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MYOFIBRILLAR ATPase ACTIVITY IN THE CARP *CYPRINUS CARPIO*: INTERACTIONS BETWEEN STARVATION AND ENVIRONMENTAL TEMPERATURE

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SUMMARY

1. The myofibrillar ATPase activity of the epaxial white muscle was measured in carp *Cyprinus carpio* L. acclimated to 10°C or 28°C. As previously reported, cold acclimation was associated with an increase in the ATPase specific activity and a decrease in the thermostability.

2. The water content of the white muscle was significantly higher in cold-acclimated fish than in warm-acclimated fish ($P < 0{\cdot}002$). Starvation for 10 weeks resulted in a significant increase in the white muscle water content of both warm- and cold-acclimated fish ($P < 0{\cdot}002$).

3. When carp were starved, the ability of the myofibrillar ATPase to show thermal compensation disappeared.

4. Previously acclimated fish, when starved, showed steady alterations of the myofibrillar ATPase activity to a level mid-way between the acclimated extremes. Refeeding resulted in a gradual return to the normal acclimated level.

INTRODUCTION

Temperature is generally regarded as the single most important environmental variable involved in the metabolic regulation of poikilothermic animals. Consequently, the effects of temperature on fish metabolism have been well documented (for reviews, see Prosser, 1967; Hochachka, 1969). The speed of shortening of muscles is, in general, related to the activity of the myofibrillar ATPase (Barany, 1967). However, these two parameters were shown to possess different temperature dependencies in fish myofibrils (Johnston & Sidell, 1984), suggesting the involvement of other interacting factors. Nevertheless, the myofibrillar ATPase is one important determinant of tail-beat frequency in fish (Wardle, 1975). Adaptation of the myofibrillar ATPase enzyme as a result of temperature change has been demonstrated in goldfish *Carassius auratus* (Johnston, Davison & Goldspink, 1975), and

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in carp and other freshwater cyprinids (Heap, Watt & Goldspink, 1985). This mechanism apparently improves the locomotory ability across a wide temperature range (S. P. Heap & G. Goldspink, in preparation), and in these species it is most probable that the activity of this enzyme would show seasonal fluctuations related to water temperature. In the warm summer months, carp can maintain relatively high levels of 'cruising' activity and have a high potential 'burst' speed as a direct result of the higher ambient temperature. In winter, however, restrictions imposed on both cruising ability and burst speed as a result of the lower temperature are lessened by the acclimatory changes.

Other seasonal variations may also be important, and one of these is food availability. Carp, for example, feed mainly on bottom-living insect larvae, vegetable matter, small snails and crustaceans (Wheeler, 1978), and the first two in particular show seasonal variations in availability. Many studies have therefore focused on the effects of starvation and restricted diets (Love, 1970, 1980). This author concluded that the majority of fish do experience severe depletion for a part of every year of their lives. The metabolic changes associated with starvation differ between species. In non-fatty species, starvation mainly affects glycogen levels in both liver and muscle, together with the insoluble myofibrillar proteins. This has been demonstrated in plaice *Pleuronectes platessa* (Johnston & Goldspink, 1973) and in cod *Gadus morhua* (Love, 1970). Where visceral fat bodies are present they are utilized first, and these have been shown to play an important role in rainbow trout *Salmo gairdneri* (Weatherley & Gill, 1981) and in pike *Esox lucius* (Ince & Thorpe, 1976). Creach & Cournede (1965) starved carp for 2 months and, by direct weight measurement of individual organs, they concluded that organs were affected in the order intestine: liver: kidney: spleen: muscle: heart. Thus it appears that the vital organs are spared as long as possible.

