Can you really use the creatine equilibrium to calculate free ADP concentrations?

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Introduction: Free [ADP] is considered to be an important regulator of cellular energy processes. Since decades it is common to calculate free cellular [ADP] in muscles and other tissues from the creatine kinase (CK) reaction by the equation: [ADP] = ([ATP]. [Cr]) /([PCr] K .H^+), which assumes that the reaction is at (near-) equilibrium[1]. The phosphocreatine (PCr) / ATP ratio and tissue pH are derived from ^{31}P MR spectra, the equilibrium constant K from *in vitro* experiments and creatine (Cr) from other assessments. As [ADP] calculated in this way is lower than [ADP] measured by biochemical means, it is considered to reflect true free cellular [ADP] which plays the key regulating role, while [ADP] measured biochemically also includes ADP, that is bound to other molecules. CK reaction rates can be assessed *in vivo* by ^{31}P saturation transfer (ST) by monitoring exchange between phosphate spins of γATP and PCr after saturation of the γATP spins[2]. As γATP and βADP spins resonate at nearly the same frequency βADP spins are cosaturated. This should have a substantial effect on the β-ATP signal according to the reverse CK reaction (ATP+Cr→ADP+PCr+H^+) and even more if the enzyme adenylate kinase (AK) contributes. ***Aim of this study:*** to evaluate the ADP→ATP exchange-mediated magnetisation transfer (MT) effect on the βATP signal in skeletal muscles using wild type (WT) mice and mice deficient in both CK and adenylate kinase (MAK$^{-/-}$) as model systems.

Materials and Methods: ^{31}P ST experiments on skeletal muscle were performed in order to determine the MT effect on β-ATP upon selective saturation of the γATP/βADP resonance. In addition the PCr signal was irradiated to obtain $k_{ADP,rev}$, the pseudo first order rate constant of the ATP→ADP β-phosphoryl exchange reaction. The ^{31}P MR experiments were carried out at 7.0 T, in a 120 mm horizontal bore, magnet (Magnex Scientific, Abingdon, UK) interfaced to an M.R.S. spectrometer (MR Solutions, Surrey, UK) operating at 121.53 MHz for ^{31}P. MR spectra were acquired from the hind leg of the mice with a 9 mm diameter solenoid coil (16). A low power continuous wave pulse was applied prior to the acquisition pulse to saturate signals for variable durations (*t*): 0.2-5.0 s. The spectra were acquired using 64 averages at a repetition time of 7 s. Signal integrals were fitted in the time domain with AMARES.

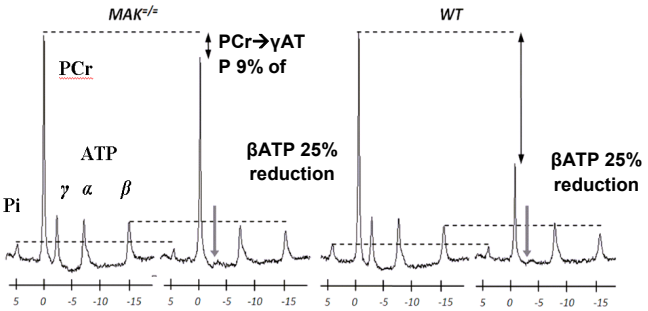

Fig. 1: ^{31}P MR spectra of skeletal muscle of MAK$^{-/-}$ and WT mice recorded in the presence and absence of a 5 sec saturation pulse at the γ-ATP(/β-ADP) resonances. *The second spectrum on the left hand side (MAK$^{-/-}$ mice) shows only a very small reduction in the PCr signal as a result of the saturation of the γ-ATP spins, while both groups show equal decreases in β-ATP signal.*

Results & Discussion: First we define the β-phosphoryl exchange by a simple two-site exchange system:

$$ATP_{free} \underset{k_{ADP,rev}}{\overset{k_{ADP,for}}{\rightleftharpoons}} ADP \qquad [1]$$

The steady state MT effect on the βATP the situation that ADP-ATP exchange occurs between the β-phosphoryls can be expressed as:

$$\frac{M_\beta(t) - M_\beta^0}{M_\beta^0} = -\frac{k_{ADP,rev}}{\rho_\beta + k_{ADP,rev}}\left(1 - e^{-(\rho_\beta + k_{ADP,rev})t}\right) \qquad [2]$$

