

³¹P-NMR STUDIES OF THE RELATION BETWEEN PHOSPHOCREATINE
DYNAMICS AND INTRACELLULAR pH IN CARP (*CYPRINUS CARPIO*)

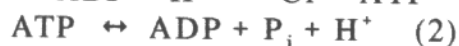
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Introduction

Intracellular phosphocreatine (PCr) concentration is maintained at high levels in resting fish muscle (van Waarde *et al* 1990), being rapidly depleted whenever oxygen demand exceeds supply. A few tail beats in a fish reduces PCr concentrations dramatically (Dobson and Hochachka 1987). Also, exposing a fish to anoxia causes a reduction in PCr (van den Thillart *et al* 1989). However PCr is not the only source of anaerobic energy products in these conditions. Anaerobic glycolysis (equation (4) below) provides ATP at the expense of a noxious end-product, lactic acid. All these reactions are shown, in summary, below:



yielding,



Creatine kinase catalyzes the hydrolysis of PCr in the forward direction (1), and the reverse, the synthesis of PCr (1). As ATP is utilized (2), PCr can be hydrolyzed to maintain ATP at resting levels. Since these reactions appear to be inter-related, at least part of the time (van Waarde *et al* 1990), elevated levels of protons should influence reactions (1) and (2). We hypothesize that the muscle acidosis that accompanies lactic acid production will slow the progression of reactions (1) and (2), which will be evident as changes in the rates replenishment of PCr stores following the anaerobic stress.

Cyprinus carpio are ideally suited to be used as a model to study this hypothesis. Carp are fairly tolerant of low environmental oxygen levels. Common carp combat the demand for ATP during anaerobic conditions by converting glycogen into lactate, unlike its close relatives, the crucian carp (*Carassius carassius*) and the goldfish (*Carassius auratus*) which utilize ethanol production or

metabolic suppression, respectively (van den Thillart and van Waarde 1985). Cyprinids are also very proficient swimmers (Smit *et al* 1971), capable of maintaining swimming velocities higher than comparably sized salmonids by using anaerobic metabolism. Cyprinids are able to provide for 70% of the power output necessary to reach their maximum swimming speed from glycolytic, white muscle fibers (Jones 1982). Lactate levels are considerably higher in exercised or exhausted muscle compared with hypoxic or anoxic muscle (Driedzic and Hochachka 1976, West *et al* 1994). This being the case, we expect production of lactate to occur during both anoxia and exercise, but considerably more following exhaustion. This will result in a larger drop in intracellular pH in exhausted than in anoxic muscle which would shift creatine kinase far from its optimum pH, resulting in a slower rate of PCr rebuilding in exercised than in anoxic white muscle.

By using Nuclear Magnetic Resonance Spectroscopy (NMRS) we can simultaneously follow any changes in PCr and ATP levels and intracellular pH in "recovering" carp white muscle. NMRS is non-destructive and non-invasive, thus allowing us to observe an individual fish sequentially under both anaerobic stresses, exhaustion and anoxia, thus serving as its own control.

Methods

Carp were caught locally, transported to and housed at the University of British Columbia in normoxic, non-recirculating, dechlorinated water at 15°C. The animals ranged from 800-1100 g body mass and 36-42 cm in length.

The fish were exercised in a Brett-style respirometer at U.B.C. (see Gehrke *et al* 1990 for description) at the maximum speed the fish could swim in a burst-and-glide pattern. The fish were considered exhausted when they could not swim against 1 BL·s⁻¹ current even when stimulated by an electrified grid located at the rear of the swim tube. After exhaustion the fish was placed on its side in a plastic box and put in the NMR. The box was flushed with 15°C normoxic water at a constant flow of 0.5 l·min⁻¹. High energy metabolites, PCr and ATP, were monitored throughout the recovery period. Following a rest period of at least one week, the fish was again placed in the box in the NMR for at least 8 hours before being subjected to anoxia. After a period of normoxia, the PO₂ of the water being flushed through the box was reduced using a series of stripping columns and a low oxygen level was maintained until PCr depletion approximated that seen following exhaustion. Levels of high energy metabolites in the carp were followed at rest, during anoxia and throughout the recovery period.