Many studies have reported an increase in the water content of the myofibrillar protein with starvation, and this has been demonstrated for plaice (Johnston & Goldspink, 1973) and carp (Creach & Cournede, 1965). Such changes were not observed in the European eel (Dave *et al.* 1975), the American eel *Anguilla rostrata* (Moon, 1983) and the pike (Ince & Thorpe, 1976). Examination of the individual muscle fibres of cod following 130 days' starvation revealed a 15% decrease in the diameter of the red fibres and a 40% decrease in the diameter of the white fibres (Walker, 1971). This preferential utilization of white muscle during starvation has also been demonstrated in plaice (Patterson, Johnston & Goldspink, 1974), in crucian carp *Carassius carassius* (Patterson & Goldspink, 1973) and in salmonids (Mommsen, French & Hochachka, 1980).

These examples demonstrate that starvation can lead to extensive alterations in the myotomal structure which must surely affect locomotory ability. Moon & Latham (1984) reported that starvation had no effect on the specific activity or activation energy of the myofibrillar ATPase enzyme of the European eel. However, the significant decrease in the muscle protein content of both red and white fibres suggested that the locomotory performance would be altered. This problem has also been studied in the black mollie, *Molliensia* sp., by Patterson (1974). Fish starved for

8 weeks showed atrophy of both red and white fibres, but the effect was greater for the white fibre types. Comparison of the locomotory performance with fed individuals showed that the sustained swimming speed was reduced from 8·8 to 6·8 L s^{-1} (L = body length), whilst the sprint speed was reduced from 12·4 to 9·8 L s^{-1}. This study provided direct evidence that swimming ability can be impaired because of fibre atrophy as a result of starvation.

In the present study, the interaction between temperature acclimation of the myofibrillar ATPase and starvation was investigated in carp. In particular, it was hoped to ascertain whether starvation leads to qualitative changes in the myofibrillar ATPase as well as to the quantitative changes which occur as a result of the reduction in the myofibrillar protein fraction.

MATERIALS AND METHODS

Immature carp, *Cyprinus carpio*, (4–8 cm) were obtained from a local supplier and maintained for 4 weeks in 1141 tanks of circulating, aerated tap water at 18°C (±1°C). All fish were fed *ad lib.* daily with a commercial pellet diet, and kept under a 12:12 h light:dark photoperiod. The water temperature was adjusted to 10°C (±1°C) or 28°C (±1°C) over a 1-week transition period, during which time the fish were not fed. At each temperature extreme, fish were divided into three groups. One group was starved for 6 weeks before feeding was commenced, a second group was initially fed before starving from 6 weeks onwards, and the third control group was fed throughout the experiment. At regular intervals during the experimental duration, the myofibrillar ATPase activity of the epaxial white muscle was measured. Thermal inactivation properties of the enzyme were studied in fish starved for 10 weeks, and those fed normally.

A further two groups of fish were maintained at 10°C and 28°C, respectively, and starved for a period of 10 weeks. Over this period individuals were weighed every 2 weeks. This allowed for the rate of weight loss at each temperature to be monitored. After 10 weeks' starvation, samples of epaxial white muscle were dissected, weighed and dried to constant weight in an oven at 60°C, together with samples from normally fed individuals.

Preparation of myofibrils

Fish were stunned by a blow to the head, followed by pithing and transection of the spinal cord. The preparation of myofibrils was a further modification of the procedure used by Penney & Goldspink (1981*a*), based on the method of Perry & Grey (1956). The white epaxial musculature was dissected and immersed in three volumes of 0·1 mol l^{-1} KCl, 5 mmol l^{-1} Tris, 1 mmol l^{-1} dithiothreitol, pH 7·0 (extraction buffer). The muscle was homogenized twice for 15 s with a cooled Polytron blender, and washed four times by centrifugation at 600 ***g*** in the extraction buffer, discarding the supernatant between washes. Myofibrils were prepared by differential centrifugation at 450 ***g*** for 30 s, discarding the residue, followed by a final wash at 600 ***g***. Protein concentration was estimated by the Folin phenol reagent as

outlined by Lowry, Rosebrough, Farr & Randall (1951), since the presence of lipid in the tissue rendered the Biuret reaction inaccurate. Bovine serum albumin was used as a standard, and concentrations were adjusted to $10 \pm 2\,mg\,ml^{-1}$.

