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ENERGETICS AND POWER OUTPUT OF ISOLATED FISH FAST MUSCLE FIBRES PERFORMING OSCILLATORY WORK

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Summary

Fast myotomal muscle fibres were isolated from the cod (*Gadus morhua* L.) and the energy cost of contraction was measured under conditions simulating swimming. Fibre bundles were subjected to sinusoidal cycles of shortening and lengthening about their *in situ* fibre length, and stimulated at selected phases in each cycle. The preparations were poisoned with iodoacetic acid and bubbled with nitrogen to block the synthesis of ATP. After an initial rapid decline over the first 10 cycles, force and net work remained steady in some cases for up to 64 oscillatory length cycles, but more commonly declined slowly after about 30 cycles. The total mechanical work performed increased largely in proportion to the number of work cycles. At the end of each experiment fibres were frozen in isopentane cooled in liquid nitrogen and metabolite concentrations determined by high performance liquid chromatography (HPLC) and enzymatic analysis. Concentrations of adenylates did not differ significantly from control values, although a significant increase in IMP concentrations at 64 cycles accounted for the maintenance of relatively high energy charge values. Creatine (C) concentrations increased and creatine phosphate (CP) concentrations decreased, implying a tight coupling of the ATP/ADP reaction to the CP/C reaction. Muscle economy was calculated as the positive work performed during a work cycle divided by the total chemical energy expended. These values (approx. 7 mJ μmol^{-1}) were found to be independent of the number of work cycles performed, although a trend to increase was observed. Muscle efficiency values, calculated assuming a Gibb's force free energy change for CP splitting *in vivo* of 55 kJ mol^{-1}, were in the range 12–23 %.

Introduction

The conversion of chemical free energy into mechanical work by living skeletal

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muscle is a complex process involving energetic costs from crossbridge cycling and from the release and sequestration of calcium (Kushmerick, 1983; Woledge *et al.* 1985; Woledge, 1989). Muscle efficiency, or the work performed per unit of free energy used, is complicated by this extra-contractile, or calcium-sequestering, component. Muscle efficiency has, however, been established either from estimates of ATP splitting (chemical method) or from measurements of total energy output as heat (calorimetry) plus work. Both approaches have been found to yield values of 30–70 % for a variety of muscles including frog sartorius muscle (Woledge, 1989). Interestingly, and probably not unexpectedly, muscle efficiency under isotonic or isometric conditions varies between species (Woledge, 1989) and between muscle types (Kushmerick, 1983; Heglund and Cavagna, 1987; Woledge, 1989).

It seems more appropriate, however, to measure overall muscle efficiency when the muscle is contracting as it would *in situ* rather than isometrically or isotonically, conditions that are not directly relevant to locomotion (Johnston and Altringham, 1988). Swimming at a steady speed by fish is associated with sinusoidal cycles of shortening and lengthening by their myotomal muscles (Hess and Videler, 1984). Muscle power output under these conditions is a complex function of the amplitude of the length changes, the number and timing of nervous stimuli relative to the onset of shortening, and the cycle frequency (Altringham and Johnston, 1990*a*,*b*). These considerations have been employed to estimate power output in a synchronous insect muscle by Josephson (1985) and in myotomal muscles from the sculpin *Myoxocephalus scorpius* by Altringham and Johnston (1990*a*).

The purpose of this study was to determine the energetic costs of contraction in cod (*Gadus morhua*) fast myotomal muscle under conditions appropriate to locomotion. Fish are eminently suited to this approach, since muscle strains are known to be sinusoidal during steady swimming (Hess and Videler, 1984) and the different fibre types are anatomically discrete, enabling easy isolation of fast muscle fibre bundles (see Altringham and Johnston, 1990*b*). The cod was selected as a typical, readily available, subcarangiform swimmer, yielding stable muscle fibre preparations (Altringham and Johnston, 1990*b*). Energetic costs are defined in terms of economy of contraction (net positive work per unit energy expended). Efficiency, as defined by Woledge (1989), has been calculated assuming a Gibb's force free energy change for creatine phosphate hydrolysis of $55\,kJ\,mol^{-1}$. This paper examines economy under anaerobic conditions where the only free energy available for contraction is from ATP and creatine phosphate (CP).

