

In vivo ^{31}P -NMR studies of speeding fish: Online monitoring of muscular energetics in Atlantic cod (*Gadus morhua*)

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ABSTRACT

Previously, energetic studies in exercising fish were carried out by invasive tissue sampling and online respirometry in swim tunnels. The use of ^{31}P -NMR techniques was restricted to "stop and go" procedures, animals being repeatedly moved into the magnet. These limitations were overcome in combined swim tunnel and NMR systems, by adequate adaptation of ^{31}P -NMR techniques as well as the triggering of NMR recordings by tail beat pressures monitored through differential pressure transducers. Online ^{31}P -NMR studies at various swimming speeds in Atlantic cod (*Gadus morhua*), combined with oxygen consumption analyses, led to the determination of critical swimming velocity, and anaerobic threshold.

INTRODUCTION

Study of muscular exercise in fishes has always provided a comparative data base for the understanding of skeletal muscle energetics and fatigue. Setting of finely tuned exercise levels *in vivo* at well defined environmental conditions are possible by subjecting fish to various water speeds in swim tunnels. Online respirometry accurately quantifies the oxygen demand of the whole organism at various exercise levels. Accordingly, our goal was to compare metabolic performance and limitations in fish permanently adapted to different environmental temperature regimes and to use online respirometric and NMR techniques at different swimming velocities.

However, until previously, detailed studies of muscle energy metabolism and acid-base status in exercising fish *in vivo* relied on invasive tissue sampling at the end of the exercise protocol and on further biochemical analyses (1). Online monitoring of changes in tissue energy and acid-base status were not possible, owing to limited applicability of MR techniques for use with mobile animals and, also, due to strong loss of radio signal during NMR recordings, esp. in moving conductive media like sea water. Therefore, the use of ^{31}P -NMR techniques was restricted to "stop and go" procedures, animals being repeatedly moved into the magnet after exposure to various exercise protocols (2).

MATERIALS & METHODS

Within the present study, we have overcome these limitations. Firstly, we have constructed a 15 cm diameter swim tunnel fed through the 40 cm horizontal bore of a 200 MHz Bruker magnet at 4.7 T, equipped with a double tunable ^1H - ^{31}P -birdcage resonator (inner diameter 19.5 cm), thereby allowing to swim Atlantic cod (*Gadus morhua*) of 0.5 to 1.1 kg body weight (35 to 52 cm body length) in the center of the magnet, in a perspex tube, up to 60 cm long and closed at both ends by grids of nylon threads. Freshly aerated and continuously filtered sea water was recirculated from a thermostatted water reservoir (1 m³) at water velocities between 0.2 and 1 m sec⁻¹. The fish usually started to swim against the sea-water flow at speeds above 0.2 m/s and maintained position in the center of the chamber. A bypass allowed to close a smaller circulation during periods when the rate of oxygen depletion was monitored by optodes, for an analysis of oxygen consumption. A birdcage resonator adapted to high loadings was used for signal excitation. An insulated inductive coil (2 cm diameter) was fixed onto the side of the fish tail and placed opposite to a watertight, passively decoupled 5 cm surface coil for signal perception. This arrangement largely improved the penetration of the RF signal and the S/N ratio. The cartilaginous tail of the animals close to the caudal fin was perforated with a stainless steel cannula which was permanently connected to one port of a differential pressure transducer adapted to the size of the fish (3). Tail beat pressures were monitored by use of a PowerLab system (ADI instruments, Hastings, UK). Voltage thresholds set within pressure pulses gated the monitoring of high energy phosphates and intracellular pH, allowing to average the signals for various time windows during and between pressure oscillations, for time resolved analyses of metabolic events (4). Swimming performance and metabolism of animals from two populations (North Eastern Arctic cod from the Barents Sea and cod from the European North Sea) permanently adapted to different temperature regimes, were compared at 10°C.

RESULTS & DISCUSSION

Online ^{31}P -NMR studies at various swimming speeds in Atlantic cod (*Gadus morhua*), combined with oxygen consumption analyses, led to the determination of critical swimming velocity, and anaerobic threshold. At low swimming speeds, starting with long term ungaited recordings and, at higher swimming velocities, using gaited recordings, spectra revealed stable concentrations of the phosphagen, phosphocreatine, regardless of exercise level. Pressure amplitudes and frequencies rose steadily with increasing swimming speed until the average signals were complemented by the onset of additional high pressure pulses which appeared at regular intervals. These high pressure pulses likely reflect the onset of strong muscular contractions with kick and glide swimming. Only when these pulses became involved, did spectra display onset of phosphocreatine depletion and the accumulation of inorganic phosphate, associated with a drop in intracellular pH (Fig. 1). These metabolic changes are interpreted to characterize the anaerobic threshold, equivalent to the critical swimming speed. In contrast to previous comparisons of the two populations at 4°C, which revealed significantly higher metabolic rates in Arctic than in North Sea cod, no difference in oxygen demand and critical swimming speed between the two populations could be detected at 10°C.

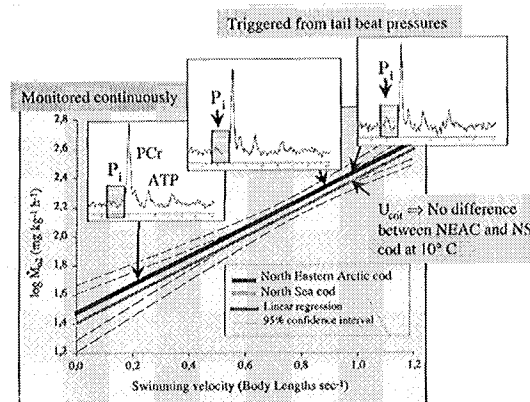


Figure 1: Online identification of the critical swimming speed U_{crit} by the onset of phosphocreatine depletion and acidosis detected by ^{31}P -NMR in swimming cod (*Gadus morhua*) at 10°C. In a comparison of cod from the North Sea (NS) and the Barents Sea (North Eastern Arctic cod, NEAC) no difference in metabolic rate M_{O_2} and U_{crit} could be found.

CONCLUSION

Baseline muscular activity in fish is characterized by the predominant use of red musculature. The critical swimming velocity U_{crit} , indicated by the onset of anaerobic energy production, is reached when strong muscular contractions, likely involving white muscle tissue, complement the more regular activity pattern. White muscle fibre recruitment and anaerobic threshold are the same in the two population, when investigated at 10°C, regardless of the permanent differences in the ambient temperature regime.

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