\alpha=15^\circ$ and $\beta=60^\circ$, and secondly with the constant flip angle experiment with the conventional correction for saturation. The simulated experimentally-determined recovery time constant was calculated by performing a least squares fit to Eq. (11) of the corrected data for the specified set of parameters and conditions across a range of TR values. Percent errors in the derived values of τ_{PCr} were calculated based on the difference between the derived values and the actual value of $\tau_{PCr} = 2.78 \text{ min}^{-1}$.

Results

Experimental results

Figure 1 shows typical spectra acquired during control perfusion, low-flow ischaemia and reperfusion with alternating flip angles of 15° and 60° . The resonances from PPA, Pi, PCr and the three phosphorus nuclei of ATP are indicated. All spectra were fit well using the AMARES procedure. Two distinct peaks of inorganic phosphate can be identified arising from the intra- and extra-cellular compartments. Only the signal from the intracellular compartment was considered in calculations.

Figure 2A shows the alternation in the directly measured signal intensities arising from the use of alternating flip angles of 15° and 60° . Figure 2B shows that as expected these alternations are eliminated in the corrected signal. Figure 2C demonstrates the failure of the dual-angle method to account for large changes in concentrations, as indicated by the discontinuity in the values for $T_{1,\text{eff}}(\text{PCr})$ obtained at the onset of ischaemia and reperfusion.

Figure 3A summarizes the changes in $T_{1,\text{eff}}$ throughout the experiment. Values were fairly well-maintained, with the largest change seen in $T_{1,\text{eff}}(\text{Pi})$, which exhibited a 70% increase during ischaemia. Figure 3B shows the variations in metabolite concentrations as determined by O-DAM correction of acquired data. While the ATP concentration was maintained to within approximately 20% throughout, there was a drop in PCr by a factor of nearly three, and a corresponding 5.5-fold increase in Pi concentration, during the ischaemic period. These latter two metabolites returned to baseline levels during the reperfusion period.

Simulation results: Accuracy of O-DAM as a function of the fractional change in PCr

The accuracy of the correction for partial saturation was calculated using input parameters appropriate for the perfused heart. Results are first presented describing the accuracy of the corrected metabolite concentrations as a function of f , the fractional decrease in PCr between the α and β periods. Figure 4 (A-C) shows results with $\alpha = 15^\circ$ and $\beta = 60^\circ$, and with TR =

0.25 s as used in the rat heart experiments presented here. Panels A, B, and C indicate percent errors in $M_0(PCr)$, $M_0(Pi)$, and the ratio $M_0(PCr)/M_0(Pi)$ (a measure of bioenergetic status), respectively. The error in PCr for the α period is much smaller than that for the β period (Fig. 4A), with the α period error remaining within 10% for fractional changes in PCr of up to 0.12, and within 20% for f up to 0.26. Fig. 4B shows that the errors in Pi during the α period are again smaller than those obtained during the β period, while Fig. 4C illustrates the errors in the ratio PCr/Pi. Clearly, the error for the β period becomes unacceptable even for relatively small values of f , while the error for the α period is much smaller, remaining within 10% for a fractional change of up to 0.05, and within 20% for f up to 0.12.

Figure 4 (D-F) shows results generated with the same parameters as in Fig. 4 (A-C), but with $TR = 2$ s. The error in PCr after correction for partial saturation is again much smaller for the α than for the β period (Fig. 4D), but the magnitudes of the errors are significantly lower than those for $TR = 0.25$ s. Indeed, for a fractional change of 0.5, the error is greater than 100% for corrected β period data but near 10% for the α period. Similar results are obtained for Pi (Fig. 4E); the error for the α period is within 10%, even for a fractional change of 0.5, while the error for the β period is 70%. Since the errors in PCr and Pi are of opposite sign, the errors in the ratio PCr/Pi are larger than those for the individual metabolites (Fig. 4F). During the α period, the error in this ratio remains within 10% for f up to 0.32, and remains less than 20% for f up to 0.5. The error in the ratio during the β period is much larger, reaching 600%, reflecting the larger magnitude of errors in the individual metabolites. Indeed, the error for the β period remains within 20% only as long as $f < 0.033$. Simulations (not shown) performed with the same parameters as for Fig. 4 (A-F) but with $TR = 1.0$, yield results which are very similar to those shown.

Extensive simulations have demonstrated that the magnitude of metabolite errors after correction for partial saturation is much smaller for the data collected with the smaller of the two flip angles used in the O-DAM measurements. As an example, Figure 4 (G-I) shows results using the same parameters as for Fig. 4 (D-F), but with $\alpha = 60^\circ$ and $\beta = 15^\circ$. As indicated, errors are much smaller during the β period, with β being the smaller flip angle in this case, than during the α period.

