Kinetics of muscle lactate and high energy phosphates measured by localised quantitative interleaved MRS during recovery from ischaemic exercise define the contributions of lactate oxidation, lactate efflux and other processes of acid efflux

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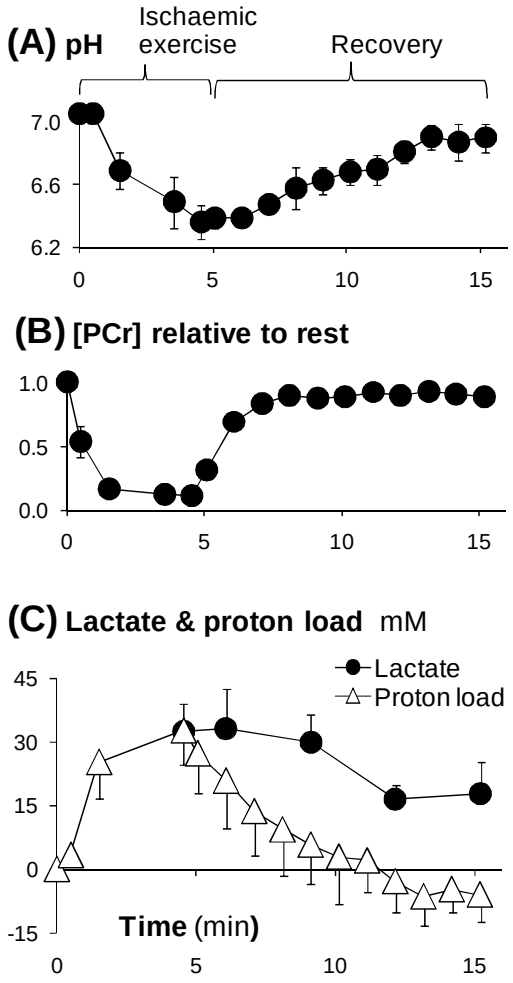
Introduction. ^{31}P MRS offers useful insight into skeletal muscle bioenergetics, but can only measure some of the processes of metabolic and physiological interest. The recently-described method for localised, quantitative ^{1}H MRS measurement of muscle lactate (1) allows detailed analysis of the production and buffering of metabolic acid during exercise and recovery (2). We used this method, interleaved with localised ^{31}P MRS, to quantify the contributions made by lactate efflux/oxidation to the recovery of pH after ischaemic exercise in human calf muscle.

Methods. Data are presented from 5 healthy young males, aged 20-27 years, from whom written informed consent was obtained. We used interleaved acquisition of double-quantum-filtered localised ^{1}H MR spectra of lactate and STEAM localised ^{31}P spectra implemented (1) on a 3 T Bruker Medspec whole-body scanner (Bruker Biospin, Ettlingen, Germany), using a double-tuned 10cm surface coil for RF transmission and reception. Absolute quantification of lactate used a phantom replacement technique, matching the coil load *in vivo*, with a small external reference to estimate coil sensitivity. Lactate amplitude was corrected for scalar coupling (J = 7 Hz) and dipolar coupling causing orientation-dependent signal modulation and multi-compartmental transverse relaxation, with calculation factors for varying voxel sizes, receiver gain and number of averages. Buffering calculations and assumptions were as in (2-6). Briefly, ^{31}P measurements of pH and PCr were used to calculate the total 'proton load' to the cytosol. During exercise this increases, being the sum of the protons which titrate the cytosolic buffers to acidic pH and those consumed as a consequence of PCr 'splitting', and should equal the protons derived (1 per lactate) from glycolytic ATP synthesis. During recovery, the load decreases and pH recovers, because processes of proton efflux (with or without lactate) outweigh proton generation as a consequence of oxidative resynthesis of PCr.

Results. As expected, pH (Fig A) and PCr (Fig B) decreased substantially during ischaemic exercise; they remained essentially constant during short resting ischaemia following exercise (omitted from Figure); they recovered after cuff release, PCr with exponential rate constant 0.83±0.07 min^{-1} ($t_{1/2}$ 0.86 min), pH more slowly ($t_{1/2}$ ~6 min). The total proton load (Fig C) increased during exercise, and decreased during recovery with rate constant 0.36±0.09 min^{-1} ($t_{1/2}$ 2.6 min). Lactate, (Fig C) having increased substantially in ischaemic exercise, decreased after cuff release with rate constant 0.07±0.02 min^{-1} ($t_{1/2}$ ~45 min) (significantly slower than proton load, P= 0.04 by paired t-test).



Discussion. Firstly**,** the agreement between measured lactate concentration and calculated proton load at the end of ischaemic exercise (1), validates the assumptions and methods. Secondly, post-exercise lactate clearance could in principle be due both to re-oxidation through the TCA cycle, and to export from the cell via the MCT1 transporter (7). If it were all exported, the kinetic rate constant would estimate a first-order permeability constant, comparable to direct measurements by invasive methods (8, 9). The kinetics of PCr resynthesis, and therefore oxidative ATP synthesis (10), are much faster than muscle lactate clearance, so clearly other processes (i.e. efflux) must be involved. An upper limit on lactate oxidation can be calculated on the assumption that this entirely fuels initial PCr resynthesis: the initial PCr resynthesis rate of 25±2 mM min^{-1} corresponds to an effective lactate clearance rate constant of 0.05±0.01 min^{-1}; although not far from the measured value, this is an overestimate to the extent that blood glucose, muscle glycogen and fatty acids derived from intramyocellular fat or plasma are also oxidised in initial recovery. Thirdly, each lactate lost either by export or oxidation amounts to a proton leaving the cytosol (11). However, lactate is cleared more slowly than the proton load (Fig C), the estimated initial rate of lactate disappearance being ~2 mM/min, smaller than the inferred initial rate of proton efflux (12±4 mM/min). Thus, as in stimulated rat leg (11), the larger part of proton efflux must be due to other processes, notably the Na^+,H^+-antiporter (11).

This methodology will allow the components of metabolic recovery to be quantified in detail, increasing the utility of MRS measurements as biomarkers of disease processes and drug responses affecting e.g. mitochondrial or microvascular function.

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