

CARDIAC ENERGY METABOLISM IN AMAZONIAN FISH

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The fish heart is a useful model system for elucidating fundamental principles of energy metabolism and contractility since it is subject to highly variable extracellular conditions within a single class of vertebrates and lends itself to studies at many levels of organization from whole animals to isolated proteins. Two of the recurring themes in this area, the importance of anaerobic metabolism and the impact of temperature are expanded upon in this investigation.

In teleosts, cardiac ATP production under aerobic conditions is usually supported by a mixed catabolism of exogenous glucose and fatty acids. In many species of north temperate teleosts, winter decreases in temperature result in an enhancement of aerobic-based fatty acid metabolism in heart (Way Kleckner and Sidell, 1985; Sephton and Driedzic, 1991; Bailey and Driedzic, 1993). The extension to this paradigm, if temperature is an important determinant of the organization of metabolism is that fish which spend their life cycles at high temperatures should have less of a reliance on fatty acids as metabolic fuels and lower levels of mitochondrial based enzymes. We tested this hypothesis by assessing the activity of marker enzymes in heart from Amazonian teleosts which have typical body temperatures of 24-28°C. Three species were selected which cover a diversity of lifestyles and physiological traits: matrinchã, a migratory species with very little tolerance for lack of oxygen; acará açu, a species which has a high level of anaerobic tolerance; and tambaqui which is intermediate in terms of sustainable high level activity and hypoxia tolerance (Val and Almeida-Val, 1995).

Matrinchã (*Brycon cephalus*) (51 ± 8 g), acará açu (*Astronotus ocellatus*) (51 ± 4 g), and tambaqui (*Collossoma macropomum*) (96 ± 13 g) were obtained from the INPA's Aquaculture Department facilities. Hearts were excised and either immediately homogenized or frozen. All assays were performed directly on total homogenates or dilutions of total homogenates. Hexokinase (HK) and phosphofructokinase (PFK) were always measured with fresh homogenates and usually within minutes of removal of the tissue. Activities of other enzymes were determined on either fresh or frozen tissue. No decreases were noted in activity levels following freezing. Results are presented as units · gm wet tissue⁻¹ where one unit equals one μmole of substrate converted to product · min⁻¹.

The table presents the maximal *in vitro* activities for cardiac enzymes selected from various metabolic pathways and includes new information for three species of Amazonian teleosts and previously published data for north-temperate teleosts.

| | Tambaqui (<i>Colossoma macropomum</i>) | Acará açu (<i>Astronotus ocellatus</i>) | Matrinchã (<i>Brycon cephalus</i>) | North Temperate Teleosts |
|----------------------|---|--|---|--------------------------------|
| Citrate synthase | 0.40 ± 0.08 (4) | 0.44 ± 0.06 (5) | 1.19 ± 0.58 (5) | 11.9 ± 1.3 |
| 3-hydroxyacyl CoA DH | 1.30 ± 0.22 (5) | 0.05 ± 0.05 (3) | 0.05 ± 0.05 (3) | 3.65 ± 0.76 |
| Hexokinase | 3.00 ± 0.64 (5) | 2.35 ± 0.30 (4) | 2.92 ± 0.35 (5) | 6.47 ± 0.87 |
| Phosphofruktokinase | 40.4 ± 5.47 (5) | 9.52 ± 1.76 (4) | 13.58 ± 2.04 (5) | 5.36 ± 1.07 |
| Pyruvate kinase | 73.46 ± 6.94 (5) | 46.2 ± 8.13 (5) | 69.3 ± 11.45 (5) | 47 ± 4.4 |
| Lactate D.H. | 573 ± 113 (6) | 134 ± 37.4 (5) | 202 ± 33 (5) | 189 ± 16 |
| Assay temperature | 25°C | 25°C | 25°C | 15°C |

Activity is expressed as units · gm wet tissue⁻¹. Values for the Amazonian species are presented as mean ± SEM with samples sizes given in parenthesis. Values for north temperate species which include both freshwater and marine representatives are taken from Sidell et al. (1987) and Crockett and Sidell (1990). Values are presented as mean ± SEM based on a sample size of 12 or 13 different species. Activity levels for north temperate species are presented for an assay temperature of 15°C. In some cases values presented in the original literature for an assay temperature of 10°C have been adjusted to 15°C using either a Q₁₀ of 2 or where provided, experimentally determined Q₁₀ values.