In which $M_\beta(t)$ represents the z-magnetization of the β-ATP spin system. ρ_β (= 0.7 s^{-1}) is the auto-relaxation rate constant for ATP, assuming a 2-spin system including dipole-dipole relaxation and chemical shift anisotropy[3]. Although the ATP-ADP reaction is mediated by CK, AK, glycolytic enzymes and ATPases, for simplicity we only incorporated the MT effect arising from the (dominating) reverse CK reaction. The PCr resonance was selectively saturated with various saturation times. From the resulting exponential decay that was observed at γATP signal we obtained a value of 1.4 s^{-1} for $k_{ADP,rev}$, Using this value in eqn 2 we calculated the expected steady state MT effect on the βADP signal due to the βATP→βADP conversion, for the case that a long saturation pulse was applied at the (γATP/)βADP resonance. Based on these calculations, we expect a 65% reduction in the βATP signal upon saturation of the βADP spins. This at odds with the experimental results shown in fig. 1, the βATP signal is reduced by just ~ 25%. Importantly, the MT effect on the βATP resonance was similar in WT and MAK$^{-/-}$ mice, while the CK activity is severely reduced in MAK$^{-/-}$ (PCr→ATP flux is 9% compared to WT). We know that the observed MT effect on the βATP signal is the result of transferred NOE effects between near-neighbor ^{31}P nuclei of ATP [3]. However, the complete absence of a exchange mediated MT effect on the βATP can only be explained if the βADP system is present in a state in which it cannot be saturated, while still being involved in the CK reaction. We propose a model in which the CK reaction proceeds via transiently free ADP, which is drawn from a pool of bound ADP that cannot be saturated (see also fig. 2):

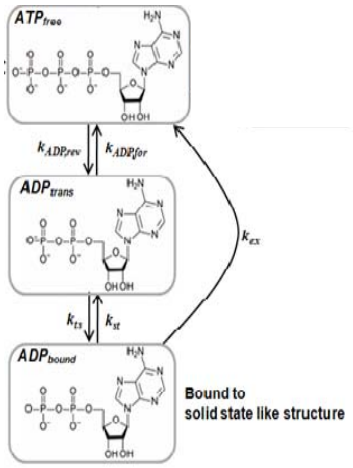

Fig. 2: Scheme of the phosphoryl exchange reactions in which cytosolic ATP and ADP are involved. *The ATP↔ADP conversion proceeds via transiently free ADP. The rate constant k_{ex} does not represent a separate path but is a combination of the rate constants in the individual reaction steps (eqn 4).*

$$ADP_{bound} \underset{k_{ts}}{\overset{k_{st}}{\rightleftharpoons}} ADP_{trans} \quad \text{and} \quad ADP_{trans} + CK \underset{k_{ADP-CK,rev}}{\overset{k_{ADP-CK,for}}{\rightleftharpoons}} ATP \qquad [3]$$

Here,k_{st} and and k_{ts} characterize the transitions between the bound and the transiently free ADP. For this situation the overall rate constant for the conversion of ADP_{bound} to *ATP* is:

$$k_{ex} = \frac{k_{st} k_{ADP-CK,for}[CK]}{k_{ts} + k_{ADP-CK,for}[CK]} \qquad [4]$$

Suppose we consider the limit that $k_{ADP\text{-}CK,for}[CK] << k_{ts}$, then the ADP to ATP transfer rate is determined by a rapid pre-equilibrium, in which ADP exchanges many times between free and bound form before it binds to CK. It is inherent here that saturation of the population of bound ADP is not possible. This condition applies, if the ADP signal is inhomogeneously broadened, e.g. when ADP is bound to a solid-state-like lattice. Due to the anisotropy of the chemical shift resonances are then spread over a region of about 180 ppm and only a negligible part of the bound population is saturated. Relatively rigid cellular structures with ADP binding capacity are components of the cell cytoskeleton, e.g. actin filaments. It is important to note that as a consequence of the inability to saturate the free ADP pool, the widely used calculations of free [ADP] from the CK equilibrium are not applicable.

Conclusion: ^{31}P saturation transfer experiments in MAK$^{-/-}$ and WT muscle demonstrated a neglegible effect of CK and AK mediated enzymatic phosphoryl exchanges between β-ADP and β-ATP. The inability to saturate the βADP spin system casts doubt on the validity of calculating the free ADP concentration from the CK equilibrium.

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References: *[1] Gadian et al. The Biochemical J 1981, [2] Brindle Prog NMR Spectroscopy 1988, [3] Nabuurs et al. #139 ISMRM2009*