Myofibrillar ATPase activity

Myofibrillar ATPase activity was measured at 20°C in an incubation medium of $50\,mmol\,l^{-1}$ Tris, $25\,mmol\,l^{-1}$ KCl, $0{\cdot}2\,mmol\,l^{-1}$ $CaCl_2$, pH 7·4, also containing $5\,mmol\,l^{-1}$ $MgCl_2$. The reaction was started by the addition of ATP to give a final concentration of $5\,mmol\,l^{-1}$ ATP, and was terminated by the addition of an equal volume of 10% trichloracetic acid (TCA). The amount of inorganic phosphate released was measured using the method of Rockstein & Herron (1951).

Thermal inactivation studies

Thermal inactivation of the myofibrillar ATPase enzyme was carried out in a water-jacketed vessel attached to a temperature-controlled water bath. The final incubation medium comprised $50\,mmol\,l^{-1}$ Tris, $25\,mmol\,l^{-1}$ KCl, $0{\cdot}2\,mmol\,l^{-1}$ $CaCl_2$, pH 7·4, together with $3{\cdot}5\,mmol\,l^{-1}$ $MgCl_2$ and approximately $4\,mg\,ml^{-1}$ myofibrils. The medium was stirred continuously, and 200-μl samples were taken at appropriate intervals and pipetted into ice-cold incubation medium to prevent further inactivation. Care was taken to standardize the myofibril concentrations, as Buttkus (1966) has shown that thermal inactivation rates depend on protein concentration. All samples were then assayed for myofibrillar ATPase activity at 20°C as described above.

Statistical analysis

Values for the myotomal muscle water content and data on myofibrillar ATPase activities were compared using two-tailed analysis of variance (Meddis, 1975). The cumulative weight loss showed a high degree of correlation with time, allowing regression analysis to be carried out and regression coefficients to be compared (Edwards, 1984).

RESULTS

When starved for a period of 10 weeks, carp at 10°C lost an average of 21·1% of the total body weight, whilst at 28°C this figure was 30·5% (Fig. 1). The cumulative weight loss was approximately linear, with correlation coefficients of 0·996 and 0·990 at 10 and 28°C, respectively. Regression analysis showed that the rate of weight loss at 28°C was significantly higher ($P < 0{\cdot}01$) than at 10°C, reflecting the increased rate of metabolism at the higher environmental temperature. The myotomal white musculature of cold-acclimated fish had a significantly higher water content than warm-acclimated muscle ($P < 0{\cdot}002$; Table 1). When starved for 10 weeks, however,

the water content of both warm- and cold-acclimated muscle showed a significant increase ($P < 0{\cdot}002$), of 5·1 % and 3·3 %, respectively.

Fish in the 'control' groups showed significantly different myofibrillar ATPase activities ($P < 0{\cdot}02$) after 2 weeks at their respective acclimation temperatures (Fig. 2). When measured at 20°C, the cold-acclimated ATPase exhibited approximately 1·5 times the specific activity of the warm-acclimated ATPase. Starvation resulted in an inability to acclimate (Fig. 3A), but there were significant differences in the ATPase activity ($P < 0{\cdot}02$) only 2 weeks after feeding was resumed. When acclimated fish were starved over a 10-week period, a gradual alteration in the myofibrillar ATPase activity was observed (Fig. 3B). Once starvation had begun, there was a gradual fall in the specific activity of the enzyme from cold-acclimated fish, but a rise in the enzyme specific activity of warm-acclimated fish. This transition occurred over a 6-week period until activity levels reached a constant level. This level was approximately equal to that observed for fish acclimated to 18°C.