Citrate synthase (CS) catalyzes the first step in the citric acid cycle regardless of the source of acetyl CoA and, therefore, is a required enzyme in aerobic energy metabolism. Maximum *in vitro* CS activities in the heart of matrinchã, acará açu, and tambaqui are low when compared to north temperate teleosts when assays are conducted at respective body temperatures. This difference is even more emphatic when adjustments are made to a common temperature.

Other indicator enzymes associated with aerobic fuel utilization are also lower in the Amazonian species investigated here than in north temperate species. HOAD which is an essential enzyme for beta-oxidation ranged from 0.05 to 1.30 units · gm wet tissue⁻¹ in Amazonian fish at 25° and is on the average 3.65 units/g wet tissue in north temperate species at 15°C. We were also unable to detect CPT activity in two specimens of tambaqui. CPT is an enzyme system involved in the movement of fatty acids across the mitochondrial membrane and is essential for fatty acid oxidation. All of the enzymes of energy metabolism measured here, which are obligatorily linked to aerobic energy production, are substantially lower in the three species of Amazonian teleosts than in the north temperate species. It has been previously contended that high levels of enzymes of aerobic fatty acid metabolism developed in hearts of species living at low temperature to compensate for reduced catalytic activity. The low activities in tropical fish reported here is consistent with this hypothesis. However, differences in enzyme activities are so substantial that it appears that the impact of temperature on catalytic activity alone cannot be the only factor involved. The above mentioned enzymes CS, HOAD, and CPT are all located in the mitochondria. Acclimation to cold temperature is associated with increased mitochondrial density in skeletal muscle of some fish species, a response which is considered in part to reduce diffusion pathlengths for molecules traversing between cytoplasmic and mitochondrial compartments and increase the area of exchange surface between these compartments (Egginton and Sidell, 1989). Low levels of mitochondrial enzymes and presumably mitochondrial density in heart of the tropical species examined here may reflect the opposite extreme of this concept. A second potential and not mutually exclusive explanation is that these Amazon species have in common a relatively low dependence on aerobic fatty acid metabolism to fuel ATP production and a relatively higher dependence upon a sustained anaerobic metabolism. This organization of metabolism may relate to adaptations to the hypoxic environment. Unfortunately, the current study did not include species which are

air-breathers and could contribute towards an understanding of temperature versus hypoxia induced responses.

The enzyme scan included four proteins required for carbohydrate metabolism. HK, which catalyzes the first step in glucose utilization ranges from 2.35 to 3 units · gm wet tissue⁻¹ in the Amazonian teleosts when assayed at 25°C. These values are similar to that of north temperate species when comparisons are made at physiological assay/body temperatures. PFK and PK are essential in both glucose and glycogen utilization while LDH is the terminal enzyme in lactate production. Activities previously reported for PFK, PK and LDH in aruanã and *Arapaima* hearts (Hochachka et al., 1978) fall within the range of that for the three species reported here. In contrast to the enzymes which are obligatorily linked to aerobic fatty acid metabolism there is no substantial difference in activities of HK, PFK, PK or LDH in comparing tropical with north temperate species. In some cases there is overlap in enzyme activity levels amongst the two groups of animals when compared at the usual body temperature. It appears that with this suite of enzymes, catalytic activity is conserved over a range of body temperatures presumably to allow carbohydrate metabolism to occur at similar rates. The high level of HK in heart of Amazonian fish and low activities of citrate synthase suggest that these hearts are supported by a high sustainable anaerobic metabolism involving glucose uptake and lactate production. Aerobic metabolism which does occur may favour carbohydrate over fat degradation since the former yields more ATP per mole of oxygen consumed than the later (Hochachka, 1993). It should be emphasized that these concepts remain tentative until tested with additional metabolic techniques.

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