Materials and methods

Mechanics

Cod, *Gadus morhua* L., were caught in the Firth of Forth during June–August, 1989, and kept in the laboratory in flow-through aquaria at 10 °C for up to 2 weeks before use. All experiments were carried out at 4±2 °C on fish 28.5–34.5 cm i

length. Fish were killed by a blow to the head and pithed. After total length and mass had been recorded, fibres were dissected from abdominal myotomes as previously described (Altringham and Johnston, 1988). Up to eight preparations were quickly dissected in chilled Ringer (composition in mmol l^{-1}: NaCl, 132.2; sodium pyruvate, 10; KCl, 2.6; $MgCl_2$, 1; $CaCl_2$, 2.7; $NaHCO_3$, 18.5; NaH_2PO_4, 3.2; pH 7.4, at 5 °C) to two or three times their final diameter, and immersed in circulating, aerated Ringer at 4 °C. Studies with isolated guinea pig cardiac muscle strips found that addition of pyruvate to the Ringer produced higher resting heat rates and contraction-related heat production than either glucose or lactate (Daut and Elzinga, 1989), although it had no effect on isometric force, the force–frequency relationship or relaxation rate in isolated mouse extensor digitorum longus (EDL) (Phillips and Woledge, 1989). Each preparation was then removed in turn to a cooled dissection platform and furnished with small aluminium foil clips on the myosepta of the adjacent myotomes at each end (Altringham and Johnston, 1988), the dissection was completed with frequent changes of Ringer, and the preparation returned to the aerated Ringer's solution. The final fast fibre preparations consisted of parallel bundles of fibres, 7–9.5 mm in length, with a mean (±S.E.) cross-sectional area of $0.838 \pm 0.04\ mm^2$. The preparations were left in this solution for at least a further 2 h. They were then transferred for at least 1.5 h to circulating Ringer's solution containing 0.5 mmol l^{-1} iodoacetic acid and bubbled with nitrogen to block both glycolytic and aerobic metabolism (Carlson and Siger, 1959).

A preparation was quickly transferred to a flow-through chamber containing the same Ringer's solution, one end was attached to a servo motor and the other to an isometric force transducer (AE 801, Sensonor, Horten, Norway) at the *in situ* rest length. Preliminary experiments established that this corresponded to the length for maximum force generation, and a sarcomere length of 2.4 μm, as measured by laser diffraction. Each preparation was then left in the chamber for 5 min before experimentation. The fibres were stimulated directly by means of two parallel platinum wire electrodes with a 2 ms supramaximal stimulus.

Kinematic analysis of swimming fish (Hess and Videler, 1984) shows that length changes of myotomal fibres during steady swimming describe a near perfect sine wave. Preparations were therefore subjected to sinusoidal length changes (strains), symmetrical about the *in situ* resting length, and stimulated at a selected phase in each cycle. Each preparation was subjected to either 8, 16, 32 or 64 oscillatory cycles, the chamber rapidly drained by aspiration at the end of the last cycle, and the preparation frozen *in situ* with a jet of liquid isopentane cooled in liquid nitrogen. A typical experiment is shown in Fig. 1A, in which a preparation is subjected to 32 cycles at a strain amplitude of ±5 % resting fibre length. By plotting force against muscle length for each cycle a loop is generated (Fig. 1B), the area of which is the net work performed during the cycle (see Josephson, 1985; Altringham and Johnston, 1990*a*). Power output is net work per cycle multiplied by frequency. The strain amplitude, the cycle frequency, the number of stimuli and the phase shift between the start of stimulation and the start of each strain

cycle were chosen to give maximum power output for preparations from each fish. These parameters were determined for different sizes of fish in a parallel series of experiments described in another paper (Altringham and Johnston, 1990*b*). Over the narrow range of fish sizes used in the present study, these parameters showed very little variation and were typically a strain of ±5 % resting fibre length, a cycle frequency of 5 Hz, and three stimuli (at 50 Hz) starting 40° after the start of each cycle (full cycle=360°, commencing at stretch from rest length). A representative optimum power output against cycle frequency curve is shown in Fig. 1C. One or two preparations from each fish were used as controls, and frozen without being stimulated or made to perform work. The remainder were subjected to 8, 16, 32 or 64 work cycles before being frozen. Any additional preparations were usually used as duplicates. Mechanical work and power output are, by convention, presented per unit wet mass; to convert to dry mass, these values should be multiplied by 7.69. The experiments were controlled through a microcomputer (IBM compatible), and the data collected and analysed on-line using in-house software.

