

## CARBOHYDRATE AND LIPID FUEL UTILIZATION IN GILL FROM FOUR SPECIES OF TELEOST FISHES

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The gill of teleost fishes is a multifunctional tissue with central roles in gas exchange, osmoregulation, acid/base balance, and nitrogen excretion. While many of these transport processes have been studied in detail, much less is known about energy supply in gill. Because the gill epithelium is well-perfused and has little intracellular energy stores (Mommensen, In Fish Physiology, v. 10. eds. Hoar and Randall. pp. 203-238, 1984), work performed by this tissue may be supported by a variety of circulating fuels. Muscular tissues, including cardiac and red skeletal muscle, from teleost fishes rely on a mixture of carbohydrate and fatty acid fuels although relative preferences for fuel type are species specific (Sidell et al., Physiol. Zool. 60: 221-232, 1987; Crockett and Sidell, Physiol. Zool. 63: 472-488, 1990). Preliminary data obtained from gill of freshwater- and seawater-acclimated eels *Anguilla rostrata*, however, suggest that capacities for mitochondrial  $\beta$ -oxidation of fatty acid fuels is limited and glucose is likely to be a preferred fuel for energy metabolism in gill (Crockett et al., Bull. MDIBL 35: 83-84, 1996).

The purpose of the present study is to evaluate the relative importance of carbohydrate and lipid fuels in gill tissues from a range of teleosts to determine if the general patterns we observed previously extend to other teleost lineages. By measuring rate-limiting enzymes indicative of maximal flux through pathways of carbohydrate and lipid catabolism (glycolysis and mitochondrial  $\beta$ -oxidation, respectively), we report capacities for ATP generation. We also assess the relative importance of aerobic and anaerobic utilization of carbohydrate.

Yellow eels were captured in freshwater (Penobscot River, Maine) and acclimated to either freshwater (recirculating well water with daily turnover) or seawater (flow-through) for one week (freshwater) or two weeks (seawater) prior to use. All other species (longhorn sculpin *Myoxocephalus octodecimspinosus*, winter flounder *Pseudopleuronectes americanus*, Atlantic mackerel *Scomber scombrus*) were collected in Frenchman's Bay and used within one week of capture. Animals were sacrificed and gill baskets removed. Gill tissues were scraped from arches on an ice-cold glass stage. Gill scrapings were homogenized and aliquoted. Carnitine palmitoyltransferase (CPT) and hexokinase (HK) were assayed on freshly prepared tissues while activities of all other enzymes (PK, pyruvate kinase; CS, citrate synthase, HOAD, hydroxyacyl-CoA dehydrogenase) were determined on aliquots that had been stored frozen (-70°C). Procedures for enzymatic analysis are those reported in Crockett and Sidell (Physiol. Zool. 63: 472-488, 1990). All assays were conducted at 15°C. ATP yields from oxidation of glucose and palmitoleoyl CoA (a CoA ester of palmitoleic acid, 16:1) were estimated from HK and CPT activities, respectively, using the conversion factors 30  $\mu$ moles ATP/ $\mu$ moles glucose and 105  $\mu$ moles ATP/ $\mu$ moles palmitoleoyl CoA.

ATP yields from glucose and palmitoleoyl CoA (16:1) suggest that glucose is the preferred metabolic fuel in gill tissues from teleost fishes (Figure 1). Capacities for ATP generation from carbohydrate fuel are at least 3.5-times greater than capacities for ATP generation with the lipid fuel

(e.g., *Anguilla rostrata*, seawater-acclimated) and as much as 32-times greater in some species (e.g., *Myoxocephalus octodecimspinosus*). Matching of CS and HK activities further supports the hypothesis that glucose is the preferred fuel for gill tissue in teleost fishes (Figure 2). As capacity for oxidative metabolism increases (over the range of CS activities among species) there is a concomitant increase in the capacity for glucose oxidation (over the range of HK activities among species) ( $r = 0.53$ ;  $P = 0.01$ ). No significant relationship with CS activity is observed for either enzyme from mitochondrial  $\beta$ -oxidation of fatty acids (CPT, HOAD) (data not shown).

