Equilibrium Transcytolemmal Water Exchange Kinetics Depend on Yeast Cell ATP Level: Potentially High Spatiotemporal Resolution in Vivo MR Access to Cellular Energetics

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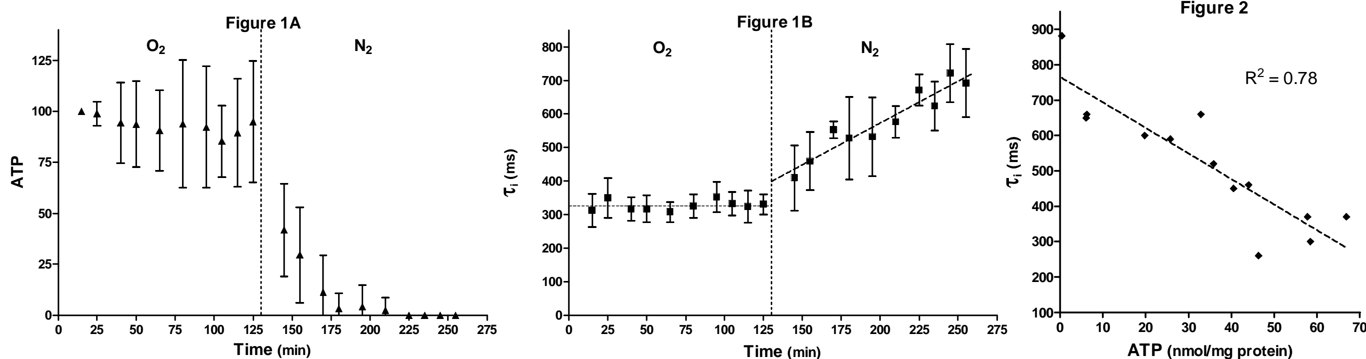
Introduction *In vivo* ^{31}P MR measurement of cellular energetics has suffered from poor spatial and/or temporal resolution. Because the *in vivo* $^{1}H_2O$ signal offers the greatest opportunities for high spatiotemporal resolution, a relation to cell metabolism could significantly improve this prospect. Intra- and extracellular water molecules undergo equilibrium exchange *via* mechanisms that include passive diffusion across the cytolemma and movement through membrane channels, like aquaporin. Longitudinal $^{1}H_2O$ MR relaxography can employ an extracellular relaxation agent, $GdDTPA^{2-}$, to distinguish intra- and extracellular $^{1}H_2O$ signals by creating a difference in their relaxation time constant (T_1) values [1-3]. Inverse Laplace transform of relaxation decay data produces the relaxogram, the relaxation time constant distribution. The *true* water mole fractions (p_i and p_e) - measures of the volume fractions v_i and v_e - can be determined if the equilibrium transcytolemmal water exchange kinetics (the mean intracellular water life time, τ_i) are quantified using two-site-exchange (2SX) analysis [3]. We test the hypothesis that τ_i correlates with cellular energetics in suspensions of yeast cells (*Saccharomyces cerevisiae*).

Methods MR measurements were acquired using a Varian Inova 9.4T spectrometer. $^{1}H_2O$ T_1 values were measured using an IR pulse sequence. $(M_0\text{-}M_z)/(2{*}M_0)$ decay data were analyzed with the 2SX analysis model [2] to extract τ_i and p_i. The ATP content was measured by ^{31}P MR and by anion exchange HPLC. Freshly isolated yeast cells (30% wt/vol) were bubbled with $95\%O_2/5\%CO_2$ (O_2, aerobic) or $95\%N_2/5\%CO_2$ (N_2, anaerobic) to alter cellular energetics and to keep the cells in suspension. Results are presented as mean (± SD) values.

Results During aerobic conditions (O_2), the cellular ATP level (Fig 1A) was high, and τ_i was 323 (± 18) ms, and constant for two hours (Fig 1B) (n=6). Switching to anaerobic conditions (N_2) resulted in a temporal ATP decline (to zero after ~50 minutes), and a linear τ_i increase - to a value of 677 (± 41) ms by ~150 minutes. In separate studies that included the MR6 yeast strain, which requires adenine supplementation for growth, ATP content could be controlled, and was varied with medium adenine concentration as well as by use of O_2 and N_2. These studies revealed that the cellular water mean lifetime τ_i was inversely linearly correlated with the cellular ATP content (Figure 2).



Conclusions: The results demonstrate that τ_i acts as a sensitive biomarker for the cellular energetic state. The latter catalyzes intra- extracellular water exchange kinetics. The mechanism(s) underlying this relationship are not yet clear but we suspect the exchange to involve one or more membrane proteins. Aquaporin is an unlikely candidate. Laboratory yeast does not express a functional aquaporin. Phenomenologically, we can imagine that the yeast cytolemmal water permeability coefficient, P_W, has an active component that is increased with cellular ATP. For the well-mixed 2SX model: $\tau_i^{-1} = P_W(A/V)$, the ratio (A/V) is that of the *individual* cell surface area and volume. The A/V could change with ATP level (say, by cell swelling or shrinking). We examined the ratio p_i/τ_i, which is proportional to P_WS, (the P_W (*total*) surface area product). We found p_i/τ_i to increase with ATP (p_i increased as ATP decreased). Since S will decrease as V (p_i) decreases (during high ATP), we conclude that P_W increases with increased ATP. The τ_i value can be determined from pharmacokinetic analyses of *in vivo* $^{1}H_2O$ DCE-MRI studies [3]. Thus, this may allow much higher resolution cellular energetics mapping than with *in vivo* ^{31}P MRI.

References:

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