Biochemical assays

Once frozen, fibres were transferred from the stimulation chamber to 1.5 ml Eppendorf tubes filled with liquid isopentane. The fibres were freeze-dried overnight, allowed to achieve room temperature under vacuum, and weighed to the nearest 10 μg using a micro balance (Mettler, ME22). The fibres (0.2 to 2.2 mg dry mass) were placed in a 1 ml Duall 20 tissue grinder, 250–300 vols of 0.3 mol l^{-1} perchloric acid was added, and the fibres homogenized on ice over a 15 min period. A known volume of the homogenate was removed to a microcentrifuge tube, neutralized with a known volume of 1 mol l^{-1} KOH (pH checked with litmus paper), a glycogen sample removed, and the remainder centrifuged for 2 min at high speed. The supernatant was immediately assayed for muscle metabolites.

Creatine (C), creatine phosphate (CP), adenine nucleotides (AMP, ADP, ATP) and IMP were assayed in a single injection using the HPLC method of Sellevold *et al.* (1986). The HPLC consisted of a Gilson pump model 303, Data Master model 620, a holochrome ultraviolet detector and an ODS-YMC (5 μm packing) 150 mm×4.6 mm column (Capital HPLC Specialists, Edinburgh, Scotland; equivalent to Supelcosil used by Sellevold *et al.* 1986). The muscle metabolites were eluted isocratically with a mobile phase consisting of 215 mmol l^{-1} potassium dihydrogen phosphate, 2.3 mmol l^{-1} tetrabutylammonium hydrogen sulphate (Sigma Chemical Co., London) and 3.5 % acetonitrile, adjusted to pH 6.25 with KOH; the solution was filtered through a 2 μm cellulose acetate filter and degassed under helium for 20 min. The flow rate was set at 1 ml min^{-1} and the detector at 206 nm; a typical elution required a 15 min run time. Each column was calibrated with a range of authentic standards and standard curves based upon peak area were established. Each peak was baseline-integrated using the Systems Manager 704 software and an Apple IIe computer. Standards were run daily, but column deterioration was slight, until just before column collapse (300–500 injections). Both pyruvate and lactate were eluted using this system, but detector sensitivity to

these compounds was low relative to that for the nucleotides and they could thus be ignored. Each muscle sample was run in duplicate, peak areas were compared to the appropriate standard curves and the separate values were then averaged (variation did not exceed 10 %) to give concentration in μmol g^{-1} dry muscle mass±S.E. (N preparations).

Glycogen was enzymatically assayed as glucose following amyloglucosidase hydrolysis (Bergmeyer, 1974). Free creatine was assayed in the muscle samples using the α-naphthol assay of Eggleton *et al.* (1943) and found to give values comparable to those reported using the HPLC method. Apparent equilibrium constants for creatine kinase (K_{CK}) and adenylate (myo)kinase (K_{AK}) were estimated under the conditions of these experiments according to Sahlin *et al.* (1975).

Statistics

To determine whether cycle number significantly modified the mean concentration of each metabolite, a single-factor analysis of variance (one-way ANOVA) followed by a Dunnett's test to isolate the specific differences between means was performed (Zar, 1974). Equilibrium constants and values of muscle economy were tested non-parametrically using the Mann–Whitney U-test.

Results

Mechanics

Records from a representative experiment are shown in Fig. 1. In Fig. 1A force and length records are shown plotted against time, and in Fig. 1B force has been plotted against fibre length for the selected loops indicated in (Fig. 1A). Both maximum force and net work per cycle decreased by about 40 % initially, before approaching a steady state after about 12 cycles. In contrast, under optimum conditions, force and net work are essentially constant for fast and slow fibres from the sculpin, *Myoxocephalus scorpius* (Altringham and Johnston, 1990*a*), and for slow fibres of the cod (*Gadus morhua*; J. D. Altringham and I. A. Johnston, unpublished observations). Force and net work in some cases remained steady for up to 64 cycles, but more commonly began to decline slowly after about 30 cycles. This is illustrated in Fig. 2A, in which work per cycle is plotted against cycle number for four preparations from the same fish, subjected to different numbers of cycles. If an experiment was interrupted briefly (4 s), force and net work recovered, often to levels close to those achieved at the beginning of the experiment, before declining quickly to pre-interruption levels and showing a steady decline thereafter (Fig. 2B).