Thermal inactivation studies on the myofibrillar ATPase of normally fed fish showed that the cold-acclimated ATPase possessed a significantly higher initial activity ($P < 0{\cdot}01$), but a significantly lower activity ($P < 0{\cdot}01$) following 60 min incubation at 37°C (Table 2). These differences in the specific activity and thermostability were not apparent following 10 weeks' starvation. Comparison of the data for cold-acclimated fish showed that starvation resulted in a significant reduction in

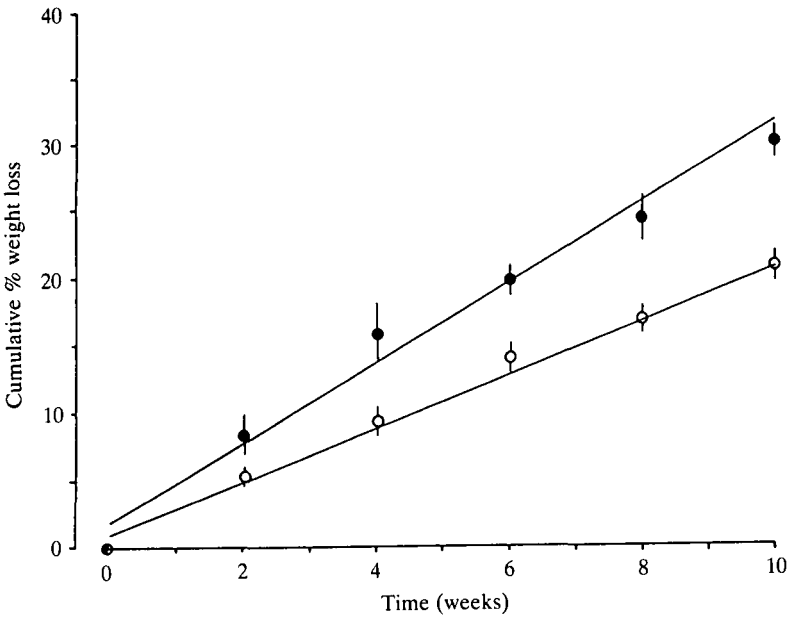

Fig. 1. The rate of weight loss in carp starved over a 10-week period. Points represent the mean of six or seven individuals (±S.E.). ●, fish at 28°C; ○, fish at 10°C. The correlation coefficients were 0·996 and 0·990 at 10 and 28°C, respectively. The rates of weight loss at 10 and 28°C were significantly different ($P < 0{\cdot}01$), and were 1·99 % per week and 2·72 % per week, respectively.

Table 1. *Alterations in the water content of the white myotomal muscle of carp as a result of starvation for 10 weeks*

Muscle sample	*N*	Mean water content (±S.E.)
Fed carp (28°C)	5	77·1 ± 0·09 †
Fed carp (10°C)	5	79·9 ± 0·13 *
Starved carp (28°C)	5	81·0 ± 0·26 †
Starved carp (10°C)	5	82·5 ± 0·25 *

* Significantly different $P < 0{\cdot}002$.
† Significantly different $P < 0{\cdot}02$.

the myofibrillar ATPase specific activity ($P < 0{\cdot}02$). For warm-acclimated fish, however, starvation resulted in a significant increase in the myofibrillar ATPase specific activity ($P < 0{\cdot}02$).

DISCUSSION

Weight loss in starved carp was linear over a 10-week period. When measured over a longer duration, Creach & Serfaty (1965) found that the weight of carp decreased asymptotically to 53% of the original value. It thus appears likely that, with prolonged starvation, a reduction in the rate of weight loss would have been observed.

Following 10 weeks' starvation, the water content of carp white muscle increased from 77·1% and 79·9% to 81% and 82·5% at 28 and 10°C, respectively. The water

