In vivo cross-relaxation in ATP in skeletal muscle measured by ^{31}P saturation transfer MRS

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Introduction Saturation Transfer (ST) is frequently applied in high resolution NMR to determine intramolecular spin-spin distances based on cross-relaxation. In contrast, *in vivo* this technique has mainly been used for the assessment of fluxes through multiple enzymatic exchange reactions involving the transfer of phosphates *[1,2]* (fig. 1). Saturation of the γATP/βADP signal in 31P MR spectra of brain and muscle results in an effect on the β-ATP resonance, which could be due to both ATP↔ADP fluxes or ^{31}P-^{31}P cross-relaxation *[3,4]*.

Aim: to differentiate among these potential causes by applying saturation at all three ATP signals in mice with deficiencies for muscle specific cytosolic CK and AK (MAK$^{-/-}$). With this knockout it is possible to resolve individual contributions of multiple exchange reactions and cross-relaxation processes to β-ATP signal decreases in muscle. Moreover, we examined the potential contribution of ^{31}P-^{31}P cross-relaxation processes and transferred Nuclear Overhauser effects (trNOE) of a bound ATP fraction by theoretical simulations.

Fig.1. Upon saturation of the γ-ATP, phosphocreatine (PCr) signals decrease due to creatine kinase (CK) activity, whereas Pi signal decreases are induced by glycolytic and mitochondrial ATP production. Decreases in β-ATP signal have been ascribed to β-ATP→β-ADP chemical exchange catalyzed by CK, adenlyate kinase (AK), glycolytic enzymes and mitochondrial ATPase activity (due to cosaturation of γ-ATP/β-ADP) or ^{31}P-^{31}P cross-relaxation [1-3].

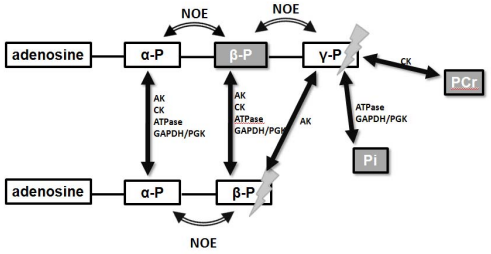
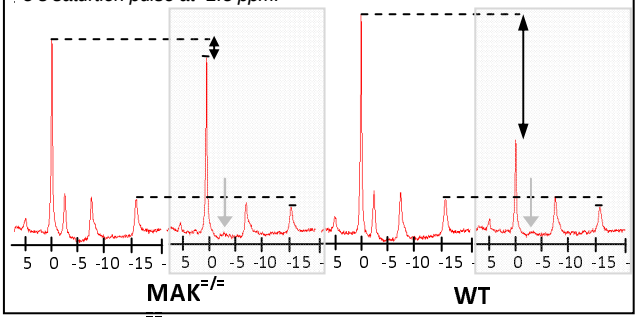
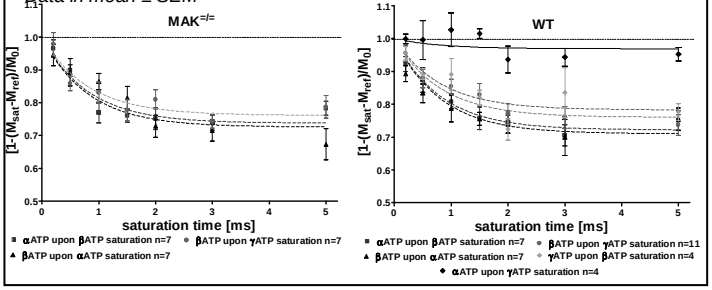


Fig. 2. ^{31}P spectra of MAK and wt mice without (left) and with (left) a 5 s saturtion pulse at -2.5 ppm.