Mean power output was not dependent on the number of work cycles performed (Table 1), and the mean maximum power output of all preparations over the first four cycles was 16.1±1.1 W kg^{-1} (N=34). Values were quite variable, ranging from 34 to 6 W kg^{-1}. Since the values were derived from dry masses, no correction was made for connective tissue content or for the presence of a small number of

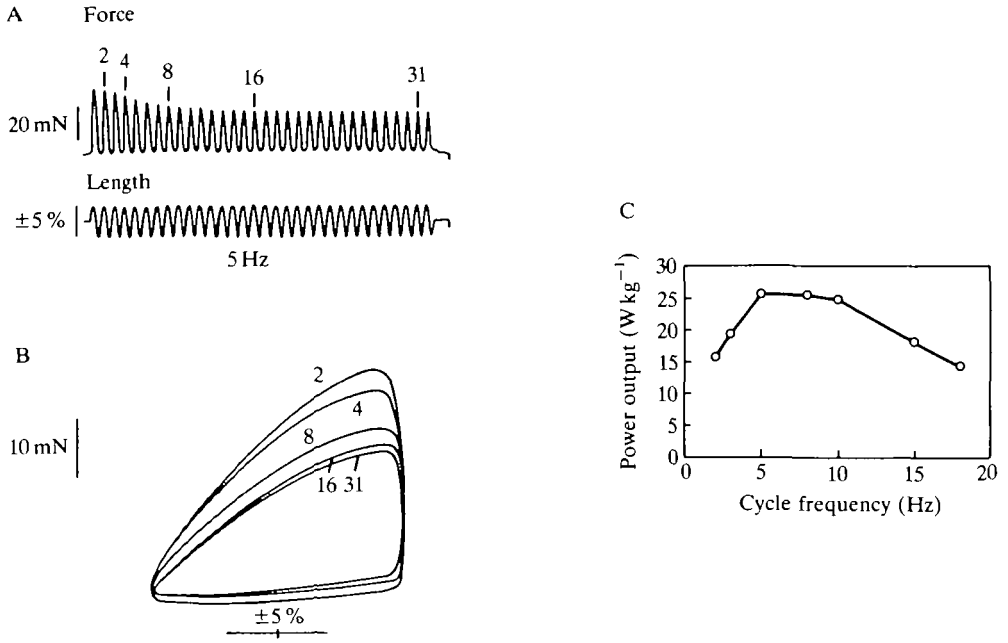

Fig. 1. The performance of oscillatory work by muscle fibre bundles from *Gadus morhua* L. (A,B) Fast muscle fibre preparation subjected to 32 sinusoidal length change cycles of ±5 % resting fibre length at 5 Hz; three supramaximal stimuli (at 50 Hz) were given 40° after the start of each cycle from rest length. In A, force and muscle length have been plotted against time; in B, force has been plotted against muscle length to produce a work loop for selected cycles indicated in A. (C) Optimum power output plotted against cycle frequency for a preparation isolated from a 28.5 cm fish.

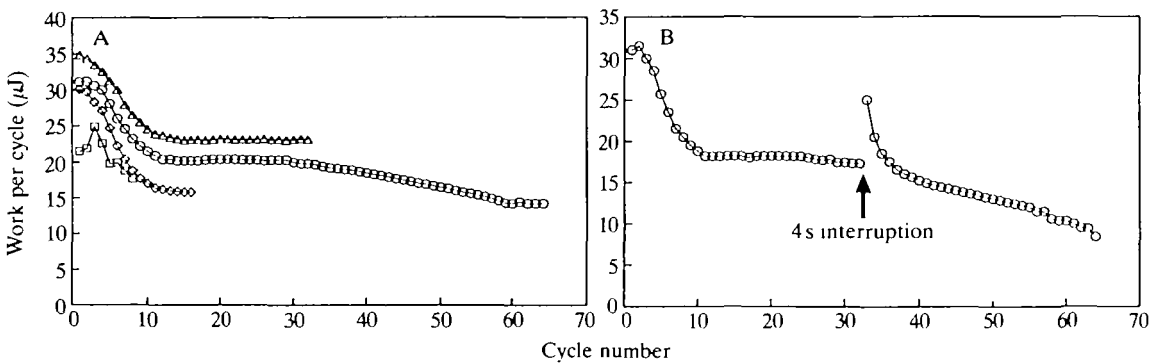

Fig. 2. (A) Work per cycle plotted against cycle number from four preparations subjected to 8, 16, 32 and 64 cycles. (B) A similar plot from a preparation subjected to 64 cycles, interrupted for 4 s after cycle 32.

Table 1. *Mechanical work, power output and glycogen content of cod fast muscle fibre bundles as a function of work cycles performed*

Cycle number	Mechanical work*	Power output†	Glycogen content‡
Control§	–	–	118.9±8.0 (9)
8	20.2±3.1 (9)	13.3±2.3 (9)	108.4±5.2 (7)
16	44.3±6.4 (9)	12.2±1.8 (9)	110.2±8.5 (7)
32	72.0±7.5 (9)	11.6±1.2 (9)	146.0±9.5 (8)
64	128.8±15 (7)	10.1±1.3 (7)	111.9±11 (6)

* $J\,kg^{-1}$ wet mass±S.E. (N), all values significantly different ($P<0.05$, Dunnett's test) from the preceding value.

