

**EXERCISE RECOVERY IN FISH: FUEL SUBSTRATE PREFERENCE  
DURING MUSCLE GLYCOGEN RESYNTHESIS**

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**EXTENDED ABSTRACT ONLY- DO NOT CITE**

**Introduction**

In rainbow trout, short-term burst exercise to exhaustion results in the activation of glycogenolysis in white muscle resulting in the depletion of glycogen, adenosine 5'-triphosphate (ATP) and phosphocreatine (PCr) stores and accumulation of lactate (Richards *et al.* 2002). Metabolic recovery of the muscle from exhaustion requires the repletion of the PCr and ATP and glycogen stores. It is currently believed that the major substrate for glycogenesis is lactate (e.g. Milligan and Girard, 1993; Richards *et al.* 2002). Since glycogenesis is an ATP-dependent process, substrates must be oxidized to provide the energy necessary for glycogen resynthesis from lactate. Blood glucose is not likely an important fuel for trout muscle given that the apparent capacity of muscle to transport glucose is limited (West *et al.* 1994). Though the major fate of muscle lactate is likely glycogenic, muscle can oxidize some for ATP synthesis. However, the most likely fuel oxidized by trout white muscle to provide ATP for glycogen resynthesis is fat. Recently, *in vivo* work suggests that lipid

oxidation is important in fueling exercise recovery in trout white muscle (Richards *et al.* 2002). However, the relative contributions of carbohydrate, lipid and protein as oxidative substrates for white muscle are unclear. Utilizing an *in vitro* muscle slice preparation, the purpose of this study was to examine fuel preference by trout white muscle undergoing glycogen resynthesis and to quantify the contribution of exogenous substrates in fueling recovery from exhaustive exercise.

## Methods

### *Tissue Preparation*

Fish were manually swum to exhaustion for 8 minutes at which point they were unresponsive to stimulation. Fish were then killed by anesthetic overdose, and muscle slices obtained from the dorsal epaxial muscle. One slice was then immediately frozen in liquid nitrogen immediately (time 0). Slices were then suspended in flasks containing 3 mL of glucose-free Cortland's saline aerated with a humidified 99.5% O<sub>2</sub>/0.5% CO<sub>2</sub> mixture, and incubated at 15°C for 1 hour. The following concentrations of substrates were added in various combinations: 5mM glucose, 5mM lactate, 0.5mM pyruvate, 2mM alanine, 1mM glycerol and 0.13mM palmitic acid. Following incubation, slices were blotted dry, frozen in liquid nitrogen and ground to a fine powder for analysis of glycogen, lactate, ATP, and PCr.

### *Statistical analyses*

Values are presented as mean  $\pm$  1 S.E.M. Statistical analysis was performed using paired, one-tailed Students t-test ( $P < 0.05$ ).

## Results and Discussion

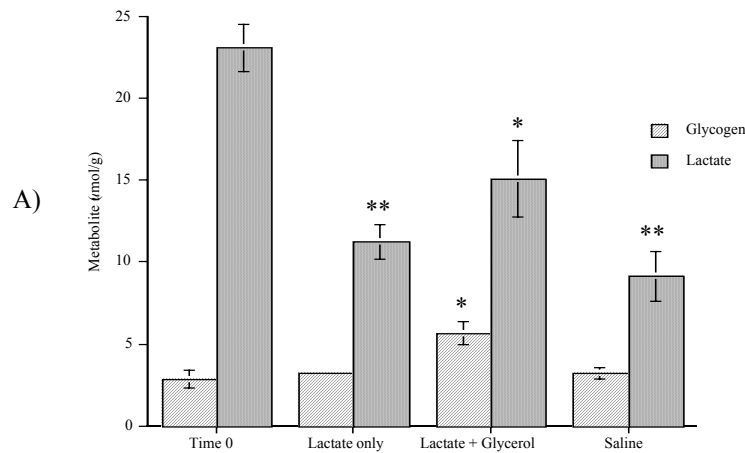
Within the one-hour incubation period, all muscle slices cleared the accumulated lactate load (Figure 1). In slices incubated in glycerol, lactate or glycerol and lactate in combination, lactate clearance was somewhat reduced but was associated with net glycogen synthesis (Figure 1). In all other incubation conditions, lactate was cleared in the absence of any net glycogen synthesis. Taken together, these observations suggest that *in vitro*, in the absence of appropriate exogenous substrates, muscle oxidizes lactate, presumably to recharge ATP and PCr stores, consequently limiting substrate supply for glycogen resynthesis. The presence of glycerol in the incubation media appears to "spare" muscle lactate for glycogenesis by providing muscle with an alternate oxidative substrate. The tendency towards higher lactate levels at the end of the

