Direct comparison of parameters of skeletal muscle energy metabolism

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Introduction

Skeletal muscle is the main contributor to energy expenditure. It is generally agreed that ATP production is demand-driven. In PCr recovery, after exercise or ischemia, it is assumed that PCr kinetics reflects ATP producing and measures mitochondrial capacity. Saturation transfer (ST) measurements determine synthesis rates of ATP in the resting state and give information about mitochondrial activity. Furthermore, ischemia-induced PCr decrease permits another independent measure of resting state ATP turnover.

Methods

Eight non-obese male volunteers (age 29.8±7.7 a, BMI 22.2±1.3 kg/m^2) were studied on a 3-T MR scanner (Bruker, Ettlingen, Germany) using a 10-cm ^{1}H/^{31}P surface coil positioned below the subjects' calf. The sensitive volume of the coil covered the gastrocnemius and soleus muscles.

The experimental Protocol consisted of three blocks: ST, ischemia–recovery, ST (Figure 1). **Saturation Transfer Experiment:** To measure the chemical exchange between Pi and ATP (T_R=15 s, pulse-acquire, 10 kHz bandwidth/200 ms acquisition), the γ-ATP resonance was saturated (continuous irradiation) and compared to a control experiment (Figure 2A), mirroring the saturation frequency about Pi and the apparent T_1 while saturating γ-ATP (measured by inversion recovery, adiabatic inversion, 8 inversion times between 0.5 s and 7 s). Spectra were processed using the XWIN-NMR software (Bruker).

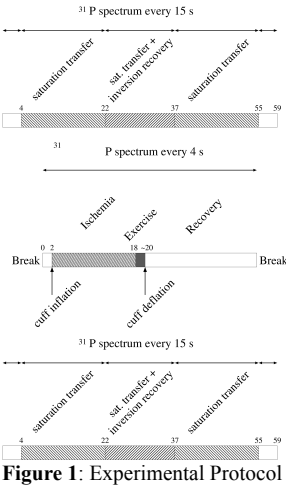

Figure 1: Experimental Protocol

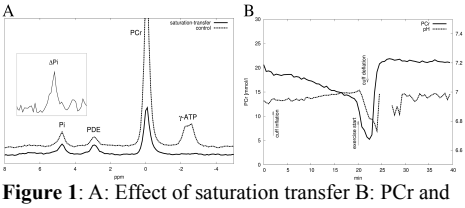

Figure 1: A: Effect of saturation transfer B: PCr and pH in ischemia-reperfusion

Ischemia – recovery experiment: ^{31}P spectra were acquired every 4 s (otherwise identical to the baseline scans of the ST blcok. After two minutes, the cuff was inflated to 200 mmHg and remained so for about twenty minutes. 18 min within ischemia, subjects were instructed to do plantar flexions on an exercise rig every 4 s until exhaustion. PCr and Pi resonances as well as pH were quantified using AMARES (jMRUI) and plotted in Figure 2B. PCr recovery was modeled as an exponential function using a non-linear least square fit (perl/PDL http://pdl.perl.org), yielding the recovery time-constant τ_{PCr}. The maximal oxidative capacity Q_{max} was derived from the linear model ($Q_{max,lin}$=$PCr_{end\text{-}ex}$./τ_{PCr}) (Meyer et al Am J Physiol 1988) and the ADP based model ($Q_{max,ADP}$=$\Delta PCr_{end\text{-}ex}$./$\tau_{PCr}$[1+$K_m$/ADP]) (Kemp et al. NMR Biomed 1993).

Net aerobic ATP syntheis rate: In ischemia, the rate of PCr breakdown represents net aerobic ATP turnover Q = δPCr/δt. Glycolytic ATP turnover (using pH) L=3/2[φ(δPCr/δt)-β(δpH/δt)]. The calculations were performed assuming β=30 and φ=0.36. The total resting net ATP synthesis rate F.=Q+L

Table 1: Results of the saturation transfer and ischemia-exercise-recovery experiments.

	Mean ± SD
Resting state (mean of pre- and post-ischemic values	
f (Pi → ATP) [mM/s]	0.212±0.037
PCr [mM]	25.15±4.36
Pi/PCr	0.155±0.033
Ischemic resting state	
Q=dPCr/dt [mM/s]	0.0079 ± 0.0015
glycolytic ATP production L [mM/s]	0.0010 ± 0.0005
Recovery	
PCr recovery time (τ_{PCr}) [s]	34.7 ± 7.0
End-exercise pH	6.97 ± 0.09
Exercise-induced ΔPCr [mM]	13.61 ± 4.23
$Q_{max,ADP}$ [mM/s]	0.57 ± 0.18
$Q_{max,lin}$ [mM/s]	0.77 ± 0.22

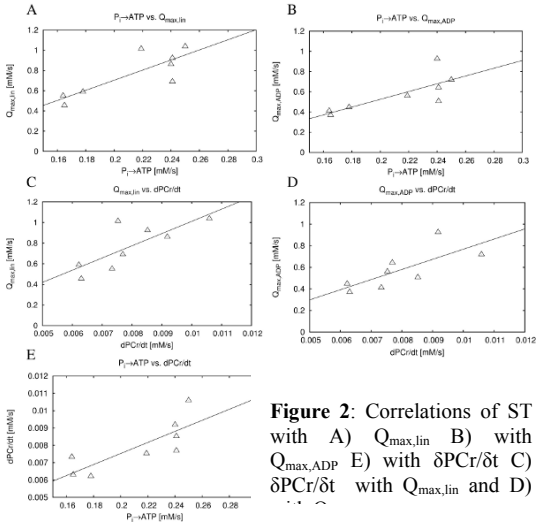

Figure 2: Correlations of ST with A) $Q_{max,lin}$ B) with $Q_{max,ADP}$ E) with δPCr/δt C) δPCr/δt with $Q_{max,lin}$ and D)

Results

Saturation transfer: ATP synthesis rates were 0.20±0.04 mM/s before ischemic exercise, 0.22±0.06 mM/s, afterwards. All metabolite concentrations were similar before and after ischemia exercise protocol, only Pi/PCr was higher afterwards (p=0.037). PCr decrease during ischemia started around 8 min after cuff inflation to decrease to 18.6±3.3 mM. Aerobic net ATP production Q=0.0079±0.0015 mM/s, F=0.0089±0.0017 mM/s, when taking glycolysis (L) into account (Table 1).

Correlations: Rates of ST significantly correlated to τ_{PCr} (P =-0.85, p=0.007), $Q_{max,lin}$ (P=0.83, p=0.01) and $Q_{max,\ ADP}$ (P=0.77, p=0.02), also to Q (P=0.80, p=0.02) and F (P=0.79, p=0.02). Further, parameters of PCr recovery (τ_{PCr}, $Q_{max\text{-}lin}$ and $Q_{max\text{-}ADP}$) correlated to Q (P=0.70, p=0.05; P=0.78, p=0.02 and P=0.76, p=0.03), respectively.

Discussion

This work clearly shows that all three parameters of ATP synthesis correlate very well (Figure 3), indicating good qualitative correspondence between the methods. Absolute values, however, differed significantly. In conclusion, (i) mitochondrial capacity, as determined by PCr recovery kinetics, correlates well with mitochondrial activity as measured by PCr depletion and by ST (ii) short-term ischemic exercise does not influence resting state ATP synthesis and (iii) ATP synthesis measured by saturation transfer may overestimate true values, but still exhibits a strong correlation with net resting aerobic ATP turnover.

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