† $W\,kg^{-1}$±S.E. (N) calculated over all cycles.

‡ $\mu mol\,g^{-1}$ dry mass±S.E. (N).

§ Nine fish in the control group; N values represent preparations from different fish, although all work cycles could not necessarily be accomplished on preparations from one individual.

dead fibres. As expected, total mechanical work increased roughly in proportion to the number of cycles performed.

Under optimum conditions, most preparations were able to relax almost completely between cycles, with force returning to zero. In some, relaxation was incomplete and minimum force during cyclical work rose to a typical plateau of 0–5 % maximum force. The maximum extent of this muscle 'tone' is illustrated by the record in Fig. 3C.

Biochemical studies

Levels of adenylates were not significantly dependent upon the number of work cycles performed (Fig. 4), even though both AMP and ATP concentrations tended to decrease with cycle number in individual experiments (Fig. 3B,D). This trend towards a decreased AMP concentration is reflected as an increase in IMP concentration, which was significantly higher following 64 oscillatory cycles (Figs 3D, 4). The apparent adenylate kinase equilibrium constant decreased, but this change was not significant at any cycle frequency compared to the control preparation. Energy charge values ([ATP]+0.5 [ADP]/[ATP]+[ADP]+[AMP]) ranged between 0.73 and 0.93, with an average of 0.86±0.05 (N=39) (data not presented).

Total creatine (C_T) concentration was not significantly affected by cycle number, but the increase in creatine (C) and the decrease in creatine phosphate (CP) were highly significantly correlated with the number of work cycles ($P<0.001$, one-way ANOVA) (Fig. 5). In addition, values for each cycle number were significantly different from controls for both C and CP, but the only differences between stimulated fibres occurred between 32 and 64 cycles ($P<0.05$, Dunnett's test). These differences in C and CP are reflected in an increase in the apparent equilibrium constant for creatine kinase, which became significantly different from the control value and 32 cycles at 64 cycles.