Table 1 shows the maximum allowable fractional decrease in PCr, f , which permits a specified degree of quantification accuracy after O-DAM correction using the same parameters as in the simulations described above. Only data from the smaller of the two flip angles is shown. A larger table entry value indicates an improved ability of O-DAM to accurately account for partial saturation; this ability evidently increases with TR . Again, in all cases correction for partial saturation is much more readily achieved for the data acquired for the smaller of the two angles used for the O-DAM measurement.

DAM-based correction during simulated cardiac ischaemia

A particularly convenient representation of the O-DAM results is shown in Figure 5. The metabolite time courses used as input to the simulations are indicated, as are the corresponding values after direct measurement and subsequent correction for partial saturation according to the conventional approach (Panel A) and the O-DAM approach (Panel B). The O-DAM correction generally yields improved accuracy as compared to the conventional correction. The resulting errors are shown quantitatively in Figure 6. As expected, the largest errors in O-DAM correction occur at the onset of ischaemia and reperfusion, where the rate of change of metabolite concentrations is the greatest. The conventional correction becomes worse throughout the ischemic period, as the system parameters progressively deviate from the control values used to calculate the saturation factor, and then improves throughout the reperfusion as the simulation parameters return to the control values. The result of this is that the conventional correction outperforms O-DAM

at the onset of ischaemia, but O-DAM is significantly more accurate as ischaemia progresses, at the onset of reperfusion, and during the reperfusion period. This is of particular significance since in many circumstances it is the recovery period that is of most interest in defining the biosynthetic capacity of tissue.

The recovery data shown in Figure 5 permits the calculation of the recovery time constant, τ_{PCr} , as indicated above. With recovery data simulated using $\tau_{PCr} = 2.78 \text{ min}^{-1}$, the value obtained from the conventional saturation correction was $\tau_{PCr} = 3.19 \text{ min}^{-1}$, representing a 14.7% error. The value obtained from the O-DAM experiment was $\tau_{PCr} = 2.83 \text{ min}^{-1}$, indicating an error of only 1.77%. The reliability of τ_{PCr} measurement as a function of TR is shown in Figure 7. The O-DAM correction is significantly better than the conventional correction for all TR's less than approximately 10 s; beyond this value, the errors in the conventional correction become negligible due to minimal saturation effects.

Discussion

Metabolite quantification is of great importance in ^{31}P studies and has formed the basis for numerous investigations of resting bioenergetic status and of the response to metabolic stress in cells, tissues, and organisms. The one-pulse experiment is widely used for such studies, and is most often performed under pulsing conditions in which one or more of the observed metabolites is partially saturated to improve SNR. The conventional correction for partial saturation relies upon a saturation factor measured during the control period of an intervention experiment. This saturation factor is then used to provide a correction for data collected even at times late in the protocol, resulting in potentially large errors as illustrated in Figures 5 and 6.

In contrast, the proposed O-DAM correction makes use of a $T_{1,\text{eff}}$ calculated locally in time, that is, directly from the data to be corrected. The experimental work presented here demonstrates the potential of the O-DAM technique for accurately quantifying metabolite concentrations in the setting of a perfused heart ischaemia-reperfusion study. However, the technique is generally applicable to any intervention experiment, including *in vivo* muscle exercise studies, and would be relatively simple to implement on clinical scanners with a multi-nuclear capability.

The simulation work presented has further demonstrated that the O-DAM technique provides a superior correction to conventional methods, except in situations of rapid concentration changes. These failures result from errors in the calculation of $T_{1,\text{eff}}$ due to the different concentrations of the metabolite during the α - and β -periods. Further, although the O-DAM scheme can be used to correct data acquired at both flip angles, we have also shown that correction for partial saturation was markedly more effective when applied to the data acquired for the smaller of the two angles due to the reduced saturation effects encountered with lower flip angle acquisitions.

As well as situations where metabolite concentrations change, the O-DAM correction scheme should help to reduce errors in the correction of partial saturation in the presence of changes in T_1 's and reaction rates. While there have been numerous studies of ^{31}P -containing metabolite concentrations during interventions, there have been relatively few studies of changes in T_1 's and k 's. A study of reaction rates in heart using saturation transfer found substantial decreases in the forward creatine kinase (CK) reaction rate and the corresponding overall CK flux in residual intact left ventricular tissue of the perfused rat heart after chronic infarction [17]. In the gastrocnemius muscle of the living rat, it was found that the T_1 's of ^{31}P -containing metabolites did not change with stimulation, while the rate of the forward CK reaction increased by over 50% [22], although overall flux through the CK

reaction was unchanged. In another study, human forearm exercise also resulted in no changes in the T_1 's of ^{31}P -containing metabolites, but up to a 50% increase in forward CK rate [23]. Using a 3-site exchange model applied to the rat heart, a nearly 100% increase in the pseudo-unimolecular rate of synthesis of γ -ATP from P_i was found to follow from an increase in cardiac rate-pressure product [19]. Newcomer [24] found changes in $T_1(\text{PCr})$, $T_1(\text{P}_i)$, and $T_1(\beta\text{-ATP})$ with exercise of the human gastrocnemius, and also provides additional references for T_1 and rate changes with muscle activation.