Phosphate metabolites were measured by *in vivo* ³¹P NMR spectra using a Nicolet spectrometer (1.89T). The coil was placed along the midline of the body above the anal fin. The signal was detected by a 2 cm diameter coil tuned for ³¹P (32.5 MHz). Homogeneity of the magnet field was optimized by shimming on the ¹H signal of the intracellular water. Spectra (1024 data points) consisted of 256 individual scans accumulated over 5 minutes with a pulse width of 42 μs and a delay between pulses of 1 s. The baseline corrected raw signals were smoothed by a Gaussian multiplication factor of 20, zero-fielded to 4096 points, Fourier transformed and phase shifted before deconvoluting the PCr, inorganic phosphate and β-ATP peaks to obtain the areas.

A carp was considered "recovered" and the experiment ended when PCr returned to 95% of its resting value. Intracellular pH of the sample was estimated by the chemical shift (σ) of the inorganic phosphate peak relative to the PCr peak. In the post-exercise and post-anoxic carp, the intracellular pH measurements were calibrated using the following equation (van Ginneken *et al* 1995):

$$\text{pH} = 6.72 + \log | (\sigma - 3.27) / (5.6 - \sigma) |.$$

By six hours into the recovery period the amplitude of the inorganic phosphate peak was too small to accurately distinguish it from background noise with a reasonable level of confidence. Thus the profile of pH_i could not be followed past this point.

Results

PCr levels were significantly lower in white muscle after anoxia than exercise. Levels at the start of the recovery periods were 19.5% and 30.6% of resting PCr concentrations in following anoxia and exhaustion, respectively.

PCr stores were replenished considerably more rapidly following anoxia than after exercise (Figure 1). Typically, the recovery of PCr was initially very quick, a large portion of the stores were rebuilt during the first hour after both exercise and anoxia. PCr synthesis slowed after one hour of recovery from both anoxia and exercise. ATP levels did not change significantly from resting levels following exhaustive exercise, or during or after anoxia.

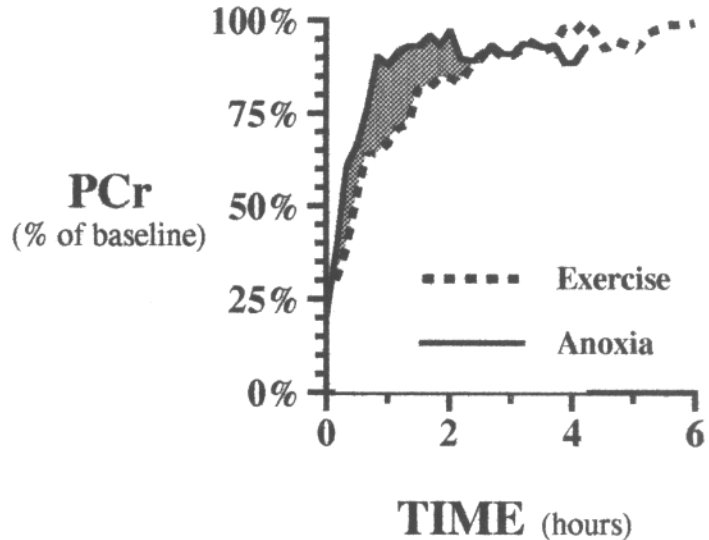


Figure 1. Relative PCr levels in a representative carp following exhaustive exercise and 1 hour of anoxia. The shaded region represents the difference in the rates of recovery between the two treatments.

Data from each fish were transformed to the time required to attain 50%, 75% and 95% of resting PCr levels. The time required to rebuild PCr to 50% and 95% was significantly shorter in post-anoxic fish than in post-exercise fish (Figure 2).