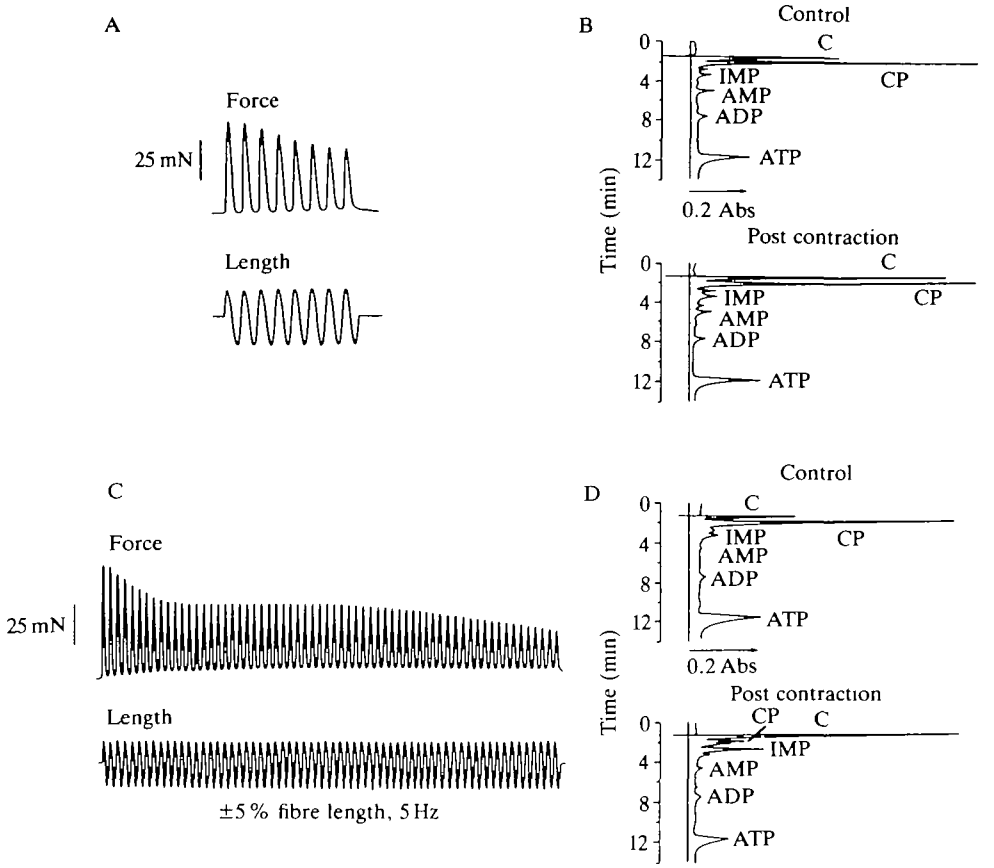

Fig. 3. (A,C) Force and muscle length records from two preparations subjected to 8 and 64 cycles. (B,D) Corresponding chromatograms from muscle fibres extracted after performing work, together with chromatograms from control preparations. 0.2 Abs, absorbance scale.

There was no significant depletion of muscle glycogen under any condition tested (Table 1), providing evidence that glycolysis was not activated under the iodoacetic acid plus N_2 block used in this experiment. Given the magnitude of the S.E. values of the glycogen estimates (Table 1), however, it is possible that the contribution of glycogen metabolism to energy expended would be undetectable.

Muscle contraction economy was calculated as the work performed per unit chemical energy expended. Since concentrations of muscle adenylates (Fig. 4) and of total creatine (Fig. 5) did not change significantly in the muscle at any work cycle value, the total chemical energy expended should be equivalent to the difference in CP concentrations between control and each cycle number (i.e. CP splitting; Fig. 5). There were, however, general decreases in muscle ATP and AMP concentrations, and increases in IMP concentration (especially at 64 cycles). The total chemical change, therefore, was calculated as the sum of the differences (number of cycles minus control) in the adenylates considering the number of

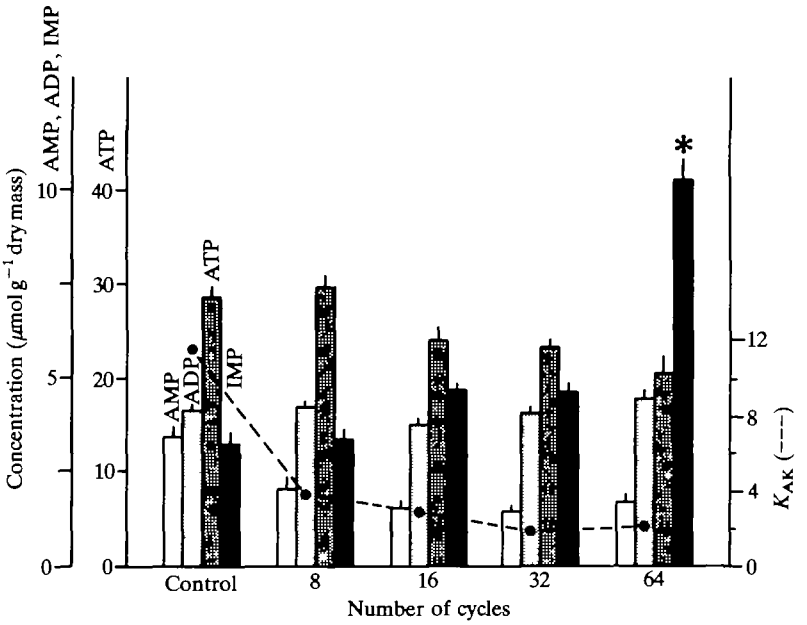

Fig. 4. Concentrations (mean±S.E.) of adenylates and IMP in control muscle and muscle fibre bundles subjected to 8, 16, 32 and 64 cycles of oscillatory work. K_{AK} is the apparent adenylate kinase equilibrium constant. * Significantly different from value at 8 cycles ($P<0.005$, Dunnett's test).

high-energy phosphates (3ATP, 2ADP, AMP) and IMP, plus the mean CP splitting (sum of changes in CP+C divided by 2). The estimates of economy are presented in Fig. 6; these values were not significantly different from those calculated using only CP lost or CP lost plus IMP gained. Muscle economy was independent of cycle number, although a trend to increased economy with increasing cycle number was noted (Fig. 6).

Discussion

This study was designed to estimate the energy cost of contraction in fish muscle under conditions approximating those found in the swimming animal. For anterior myotomes, maximum force is thought to coincide with the onset of shortening and positive work is performed throughout each cycle (Hess and Videler, 1984). The strain amplitude, stimulation phase and cycle frequency required to produce maximum positive work for cod fast muscle fibres were determined as previously described (Altringham and Johnston, 1990*a*,*b*). These studies have shown that twitch duration is reduced and force is higher with oscillatory contractions than under isometric conditions (Altringham and Johnston, 1990*b*).