Method and study limitations

With the O-DAM procedure, two successive spectra, taken at different angles, are required to obtain a single concentration measurement; in effect, this decreases the temporal resolution of the experiment by a factor of two, with the offsetting advantage of substantially more accurate quantification. However, it would be possible to partly regain the lost temporal resolution by more sophisticated data analysis methods in the O-DAM scheme. Two such data analysis methods are:

Correction Scheme 1—each acquired spectrum is corrected by basing the effective T_1 calculation on the ratio of its own amplitude with the average of the spectra acquired either side of it. Thus, if the spectra are acquired as $(\dots, \alpha_{n-1}, \beta_{n-1}, \alpha_n, \beta_n, \alpha_{n+1}, \beta_{n+1}, \dots)$, the spectrum acquired with β_n would be corrected using an effective T_1 derived from the amplitudes β_n and $(\alpha_n + \alpha_{n+1})/2$.

Correction Scheme 2—each successive α, β or β, α pair is used to calculate an effective T_1 and to generate a concentration measurement which would have a temporal position between the two measurements. Thus, if the spectra are acquired as $(\dots, \alpha_{n-1}, \beta_{n-1}, \alpha_n, \beta_n, \alpha_{n+1}, \beta_{n+1}, \dots)$, an effective T_1 measurement derived from the spectra acquired with α_n and β_n would be used to calculate a corrected concentration value that lies temporally between α_n and β_n , as in the normal O-DAM scheme. However, the spectra acquired with β_n, α_{n+1} would be used to generate a second corrected concentration measurement with a temporal position halfway between β_n and α_{n+1} .

Either of these schemes would improve the temporal resolution of the generated data in that the acquisition of N spectra (half with flip angle α and half with flip angle β) would generate N distinct values of effective T_1 and N distinct concentration measurements. However, as each effective T_1 would no longer be fully independent of the neighbouring values, this return of full temporal resolution is provided at the expense of a degree of temporal smoothing in the data.

Another potential limitation of the O-DAM scheme is a reduction in achievable SNR that occurs due to the use of excitation flip angles which deviate from the optimum Ernst angle excitation. In their original manuscript on the dual angle method for T_1 measurement [13], Bottomley and Ouwerkerk point out that a dual angle measurement necessarily involves a compromise between achievable SNR and accuracy in the measurement of T_1 . Based on extensive simulations, they recommend acquisition with a flip angle pairing of 15° and 60° (as used here), as this provides 70-79% of the maximum achievable SNR and a nearly linear dependence of the signal ratio, R , on T_R/T_1 over the range $0.1 \leq T_R/T_1 \leq 1.0$. They also discuss in detail the propagation of errors in the T_1 measurement due to reduced SNR, RF field inhomogeneities and incorrect setting of the excitation flip angles. We refer the reader to this work for a detailed discussion of such errors [13].

For simplicity, in our simulations we have implicitly assumed that the changes in chemical-kinetic parameters occur instantaneously after the last pulse of the α period and before the first pulse of the β period. This assumption could be relaxed by defining the number of

averages at each flip angle and the functional form of the temporal dependence of changes to any of the parameters. The observed magnetization could then be calculated by solving the time-dependent Bloch-McConnell equations for each average at a particular flip angle. The values obtained from the Bloch-McConnell equations could then be summed appropriately for the α and β observation periods to give the observed magnetizations during those periods. Aside from the additional complexity of this average-by-average analysis in comparison to the steady-state analysis implemented above, the average-by-average analysis introduces two additional free parameters, namely, the number of averages and the (unknown) functional form of the temporal change in metabolite concentrations (or other parameters). In contrast, the approach taken here, based on observed steady-state amplitudes in a signal-averaged spectrum after repetitive pulsing [6], permits a substantially simpler analysis with fewer free parameters. The analysis incorporates what are in effect mean changes in all parameters over the relatively short time period of the acquisition of a single spectrum, so that the departures from the actual, temporally dependent, changes will be small.

Furthermore, our analysis also suggests that the improved accuracy of metabolite estimates leads to significant improvements in the accuracy of the calculated [PCr] recovery time constant across a wide range of TR values. As a result, application of O-DAM correction may provide significant advantages in the quantification and analysis of mitochondrial function in muscle and other tissues [1·25].