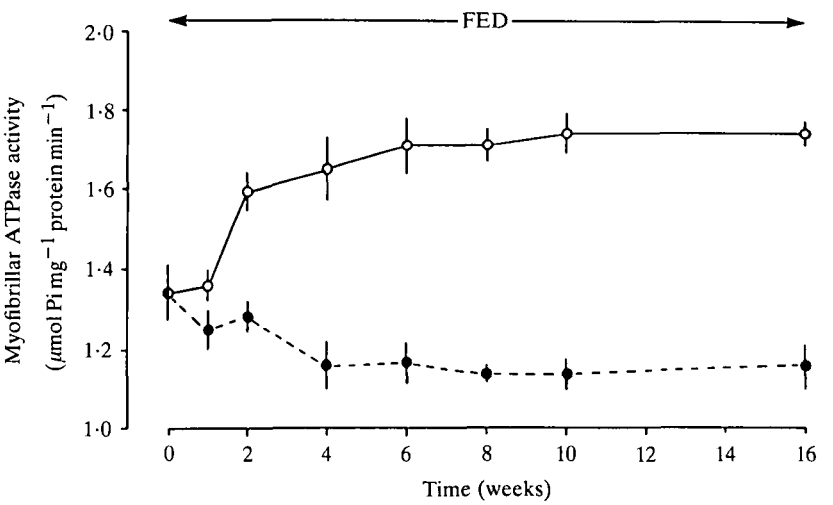

Fig. 2. Myofibrillar ATPase activity of carp white myotomal muscle from fish fed *ad lib.* daily throughout the 16-week experiment. Values are the means of five individuals (±S.E.) for fish maintained initially at 18 C (◐), before transfer to 10°C (○) or 28°C (●). Enzyme activity was measured at 20°C, and is expressed as μmol Pi mg^{-1} protein min^{-1}.

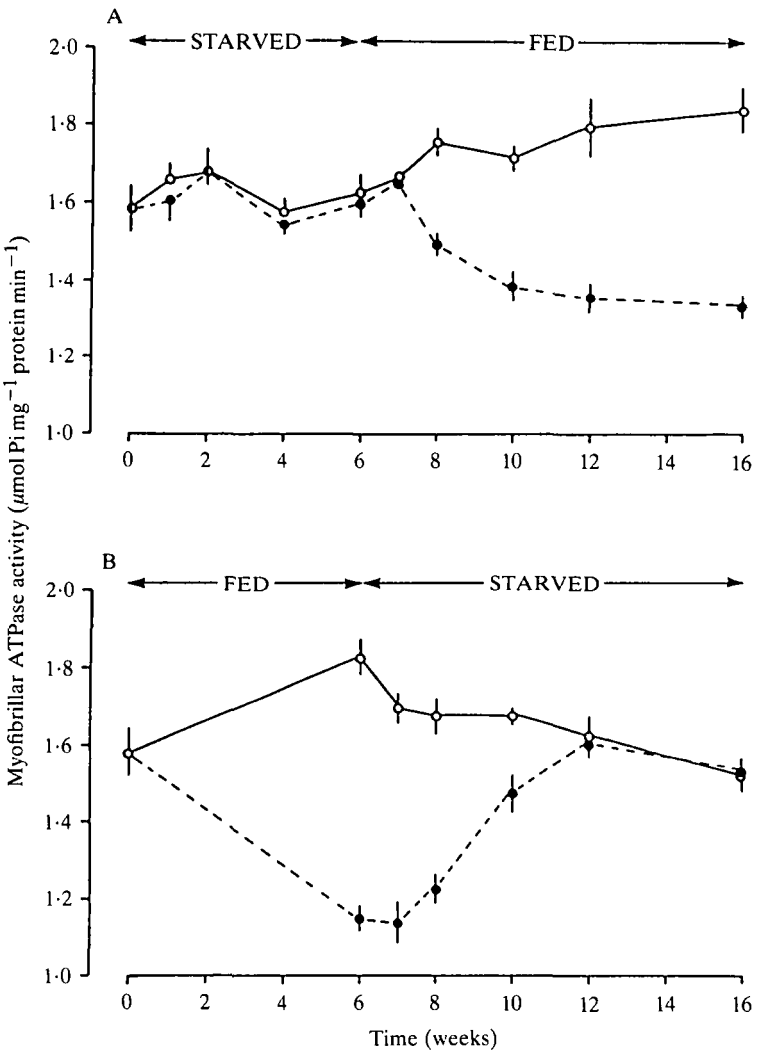

Fig. 3. Alterations in the myofibrillar ATPase activity of the white myotomal musculature of carp as a result of enforced starvation. Enzyme activity levels are expressed as μmol Pi mg^{-1} protein min^{-1}, for the mean of five individuals (±S.E.). (A) Fish starved for 6 weeks at either 10°C (○) or 28°C (●), before being fed for a further 10 weeks. (B) Fish fed normally for 6 weeks at either 10°C (○) or 28°C (●), before being starved for a further 10 weeks.