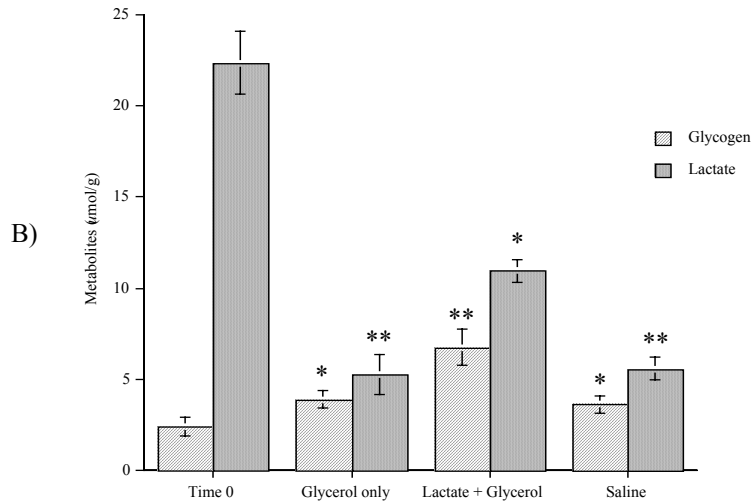
hour in these slices suggests that muscle is shifting away from lactate oxidation towards glycerol oxidation. Interestingly, the greatest net glycogen synthesis was seen in slices incubated in saline containing both glycerol and lactate (Figure 1). This suggests that when provided with adequate oxidative substrate, muscle will utilize exogenous as well as endogenous lactate for glycogenesis.

The rapid (within one hour after exercise) lactate clearance and glycogen resynthesis seen in the *in vitro* muscle slices in the present study is at odds with most *in vivo* observations, where typically lactate clearance and glycogen resynthesis are not seen until 4-6 hours after exercise (Richards *et al.*, 2002). This tends to suggest that there is/are some, as yet unidentified, mitigating factors *in vivo* that act to retard muscle metabolic recovery.

In summary, these results suggest that to recover from a bout of exhaustive, burst exercise, trout white muscle requires an exogenous oxidative substrate, and appears to prefer glycerol.

Figure 1. Glycogen (expressed as  $\mu\text{mol}$  of glucosyl units/g) and lactate ( $\mu\text{mol/g}$ ) levels in tissue incubated with various substrates. A)  $N = 7$  B)  $N = 8$ . Results are expressed as mean  $\pm$  1 S.E.M. \* indicates a significant difference from the corresponding time 0 value ( $P < 0.05$ ); \*\* indicates a significant difference from the corresponding time 0 value ( $P < 0.01$ ).





## References

- Milligan, C.L. and Girard, S.S. 1993. Lactate metabolism in rainbow trout. *J.Expt.Biol.*180:175-193
- Richards, J.G., Heigenhauser, G.J.F., and Wood, C.M. 2002. Lipid oxidation fuels recovery from exhaustive exercise in white muscle of rainbow trout. *Am.J.Physiol.*282:R89-R99
- West, T.G, Schulte, P.M. and Hochachka, P.W. 1994. Implications of hyperglycemia for post-exercise resynthesis of glycogen in trout skeletal muscle. *J.Expt.Biol.*189:69-84