Summary

In conclusion, we have experimentally implemented and simulated an ongoing dual-angle correction for partial saturation of metabolite resonances in the isolated perfused rat heart. The analysis fully incorporated the effects of changing M_0 's, T_1 's, and reaction rates. We found that correction using O-DAM was clearly superior to that using the conventional approach, except at the onset of ischaemia. In addition, the improved quantification throughout the metabolic recovery period available through use of O-DAM leads to substantial improvement in the calculation of metabolite recovery time constants, a marker for mitochondrial function.

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Figure 1.
 ^{31}P NMR spectra of the isolated perfused rat heart obtained using a 500 MHz spectrometer. Data were acquired during baseline perfusion, low-flow ischaemia, and reperfusion.

Figure 2.

A: Directly observed values of the PCr signal intensity during the perfusion and ischaemia protocol. Variations in signal amplitude due to the use of two alternating flip angles are evident. **B:** PCr signal intensities corrected for partial saturation in accordance with the dual-angle procedure. Amplitude variations are no longer seen. **C:** Values of $T_{1,\text{eff}}$ (PCr) derived according to the dual-angle procedure. Values are largely consistent throughout the protocol except during periods of rapid metabolite concentration changes.

Figure 3.

A: Variations in the $T_{1,\text{eff}}$ of ATP, PCr, and Pi as determined by the dual-angle procedure described in the text. The most significant change seen was the increase in $T_1(\text{Pi})$ during low-flow ischaemia. **B:** Variations in metabolite concentrations as determined by O-DAM correction of the acquired data. The most significant changes were the decrease in PCr and the increase in Pi during low-flow ischaemia.

Figure 4.

Simulation results for the errors in $M_0(PCr)$, $M_0(Pi)$, and $M_0(PCr)/M_0(Pi)$ during both the α and β periods after correction according to O-DAM. A linear three-site model was used to describe the exchange among metabolites. For both the α period and the β period, $T_1(PCr) = 2.78$ s, $T_1(\gamma\text{-ATP}) = 0.64$ s, $T_1(Pi) = 2.4$ s, $k_{PCr \rightarrow \gamma\text{-ATP}} = 0.7$ s⁻¹, $k_{Pi \rightarrow \gamma\text{-ATP}} = 0.37$ s⁻¹, $M_0(PCr, \beta) = M_0(PCr, \alpha)(1 - f)$, $M_0(\gamma\text{-ATP}) = 4.3$, and $M_0(Pi, \beta) = M_0(Pi, \alpha) + fM_0(PCr, \alpha)$. For panels A-C, the experimental parameters were $\alpha = 15^\circ$, $\beta = 60^\circ$, TR = 0.25 sec, for panels D-F, the experimental parameters were $\alpha = 15^\circ$, $\beta = 60^\circ$, TR = 2.0 s and for panels G-I, the experimental parameters were $\alpha = 60^\circ$, $\beta = 15^\circ$, TR = 2.0 s.

Figure 5.

Simulation input and corrected metabolite concentrations representative of a perfused rat heart experiment with $TR = 1$ sec, and with changing T_1 's and exchange rates as described in the text. **A:** Input concentrations, and concentrations resulting from the conventional saturation factor correction. A flip angle of 60° was used. **B:** Input concentrations and concentrations resulting from O-DAM correction using flip angles $\alpha = 15^\circ$ and $\beta = 60^\circ$.

Figure 6.

Percent error in the corrected measurements of **A:** [PCr], **B:** [Pi] and **C:** [PCr]/[Pi] obtained from conventional and O-DAM corrections as shown in Fig. 7. The O-DAM procedure clearly leads to more accurate results than does the conventional correction, except at the onset of ischaemia.

Figure 7. Percent error in the estimated time constant τ_{PCr} of the [PCr] recovery curve as a function of TR. The time constant τ_{PC} is determined by fitting Eq. (11) to the corrected [PCr] values as determined from conventional and O-DAM corrections. The O-DAM procedure is clearly superior except as TR becomes sufficiently large that saturation effects are minimal.

Table 1

Maximum values of f , the fractional decline in PCr, for which quantification errors are within 10%, 20%, or 30% after correction for partial saturation in accordance with the dual-angle procedure. Simulation parameters are as specified in the text for a typical isolated perfused heart preparation. A larger value indicates that accuracy is maintained through a greater range of PCr decrease. In all cases data are shown based on the lower flip angle (15°), alpha period.

TR=0.25 s	PCr	Pi	PCr/Pi
10 %	0.12	0.15	0.05
20 %	0.26	> 0.50	0.12
30 %	0.35	> 0.50	0.20

TR=1.0 s	PCr	Pi	PCr/Pi
10 %	0.43	> 0.50	0.022
20 %	> 0.50	> 0.50	0.045
30 %	> 0.50	> 0.50	> 0.50

TR=2.0 s	PCr	Pi	PCr/Pi
10 %	> 0.50	> 0.50	0.32
20 %	> 0.50	> 0.50	> 0.50
30 %	> 0.50	> 0.50	> 0.50