Do adenylate and creatine kinase activity contribute to saturation transfer effects among ADP and ATP resonances in 31P MRS of skeletal muscle?

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Introduction Saturation Transfer (ST) approaches based on *in vivo* ^{31}P MR spectroscopy are used for a quantitative assessment of fluxes through several enzymatic reactions involved in the transfer of high energy phosphates, i.e. ATPsynthase/ATPase, creatine kinase (CK) and adenylate kinase (AK)*[1,2].* The determination of the CK flux upon saturation of the γ-ATP and PCr resonances is well established. The ST effect commonly seen on the β-ATP resonance after saturating the γ-ATP/β-ADP peak however has been ascribed to several enzymatic reactions i.e. CK, AK or ATPsynthase/ATPase *[2,3]* catalyzing ATP↔ADP exchange. In principle, the β-ATP reduction could also be due to a Nuclear Overhauser effect (NOE) *[4].* Recently we have generated mice that lack both cytosolic CK and AK (MAK$^{-/-}$) *[6].* Hence, these mice are ideal to investigate whether the CK and AK activities significantly contribute to the ST effect seen on the β-ATP resonance.

Materials and Methods Transgenic MAK$^{-/-}$ mice (n=4) were compared with age matched control littermates (wt, n=4). The mice were anesthetized with 1.2 - 1.5% isoflurane and body temperature was maintained by a warm water blanket during the experiments. MRS measurements were performed on a 7T, horizontal bore magnet. An Alderman-Grant ^{1}H coil was used for localisation and shimming, a three turn solenoid coil enabled ^{31}P MRS measurements. A selective saturation pulse at the γ-ATP/β-ADP resonance was applied for 0, 0.4, 1.2, 3.2 and 5 s prior to acquisition (TR=6750ms, 64 averages). Subtraction the ST spectra from reference spectra obtained with a similar saturation pulse mirrored around the β-ATP enabled correction for off-resonance saturation. Signals were fitted with AMARES using jMRUI (Gaussian line shapes, fixed first order phase, line width Pi = 1.5 times PCr) and normalized to signals without irradiation. After subtraction of the control spectra, β-ATP signal intensities were fitted to a mono-exponential function *[1,5]* using a least squares method *[5]* to determine the intrinsic spin-lattice relaxation time (T_1) of β-ATP and the pseudo-first-order unidirectional rate constant (k) for β-ADP synthesis (sum of rate constants of all enzymatic reactions and/or negative NOE to produce an effect on β-ATP) upon saturation of the γ-ATP resonance. An F-test was used to test if k values were statistically different ($p<0.05$).

Results Both the MR spectra of the MAK$^{-/-}$ and their controls showed similar decreases in β-ATP in skeletal muscle upon saturation of γ-ATP/β-ADP (Fig 1 a+b). Quantitative analysis of the spectra showed no statistical difference in k between MAK$^{-/-}$ (0.4 ± 0.2 s^{-1}) and their controls (0.3 ± 0.1 s^{-1}) (Fig 1 c). Although a larger effect was seen in wt, also MAK$^{-/-}$ mice showed a reduced PCr signal after γ-ATP/β-ADP saturation, which is completely due to an off resonance saturation effect.

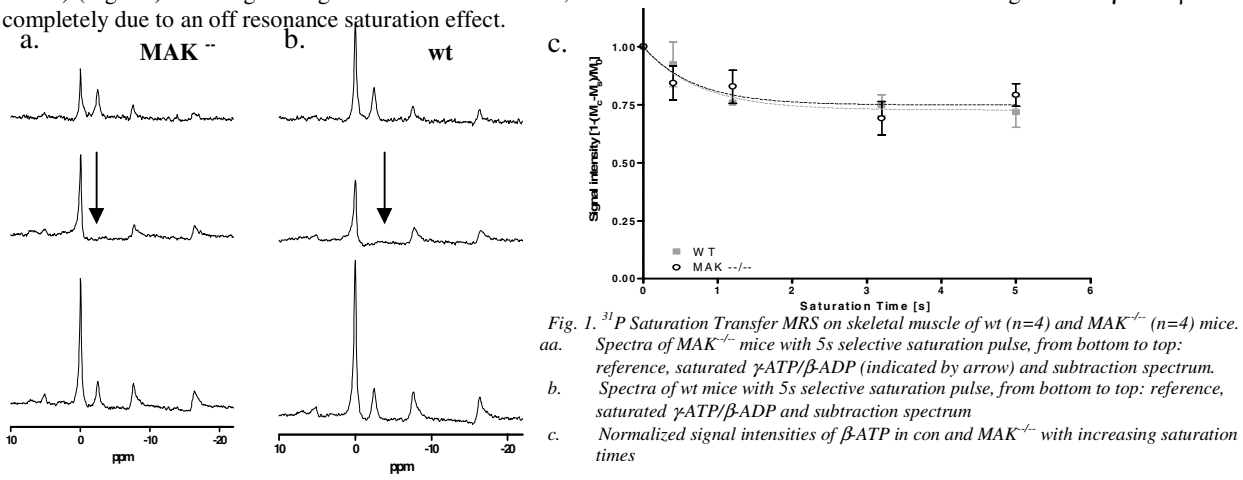

Fig. 1. ^{31}P Saturation Transfer MRS on skeletal muscle of wt (n=4) and MAK$^{-/-}$ (n=4) mice.
aa. Spectra of MAK$^{-/-}$ mice with 5s selective saturation pulse, from bottom to top: reference, saturated γ-ATP/β-ADP (indicated by arrow) and subtraction spectrum.
b. Spectra of wt mice with 5s selective saturation pulse, from bottom to top: reference, saturated γ-ATP/β-ADP and subtraction spectrum
c. Normalized signal intensities of β-ATP in con and MAK$^{-/-}$ with increasing saturation times

Discussion Our results show a similar k in both MAK$^{-/-}$ and wt mice. As skeletal muscles of MAK$^{-/-}$ mice have only about 8% of the CK activity remaining and β-ATP turnover due tot AK has decreased to 15% *[6],* it is very unlikely that the reduction is the result of AK and CK. This is supported by previous studies where no ST effect on the PCr resonance was observed after selective saturation of γ-ATP *[7,8].* In wt mice, β-ATP signal reduction could also result from additional exchanges of β-ADP↔β-ATP due to CK activity or ATPsynthase rates. However, the small ADP concentration (~0.04mM *[9]*) and CK rate (0.39 s^{-1}*[5]*) will only result in a maximal reduction of ~1% β-ATP signal after 5 seconds of β-ADP saturation. A final enzymatic reaction that could explain a decrease in β-ATP in both MAK$^{-/-}$ and wt is ATPsynthase activity. However, its rate (<0.004s^{-1}*[5]*) is so small in muscle during resting conditions that the resulting < 1% reduction in β-ATP is also beyond detection. Although validation of the actual contribution of ATPsynthase activity in the MAK$^{-/-}$ has to be assessed, we conclude here that all known enzymatic exchanges of high phosphor compounds in MAK$^{-/-}$ are too low too be visible using ^{31}P ST MRS methods. Our value for the overall k is in well agreement with that of resting rat muscle *[2],* where an important role for AK and ATPase/ATPsynthase in addition to CK is assumed. However, our study indicates that these activities cannot contribute significantly to the β-ATP decrease.

Conclusion In this study we demonstrate that AK and CK have a negligible contribution to the decrease in β-ATP upon γ-ATP saturation using ST methods in ^{31}P MRS. This suggests an important role for negative NOE in the reduction of the β-ATP signal.

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