Estimates of mitochondrial capacity derived from phosphocreatine recovery kinetics in human calf and thigh muscle differ systematically from published measurements using invasive methods

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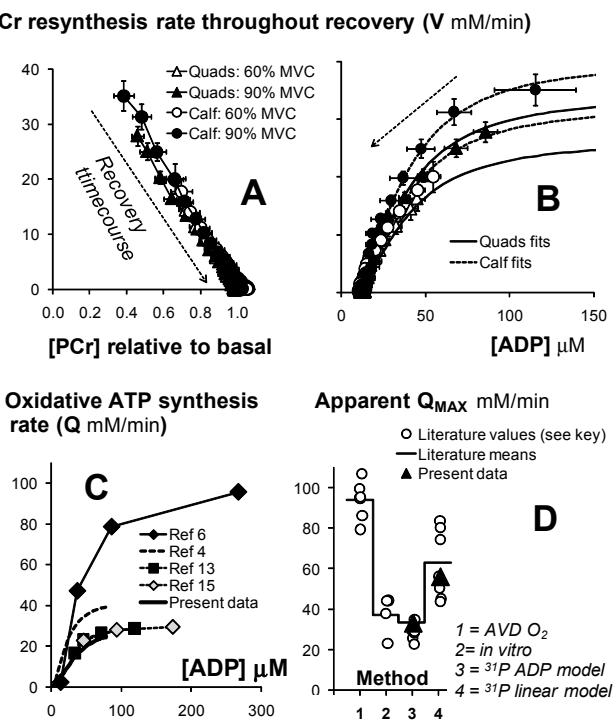
Introduction. Analysis using ^{31}P MRS of the kinetics of phosphocreatine (PCr) recovery provides information about muscle mitochondrial function *in vivo* hard to obtain any other way. In analyses of PCr recovery as a linear system (*1*) its rate constant k_{PCr} is approximately proportional to 'mitochondrial capacity' (Q_{MAX}, a function of mitochondrial numbers, function and substrate/O_2 supply) (*2*), but the argument depends on extrapolation to 'complete' PCr depletion, which is likely to be complicated by e.g. glycolytic pH change and vascular O_2 limitations. Another approach is based on the roughly hyperbolic relationship of oxidative ATP synthesis rate (Q, ≈ PCr resynthesis rate, V) to free [ADP] (calculated from pH and [PCr] assuming the creatine kinase equilibrium), resembling that obtainable with mitochondria *in vitro*, consistent with [ADP] as a feedback signal matching ATP supply to demand: $Q_{MAX} \approx V$ extrapolated to 'infinite' [ADP] (*2*). Such inference depends on the shape of the V-[ADP] relationship, of which dynamic range considerations require a degree of cooperativity (Hill coefficient $n_H \approx 2$) (*3*), consistent with results of detailed kinetic simulation (*4*). Variability of these relationships with e.g. muscle, exercise mode and intensity, and their relations to other mitochondrial measures, are relatively unexplored. Here we compare the V-[ADP] relationship and apparent Q_{MAX} in two muscles, quadriceps (vastus lateralis) and calf (gastrocnemius/soleus), at two exercise intensities, and with estimates of Q_{MAX} inferred from some published measurements.

Methods. 11 healthy subjects aged 20-26 years were studied in a 3T Trio scanner (Siemens, Germany) using a 18 cm dual-tuned surface coil (Rapid Biomedical, Germany) and purpose-built rigs for isometric plantar flexion and knee extension exercise, performed on separate occasions. After resting acquisition (TR = 10 s), data were acquired with TR=2 s during 1 min rest, 3 min 60% maximal voluntary contraction force (MVC) exercise (0.25 Hz, 50% duty cycle), 5 min recovery, 2 min 90% MVC exercise and 5 min recovery. Data were quantified by jMRUI-3.0 and analysed by monoexponential PCr recovery fit, then analysing the V-[ADP] relationship throughout recovery, constraining $n_H = 2$ (*4*) and Km to be identical for both exercise intensities. Comparative estimates of Q_{MAX} for quadriceps were obtained, recalculated or inferred from published O_2 consumption measured by arteriovenous difference (AVD) during knee extension (*5-12*), ^{31}P MRS studies (*13-19*) and studies in which mitochondrial O_2 consumption *in vitro* was extrapolated *in vivo* (*10, 11, 20, 21*).



Results & Discussion. Figs A & B show experimental data. pH changes were small (overall mean = -0.05±0.02). Between exercise intensities or muscles there were no differences in k_{PCr} (proportional to the slope in Fig A: overall mean 1.7±0.2 min^{-1}) or Km (mean 28±2 μM). 'Linear model' Q_{MAX} (by extrapolation in Fig A) was 56±4 μM. There was a small difference ($P<0.005$ by paired t-test) between 'ADP-model' Q_{MAX} for 60% and 90% MVC in quadriceps (26±2 vs 34±2 mM min^{-1}, respectively) and calf (32±4 *vs* 37±3 mM min^{-1}); overall mean was 33±2 mM min^{-1} (Fig B). Figs C & D show the comparison with the literature. The present results are consistent with the few ^{31}P MRS studies covering a wide range of [ADP] (*13-15*), including one comparing ^{31}P MRS with a detailed kinetic model (*4*) (Fig C), and with studies in which end-exercise [ADP] and initial-recovery V are used to estimate Q_{MAX} (Fig D). ADP-model values are substantially lower than AVD estimates from exhausting knee extension (which activates rate-limiting TCA cycle enzymes maximally (*5*)) (Fig D), and a study (*6*) in which such values can be related to [ADP] during submaximal exercise (Fig C). Linear-model values are less discrepant, but arguably less conceptually satisfactory. As noted (*20*), AVD estimates are substantially greater than estimates from *in vitro* data, perhaps due to 'parallel activation' mechanisms (*22*) missing *in vitro*. The origin of the similar discrepancy identified here may be similar, i.e. in different degrees of activation of such mechanisms in different kinds of exercise. Alternatively interactions between O_2 supply and demand and cellular PO_2 (*6*) may be complicated.

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