As previously reported (Altringham and Johnston, 1990*b*), in cod fast muscle fibres the amount of positive work per cycle decreased rapidly over the initial

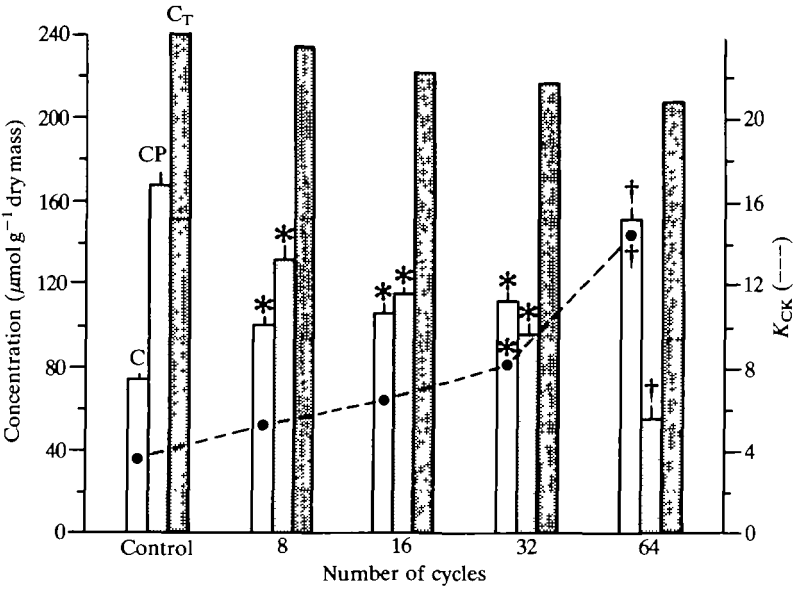

Fig. 5. Concentrations (mean±S.E.) of creatine (C), creatine phosphate (CP) and total creatine (C_T) in control muscle and muscle fibre bundles subjected to 8, 16, 32 and 64 cycles of oscillatory work. K_{CK} is the apparent creatine kinase equilibrium constant. * Significantly different from control ($P<0.01$, Dunnett's test for C and CP; Mann–Whitney U-test for K_{CK}); † significantly different from control and 32 cycles ($P<0.01$, Dunnett's test for C and CP; Mann–Whitney U-test for K_{CK}).

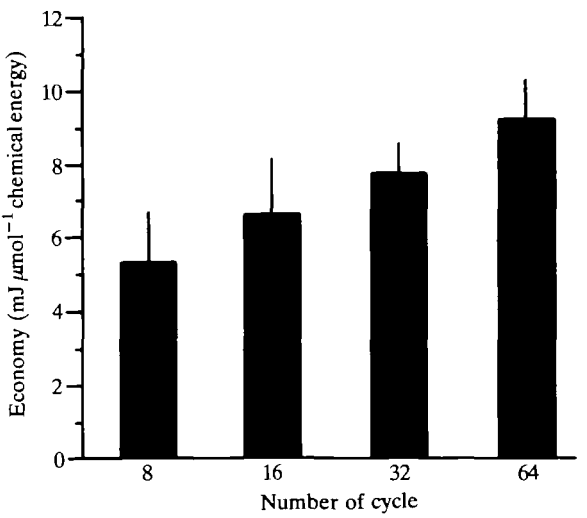

Fig. 6. Economy, defined as positive work (mJ) per total chemical energy (μmol) expended (see Results) as a function of the number of work cycles performed in cod fast fibre bundles. N values are 7 for 8, 32 and 64 cycles, and 6 for 16 cycles. Bars represent S.E.

cycles and then showed a slow decline (Fig. 2A). Force is relatively stable between the tenth and thirtieth cycle. Why do force and net work decline over the initial work cycles in cod fast fibres, in contrast to the results obtained from other preparations (Altringham and Johnston, 1990*a*; J. D. Altringham and I. A. Johnston, unpublished observations on cod slow fibres)? The rapid recovery after a brief interruption to the work cycles, and the subsequent rapid decline (Fig. 2B) suggest a mechanical rather than a metabolic mechanism. The decline is not simply fatigue; at low cycle frequencies (0.5–2 Hz), with up to 12 stimuli per cycle, high values of force and net work per cycle are maintained. The rate of decline of force increases with cycle frequency (see Altringham and Johnston, 1990*b*), and may be related to a velocity-dependent, shortening deactivation.