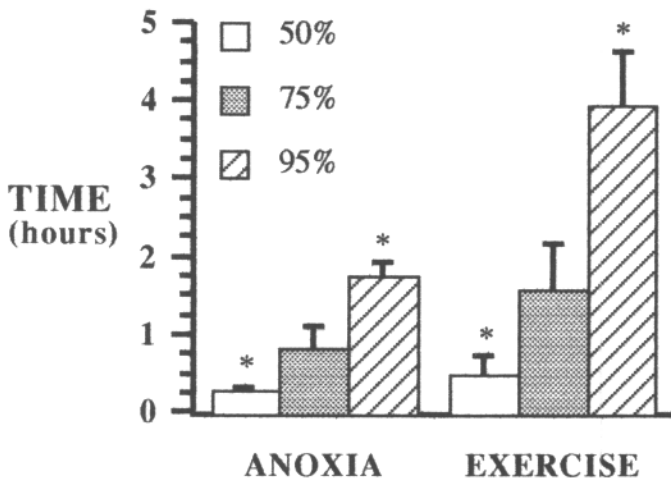


Figure 2. The time required to replenish phosphocreatine stores to 50%, 75% and 95% of resting levels in carp acclimated to 15°C after anoxia (n=2) and exhaustive exercise (n=6). * indicates a significant difference between treatments ($P \leq 0.05$).

In fact, it took more than twice as long to rebuild 95% of resting PCr levels following exhaustive exercise, 237 ± 41.4 minutes, than it did after anoxia, 106 ± 11.7 minutes.

After anoxia, the intracellular pH of carp white muscle was significantly higher than in exhausted muscle (Figure 3). Following both conditions, the pH_i of white muscle continued to fall until about 90 minutes into the recovery period. Following anoxia, it took less than 4 hours for white muscle to return to a resting pH_i of 7.3, whereas after 6 hours, exhaustively exercised muscle has only returned to a pH_i of 6.7 (Figure 3).

Discussion

Despite the difference in the PCr levels at the start of each recovery period, PCr recovery from anoxia was much faster than after exercise (Figure 1). During the first hour of recovery from anoxia the white muscle pH_i was around 6.8, which is the pH optimum for the "reverse" creatine kinase reaction (Helger 1981). Therefore, rebuilding of PCr at this pH is more rapid than occurs in the first hour of recovery from exhaustive exercise (Figure 3).

The continuous drop in pH_i in the early period of recovery in white muscle has been attributed to ATP replenishment (Dobson and Hochachka 1987, van den Thillart *et al* 1989). Our data follows the same trend, but ATP remained at resting levels following exercise and anoxia and throughout the recovery periods. Since [ATP] is constant the creatine kinase reaction is free to proceed in the reverse direction, to rebuild PCr. Intracellular pH values remain low for as long as they do, 1.5 hours following anoxia and 3.5 hours following exhaustive exercise, because the resynthesis of PCr yields a H^+ . Thus it is not until PCr levels have been restored that pH_i begins to rise.

Presently, NMRS determination of [lactate] in recovering exhausted muscle and during and after anoxia has not been completed. If we assume that there is a 1:1 stoichiometric ratio between lactate and H^+ (Hochachka and Mommsen 1983), there would be twice the amount of lactate in exhausted muscle compared with anoxic muscle. This estimation is comparable with results obtained in two previous studies (Driedzic and Hochachka 1976, West *et al* 1994). This two-fold difference in lactate, reflected in the relatively less acidic pH_i in post-anoxic muscle compared with post-exercise muscle, allows PCr to recover more rapidly following anoxia than exercise.

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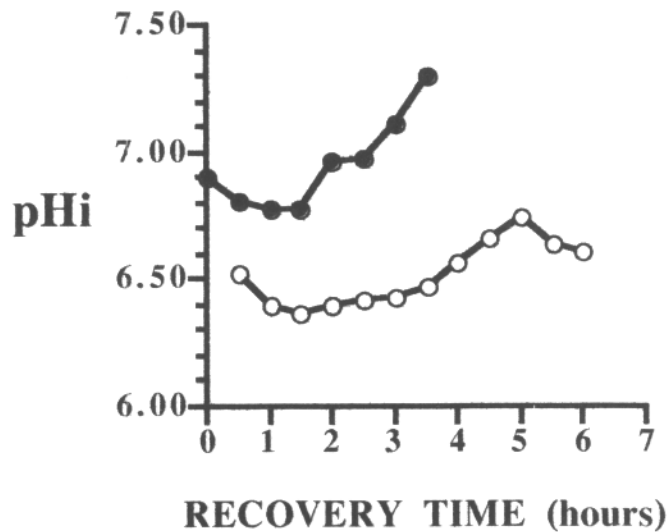


Figure 3. White muscle intracellular pH in carp following exhaustive exercise (○) or anoxia (●). Post-exercise fish muscle was significantly more acidic than that of post-anoxic fish ($P < 0.01$).

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