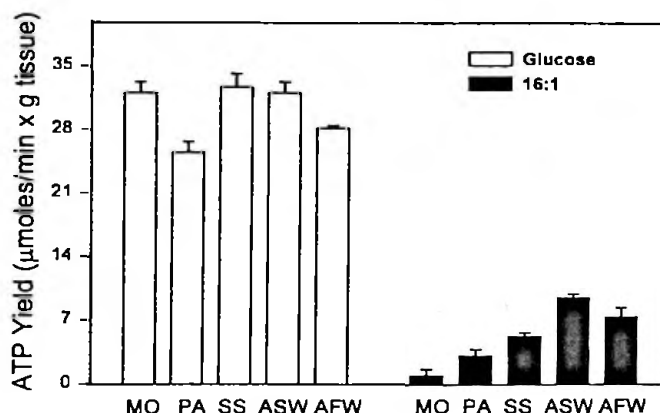


Figure 1. Capacities for ATP Generation from Glucose and Palmitoleoyl CoA (16:1). Activities of HK and CPT were used to calculate ATP yields from glucose and palmitoleoyl CoA, respectively. MO = *Myoxocephalus octodecimspinosus*, PA = *Pseudopleuronectes americanus*, SS = *Scomber scombrus*, ASW = *Anguilla rostrata*, seawater-acclimated, AFW = *Anguilla rostrata*, freshwater-acclimated.  $n = 4$ . Error bars represent SEM.

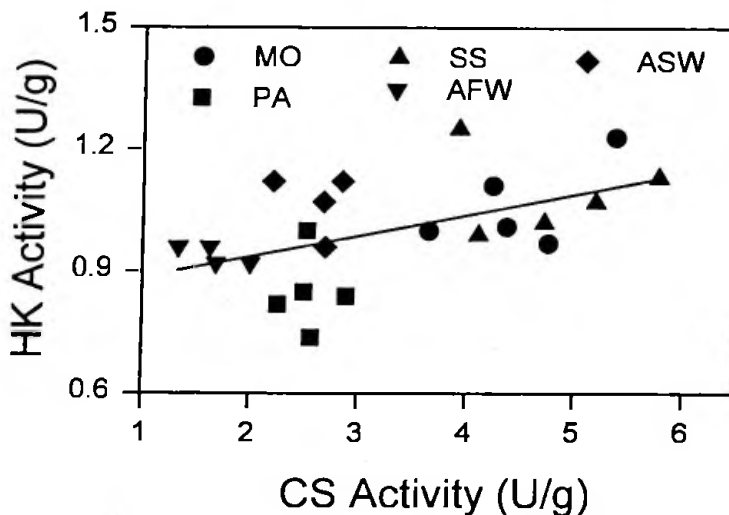


Figure 2. Relationship of Citrate Synthase (CS) and Hexokinase (HK) Activities. Data are expressed as Units (U =  $\mu$ moles product/min) per gram wet weight of tissue. MO = *Myoxocephalus octodecimspinosus*, PA = *Pseudopleuronectes americanus*, SS = *Scomber scombrus*, ASW = *Anguilla rostrata*, seawater-acclimated, AFW = *Anguilla rostrata*, freshwater acclimated. ( $r = 0.53$ ;  $P = 0.01$ )

ATP generation via anaerobic metabolism is probably of less importance than aerobic pathways of energy metabolism in gill, a tissue that acquires oxygen from the environment. Activity ratios of PK (indicative of anaerobic metabolism) to HK (indicative of aerobic metabolism) are, if anything, lower for gill than cardiac muscle from freshwater- and seawater-acclimated eels (Figure 3). Since hearts from teleost fishes rely to a large extent on aerobic metabolism to support power development (Sidell et al., *Physiol. Zool.* 60: 221-232, 1987) comparable PK/HK activity ratios suggest that gill, like cardiac tissues, largely depend on aerobic pathways of metabolism. PK/HK activity ratios for hearts range between 2 and 15 in a wide variety of teleosts (Sidell et al., 1987), while in the teleosts we surveyed, PK/HK ratios for gill fall between 4 (sculpin) and 11 (eel, freshwater-acclimated).

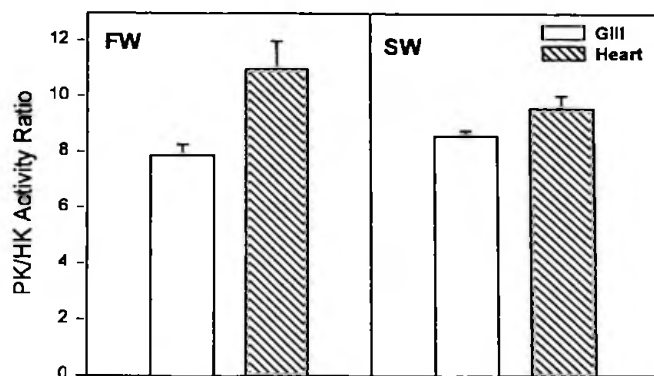


Figure 3. PK/HK Activity Ratios for Gill and Heart Tissues from Freshwater- and Seawater-Acclimated Eel. ( $n = 4$  hearts,  $n = 3$  gill/freshwater,  $n = 2$  gill/seawater). Error bars represent SEM.

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