Determination of ATP synthesis exchange rates in human liver and skeletal muscle using ^{31}P magnetization transfer

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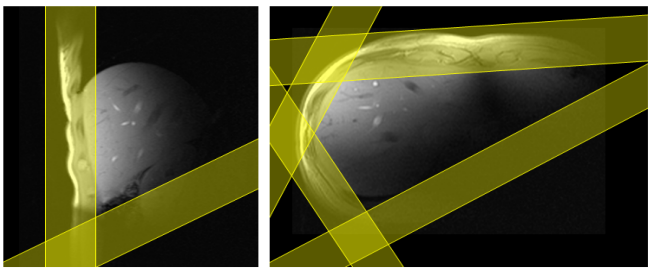
Introduction: ^{31}P-MRS saturation transfer (ST) and inversion transfer (IT) allow to follow spin exchange in moderately rapid biochemical reactions and to determine exchange rate constants and reaction fluxes. Particularly interesting are reactions in mitochondria, which could lead to a better understanding of insulin-resistance and ageing [1]. While magnetization transfer experiments have already been introduced a few decades ago, applications in human and in particular in moving organs still need considerable development [2]. We evaluated ST and IT experiments on a 3 T MR system in skeletal muscle and liver for a non-invasive characterization of mitochondrial activity measured by the forward exchange rate constant *k* of the ATP synthesis (ADP + Pi → ATP). While ST vs. IT in skeletal muscle allowed a comparison with data obtained in a static organ without volume selection, the measurements in liver had to be developed for reliable volume selection and motion-insensitivity.



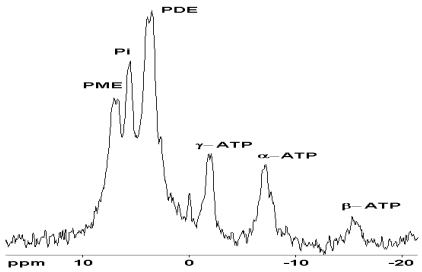


Fig. 1: a) Sagittal and axial localizer images of the liver with three regional saturation bands for the abdominal wall muscles and one for the bowel.
b) Liver spectrum with negligible PCr-signal at 0 ppm (TR = 5s, 48 averages, apodization 15Hz). The β-ATP signal is reduced due to the limited bandwidth of the 90° adiabatic excitation pulse.

Methods: 10 healthy young volunteers were measured twice in a saturation transfer [3] and inversion transfer [4] experiment in the thigh muscle or the liver. The experiments took 1 – 1.5h each and were done on a 3 T MR system (SIEMENS TRIO) using a ^{1}H/^{31}P flexible surface coil (RAPID Biomedical GmbH). The product pulse sequence was extended with 90° adiabatic excitation pulses (2.56ms), selective and non-selective inversion pulses (100ms and 10ms), regional saturation bands, and long selective saturation pulses (T_{sat}). The volume selection was achieved by the surface coil excitation and additional regional saturation bands to suppress the signals from muscles of the abdominal wall. In both experiments the magnetization of γ-ATP is transferred to Pi, which allows the calculation of the ATP synthesis exchange rate constant *k* from the signal change of Pi and/or γ-ATP:

ST: selective saturation (1) of γ-ATP (~-100Hz) or (2) at mirror frequency (~630Hz), (3) T_1 of Pi under selective saturation of γ-ATP
Muscle: (1), (2) TR=15s, avg 32, T_{sat}=13.9s, T_{presat}=0.1s (3) TR=15s, avg 8, TI_{sat}=13 times between 0.1-13.9s (total saturation of 14s),
Liver: (1), (2) TR=8s, avg 128, T_{sat}=7s, (3) TR=5s, avg 48, TI_{sat}= 10 times between 0-4.2s (total saturation of 4.2s),

IT: selective inversion of γ-ATP with 14 different inversion times (TI)
Muscle: TR=15s, avg 16, 15 TI times between 0-12s, Liver: TR=5s, avg 48, 14 TI times between 0-4.5s

Quantitation: For data fitting the JMRUI software with the AMARES algorithm was used. The kinetic parameters in the IT experiment (*k* forward/backward, T_1 of γ-ATP and Pi) are determined by least square curve fitting of the signals γ-ATP and Pi, which are described by the Bloch-McConnell equations that include terms describing the biochemical exchange.

Results: The volume selection of the liver was successful with only a small contamination by PCr that originates from muscles in the abdominal wall. The average exchange rate constant *k* in the thigh muscle is 0.082±0.007s^{-1} (ST) and 0.069±0.009s^{-1} (IT), resp. 0.30±0.06s^{-1} (ST) and 0.36±0.07s^{-1} (IT) in the liver. Bland-Altman analysis showed no significant difference between ST and IT measurements in muscle and liver (intra-subject variability: coefficient of variance (CV) of 37% in muscle and 27% in liver). The comparison between subjects resulted in a CV of 19% (ST) and 30% (IT) for muscle and 42% (ST) and 40% (IT) for liver (inter-subject variability).

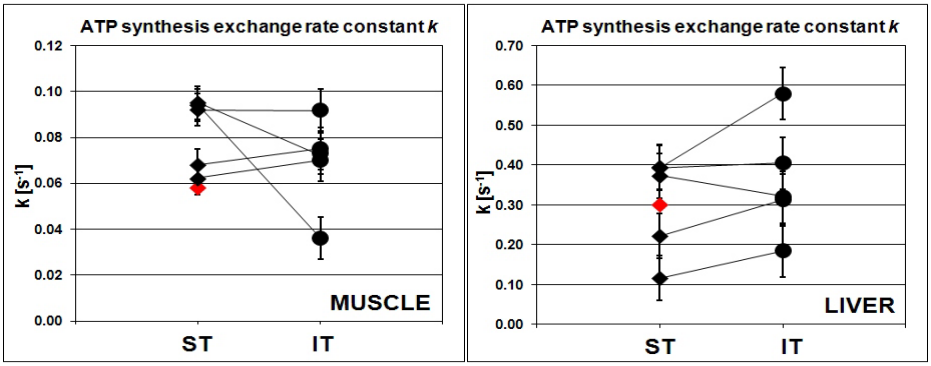


Fig. 2: ATP synthesis exchange rate constant *k* measured with the saturation (ST) and inversion (IT) transfer experiment in a) human thigh muscle (n=5, two male, three female) and b) human liver (n=5, two male, three female). The values in red are literature values [2, 5].

Conclusions: Despite the challenges of volume selection and motion of the organ, our implementation of IT and ST in the liver was reliable, with reasonable signal-to-noise, and with only little contamination from abdominal wall muscles. The agreement between IT and ST was even better in the liver than in skeletal muscle, may be due to the larger physiological range in the liver. Further experiments are needed to determine the reproducibility of the methods.

References: [1] K. F. Petersen et al. N.Engl.J.Med. 2004, 350:664 [2] A. I. Schmid et al. NMR Biomed. 2008, 21:437 [3] S. Forsen et al. J.Chem.Phys. 1963,39:2892 [4] T. R. Brown et al. Proc.Natl.Acad.Sci. USA 1977, 74(12):5551 [5] D.E.Befroy et al. ISMRM 2008, 16:2565

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