content has been observed to rise as high as 91 % following 8 months' starvation at 20 °C (Creach & Cournede, 1965). Differences in the magnitude of response between the red and white muscle fibres (Johnston & Goldspink, 1973; Patterson *et al.* 1974) have been related to the locomotory function of the two types. Thus, preferential utilization of the white muscle during starvation would minimize the effect on 'normal' swimming activity. A less complicated theory suggests that preferential utilization of the white muscle may occur because of its increased abundance (Johnston & Goldspink, 1973). Mommsen *et al.* (1980) showed that in sockeye

Table 2. *Thermostability of the myofibrillar ATPase from starved or fed carp, following a 10-week period at either 10°C or 28°C*

Muscle sample	*N*	ATPase activity Mean ± S.E.	% Activity remaining after 60 min incubation at 37°C Mean ± S.E.
Fed carp (28°C)	5	1·14 ± 0·04 *	38 ± 2 *
Fed carp (10°C)	5	1·79 ± 0·06 †	10 ± 2
Starved carp (28°C)	5	1·54 ± 0·04 †	27 ± 4
Starved carp (10°C)	5	1·47 ± 0·07	29 ± 7

* Significantly different $P < 0{\cdot}01$.
† Significantly different $P < 0{\cdot}02$.
Enzyme activity is expressed as μmol Pi mg^{-1} protein min^{-1}, and was measured at 20°C.

salmon, *Oncorhynchus nerka*, proteolytic enzymes of the white muscle but not the red muscle are activated during starvation. This may be a further reason for the tissue specificity.

In this study, it has been shown that starvation can markedly affect the ability of carp to exhibit thermal compensation of the myofibrillar ATPase. When carp were starved, the ability to acclimate was apparently 'shut off', but refeeding resulted in an immediate shift towards levels normally associated with the particular environmental temperature. Regulation of the myofibrillar ATPase has been attributed to the regulatory proteins, troponin and tropomyosin (Johnston, 1979; Penney & Goldspink, 1981*b*). Removal of this fraction from the myofibrillar ATPase complex of warm- and cold-acclimated goldfish resulted in the disappearance of the differences normally associated with thermal compensation. This suggests that starvation may lead to an alteration in the function of the regulatory proteins. In rainbow trout (Loughna & Goldspink, 1984) and carp (P. W. Watt, unpublished data), starvation is associated with a large reduction in the rate of protein synthesis, especially in the white muscle. Thus during starvation, synthesis of a particular protein may be either reduced or cease altogether. This could be an important factor in explaining the observed changes in the myofibrillar ATPase complex.

Acclimation of the myofibrillar ATPase system to low temperatures is apparently a mechanism to enhance the locomotory ability at these temperatures. The benefits offered by the warm-acclimated system are less clearly understood, although the improved thermostability is presumably an important factor. The finding that starvation significantly altered the specific activity of both the warm- and cold-acclimated myofibrillar ATPase suggests that both are regulated forms of the enzyme system, with the 'unregulated' condition being at an intermediate level. The time course for the alteration of the myofibrillar ATPase specific activity as a result of starvation was approximately 6 weeks. This is longer than the period of 4 weeks observed for the compensation of the myofibrillar ATPase as a result of temperature change (Heap *et al.* 1985).

Despite the significant alterations to the myofibrillar ATPase specific activity, significant changes to the enzyme thermostability as a result of starvation were not

observed. This indicates that catalytic efficiency and thermostability may not be as closely interrelated as was previously thought.

In summary, it appears that starvation results in suppression of the regulation of the myofibrillar ATPase activity associated with temperature acclimation. Hence, the mechanism of adaptation in response to temperature change is apparently dependent on the food supply, and starvation results in an inability to adapt in this way. This is likely to affect the locomotory ability of this species, and in particular a reduction in the burst speed would be expected.

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