Materials and Methods ^{31}P ST measurements on hind limb of MAK$^{-/-}$ and wild type mice were performed at 7T by selective saturation at the γ-ATP/β-ADP resonance (t^{sat} =0.2-5 s, TR=6.7 s, nsa=64). Signals were fitted with AMARES, corrected for off-resonance saturation and normalized to signals without irradiation. Decreases in PCr, Pi and β-ATP signals were fitted to a mono-exponential function to determine the pseudo-first-order unidirectional rate constants *(k)* of the chemical exchange reactions and fluxes of PCr→ATP, Pi→ATP and ATP→ADP, respectively*[1,2]*. Potential cross-relaxation processes were examined by comparing MT effects between β-α , β-γ and γ-α ATP signals. Simulations were performed in MATLAB based on the Solomon equations for relaxation processes in a 2-spin system that include auto-relaxation (ρ), dipolar relaxation (σ) and chemical shift anisotropy (R_{CSA}). For free ATP a rotation correlation rate constant (τ_c) of 0.3 ns was used *[5]*. Potential presence of trNOE was investigated according to the equations given by Clore and Gronenborn *[6]*. We assumed that free ATP is in fast chemical exchange with a bound ATP fraction accounting of ≤5%. For the bound ATP τ_c varied between 35 and 300ns *[7]*. Other used parameter values: the distance between two neighbouring ^{31}P spins r = 3e^{-10} *[8]*.

Fig.3. Saturation transfer effects between ^{31}P spins within ATP. ST effects between α-β and β-γ showed similar mono-exponential curves for both WT and MAK$^{-/-}$mice, whereas ST effects between α-γ (presented by x) were minimal. Data in mean ± SEM



Results Upon γ-ATP saturation, the PCr→ATP flux in skeletal muscle of MAK$^{-/-}$ (0.84±0.24mM/s) was only 8% as compared to that of wt (10.2±1.5mM/s), whereas decreases in β-ATP were equal both groups (fig. 2.), as well as Pi→ATP fluxes (MAK$^{-/-}$ 0.47±0.18mM/s, wt 0.64±0.13mM/s). ST effects between α-β and β-γ showed similar mono-exponential curves with σ ranging from 0.28 to 0.34 and SD of <0.04s^{-1} for both groups. In contrast, ST effects between α-γ were hardly present (Fig.3 only measured in WT). The calculated constants for ρ, σ and R_{CSA} (table 1), show that cross-relaxation in free ATP is negligible, but binding of ATP to a large immobile molecule can result in large negative NOE in steady state saturation (σ/(ρ+R_{CSA})).

*Table 1: Calculated constants for auto- relaxation , cross-relaxation and CSA relaxation for ATP in a free and bound state and their corresponding steady state MT effect (**σ / (ρ+R_{CSA})** in a steady state saturation experiment.*

	τ_c	ρ	σ	R_{CSA}	σ / (ρ+R_{CSA})
Free ATP	0.3 ns	0.0056 s^{-1}	0.0025 s^{-1}	0.72 s^{-1}	0.0035
Bound ATP	35 ns	0.074 s^{-1}	-0.073 s^{-1}	0.12 s^{-1}	-0.37
Bound ATP	300 ns	0.632 s^{-1}	-0.632 s^{-1}	0.014 s^{-1}	-0.98

Discussion Our results demonstrate that the highly reduced CK (8%) and AK (1-2%) *[9]* activities in skeletal muscles of MAK$^{-/-}$ mice do not affect the decrease in β-ATP signal. Thus neither of these 2 enzyme activities are significantly contributing *[5]*. Major adaptations in glycolytic and mitochondrial ATP production are excluded since Pi→ATP fluxes in MAK and wt were equal. Also the reverse flux (ADP+Pi→ATP) through glycolytic and mitochondrial ATP synthesis is not contributing significantly in β-ATP signal intensity reductions as it would be too slow. Thus our experiments show that all proposed chemical exchange reactions are not likely to cause a β-ATP signal decrease. Moreover, the strikingly similar ST effects between neighboring ATP phosphoryls and absence of such an effect between γ-α ATP suggest that cross-relaxation must be the predominant cause for the β-ATP reduction in muscle. However, the simulations showed that for free ATP in solution cross-relaxation processes between ^{31}P spin systems are too slow to explain these MT effects between neighboring ATP phosphor spins. On the other hand, our simulations show that the saturated magnetization can be transferred from γATP to βATP in free ATP via an enzyme complex formation in fast chemical exchange. The measured 31P spin interaction thus may be used to asses cellular ATP interactions.

References*[1] Brindle KM. Prog NMR spectroscopy 1988; [2] Ugurbil, JMR 198;[3] LeRumeur et al. NMR in Biomed 1997; [4] Du et al, PROG ISMRM 2008; [5] Landy et al. Eur J Biochem 1992; [6]Jarori et al. Eur J Biochem 1995; [7] Clore &Gronenborn,JMR 1982; [8] Potrzebowski et al. 200; [9] Janssen J Biol Chem 2003.*