Concentrations of ATP, ADP and IMP measured using HPLC analysis were within the range reported for fast fibres of other teleosts using conventional chemical techniques (e.g. Johnston *et al.* 1983; Dobson and Hochachka, 1987; van Waarde *et al.* 1990). AMP levels tended to be higher, even though the HPLC analysis was equally sensitive to all of these compounds. Creatine (C), CP and C_T values were similar to values reported by van Waarde *et al.* (1990) in carp, goldfish and tilapia using ^{31}P nuclear magnetic resonance (n.m.r.) and chemical estimates of C_T. Estimates of CP exceeding 70 % of C_T, as in this study and that of van Waarde *et al.* (1990), are very much higher than the 40 % reported in other studies (e.g. tilapia, Johnston *et al.* 1983; rainbow trout, Dobson and Hochachka, 1987). Such differences are generally related to the active muscle creatine kinase enzyme and the lag time in freezing tissues during *in vivo* experiments (e.g. Dobson and Hochachka, 1987; van Waarde *et al.* 1990), problems not encountered with the much faster freezing quench used in the present study. These high values of CP and adenylates provide support for the biochemical competence of these cod fast fibre preparations.

Oscillatory work resulted in a work-dependent increase in muscle fibre C content and decrease in CP without any significant change in C_T (Fig. 5). As the number of cycles exceeded 32, a sharp increase in the apparent K_{CK} occurred. Sahlin *et al.* (1975) reported a linear decrease in muscle pH (m. quadriceps femoris of man) as $\log K_{CK}$(apparent) increased; since there is no significant change in either ATP or ADP in our study (Fig. 4), we can speculate that this increase in the apparent K_{CK} may have resulted from an acidification of cod muscle fibres during oscillatory work. Metabolic acidosis linked to CP hydrolysis has been observed during exercise *in vivo* in several teleost species (e.g. Dobson and Hochachka, 1987). Van Waarde *et al.* (1990) also demonstrated using ^{31}P n.m.r. that CP and pH are linked during anoxia and recovery from anoxia in carp, goldfish and tilapia.

No significant changes in adenylate concentrations were observed in the isolated cod fast fibre preparation even at 64 cycles (Fig. 4), where CP falls to 26 % compared to the control value of 70 % of C_T (Fig. 5). The apparent K_{AK} decreased, but any significant change was obscured by the variability in the individual adenylate concentrations. These data suggest that, under these conditions, creatine kinase buffers the ATP pool or, at least, that changes in the rate

of ATP utilization do not exceed the capacity of this enzyme to meet ATP demands in these isolated fibres. This buffering capacity is species-dependent, as recently shown by van Waarde *et al.* (1990), being highest in goldfish and tilapia, but much lower in carp. The ability of cod fast fibres to buffer ATP changes may reflect the continuous swimming behaviour of this species, but it also demonstrates that the work performed by these fibres does not lead to exhaustion. Studies of teleosts exercised to exhaustion show significant changes in muscle ATP content (e.g. Dobson and Hochachka, 1987).

IMP content rose after 64 cycles of work (Fig. 4), implicating a functional 5′-AMP deaminase in the cod fast fibre preparation. A number of studies have demonstrated a 1:1 stoichiometry between increases in IMP concentration and decreases in the concentration of this adenylate pool. Generally this is related to the maintenance of energy charge and/or glycolytic flux (Driedzic and Hochachka, 1976). Since these cod fibres were poisoned, changes in IMP levels provide a mechanistic explanation for the maintenance of energy charge.

Muscle economy, as defined in this study, was independent of the number of work cycles performed, although a trend to increase was apparent (Fig. 6). Heglund and Cavagna (1987) defined economy as the 'normalized tension–time integral divided by weight specific net energy expenditure' and they reported that rat soleus was about twice as economical as the EDL over the range of shortening velocities tested. The values reported here cannot be compared with those of Heglund and Cavagna (1987), given the different methods of estimation, or with those for any other muscle, simply because values are not available. It is possible, however, to calculate efficiency (positive work done divided by the free energy input) using a value for the Gibbs force free energy change for CP hydrolysis *in vivo* ($55\,kJ\,mol^{-1}$ at pH 7, pMg 3; Woledge and Reilly, 1988). Values are 12.4, 22.8, 19.4 and 20.9 % for 8, 16, 32 and 64 cycles, respectively. These values are lower than values for maximum efficiency derived from heat and work measurements of tortoise (67 %), frog (37 %) and dogfish (30 %) fast muscles during isotonic shortening (Woledge, 1989). The rather different conditions of the present study were chosen for maximum power output, and are almost certainly different from those for maximum efficiency during oscillatory contractions.

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