The Journal of Experimental Biology 205, 697–706 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
JEB3810

How the efficiency of rainbow trout (*Oncorhynchus mykiss*) ventricular muscle changes with cycle frequency

Claire L. Harwood*, Iain S. Young† and John D. Altringham

School of Biology, University of Leeds, Leeds LS2 9JT, UK

*Present address: Department of Biology, Leidy Laboratory, University of Pennsylvania, Philadelphia, PA 19104, USA
Author for correspondence (e-mail: charwood@sas.upenn.edu)

†Present address: Department of Veterinary Preclinical Science, Faculty of Veterinary Science, University of Liverpool, Liverpool L69 7ZJ, UK

Accepted 17 December 2001

Summary

Different species of animals require different cardiac performance and, in turn, their cardiac muscle exhibits different properties. A comparative approach can reveal a great deal about the mechanisms underlying myocardial contraction. Differences in myocardial Ca^{2+} handling between fish and mammals suggest a greater energy cost of activation in fish. Further, while there is considerable evidence that heart rate (or cycle frequency) should have a profound effect on the efficiency of teleost cardiac muscle, this effect has been largely overlooked. We set out to determine how cycle frequency affects the power output and efficiency of rainbow trout (*Oncorhynchus mykiss*) ventricular muscle and to relate this to the heart's function in life. We measured power output and the rate of oxygen consumption ($\dot{V}_{O_2}$) and then calculated efficiency over a physiologically realistic range of cycle frequencies.

In contrast to mammalian cardiac muscle, in which $\dot{V}_{O_2}$ increases with increasing heart rate, we found no significant change in $\dot{V}_{O_2}$ in the teleost. However, power output increased by 25 % as cycle frequency was increased from 0.6 to 1.0 Hz, so net and total efficiency increased. A maximum total efficiency of 20 % was achieved at 0.8 Hz, whereas maximum power output occurred at 1.0 Hz. We propose that, since the heart operates continuously, high mechanical efficiency is a major adaptive advantage, particularly at lower heart rates corresponding to the more commonly used slower, sustainable swimming speeds. Efficiency was lower at the higher heart rates required during very fast swimming, which are used during escape or prey capture.

If a fixed amount of Ca^{2+} is released and then resequestered each time the muscle is activated, the activation cost should increase with frequency. We had anticipated that this would have a large effect on the total energy cost of contraction. However, since $\dot{V}_{O_2}$ remains constant, less oxygen is consumed per cycle at high frequencies. We suggest that a constant $\dot{V}_{O_2}$ would be observed if the amount of activator Ca^{2+} were to decrease with frequency. This decrease in activation energy is consistent with the decrease in the systolic intracellular Ca^{2+} ($[Ca^{2+}]_i$) transient with increasing stimulation frequency seen in earlier studies.

Key words: cardiac muscle, ventricle, muscle, fish, rainbow trout, *Oncorhynchus mykiss*, efficiency, work, power, frequency, oxygen consumption.

Introduction

Heart rate has a profound effect on cardiac muscle performance. Among others, Layland et al. (1995) demonstrated that power output increases with cycle frequency (analogous to heart rate) in mammalian cardiac muscle. Power output has also been shown to depend on cycle frequency in isolated trout ventricular preparations undergoing cyclic contractions (Harwood et al., 1998; Shiels et al., 1998) and in perfused fish hearts under conditions chosen to 'maximise cardiac performance' (Farrell et al., 1989). Harwood et al. (1998) found that maximum power output occurred at approximately 1.1 Hz or 66 beats min^{-1}. This was consistent with observations of 'peak cardiac performance' at approximately 70 beats min^{-1} in an *in situ* trout heart (Farrell et al., 1996). In mammalian cardiac muscle, efficiency has been shown to co-vary with power output as cycle frequency increases. However, while there have been many studies that demonstrate that the rate of energy consumption increases with heart rate in mammalian cardiac muscle (e.g. Van Beek and Westerhof, 1991), the effect of heart rate on the efficiency of teleost cardiac muscle has not been studied. Further, there is conflicting information regarding the relationship between cardiac power output and efficiency in teleost cardiac muscle.

Several studies have looked at the relationship between power output and oxygen consumption of perfused fish hearts. Typically, the rate of oxygen consumption increased linearly with cardiac power output. Graham and Farrell (1990) found

the mechanical efficiency (25 %) of the trout heart to be largely independent of power output at 15 °C; however, some interdependence was demonstrated at lower temperatures (5 °C). In contrast, Farrell et al. (1985) found that mechanical efficiency increased from 15 % to a maximum of 21 % as power output was increased by increasing the preload (volume loading) of the heart. Similarly, Davie and Franklin (1992) found that the mechanical efficiency of the dogfish heart increased before levelling off at high cardiac power outputs.

The comparison between energetic cost in mammalian and teleost cardiac muscle is particularly tempting because of their differences, particularly in their ultrastructure and the way that each regulates $[Ca^{2+}]_i$. Fish cardiac muscle has a less extensive sarcoplasmic reticulum (SR) and no T-tubules (Santer, 1985). This structural difference is reflected by the physiology; in mammals, up to 95 % of the activator Ca^{2+} is released from the SR (Bers, 1991). However, in fish, Ca^{2+} influx across the sarcolemma (SL) could account for around 85 % of activator Ca^{2+} (Aho and Vornanen, 1999; Harwood et al., 2000). Activation metabolism has been examined in amphibian cardiac muscle which, like fish muscle, has a relatively sparse SR, and the energy cost of activation was found to be higher than in mammalian cardiac muscle (Holroyd and Gibbs, 1993). Holroyd and Gibbs (1993) proposed that the higher cost of activation, which accounts for between 10 and 30 % of the total energy cost of contraction (Gibbs, 1995), was higher because the SL Ca^{2+}-ATPase utilises one ATP per Ca^{2+} removed, whereas the SR removes two Ca^{2+} per ATP.

Myocardial efficiency has been measured in intact heart preparations in a variety of species from diverse classes of animals such as rat (Neely et al., 1967), octopus (Agnisola and Houlihan, 1991) and sea raven *Hemitripterus americanus* (Farrell et al., 1985). In intact preparations, mechanical efficiency is usually determined by dividing cardiac power output by the rate of myocardial oxygen consumption. While these studies provide valuable information about the mechanical and metabolic performance of the working heart, they provide only indirect information about the muscle itself. Precise measurements of muscle energetics can only be made using isolated preparations (Barclay, 1999). The energetics of isolated mammalian cardiac muscle has been studied using measurements of oxygen consumption (e.g. Whalen, 1961; Hisano and Cooper, 1987), heat production (e.g. Gibbs et al., 1967; Holroyd and Gibbs, 1993; Mellors et al., 2001) and fluorometry (Chapman and Gibbs, 1975; Ebus and Stienen, 1996). Generally, these studies have used isometric (Hisano and Cooper, 1967), isovelocity (Holroyd and Gibbs, 1993) or isotonic (Whalen, 1961; Holroyd and Gibbs, 1993) contraction protocols. Most of these studies found mechanical efficiencies during shortening of between 10 and 30 % (for reviews, see Gibbs, 1978; Suga, 1990).

Several studies have measured the efficiency of skeletal muscle using physiologically realistic protocols in which the muscle is exercised using the work-loop technique (Josephson, 1985). The efficiencies of isolated frog and rat locomotory muscles undergoing repeated 'stretch–shortening' cycles (Heglund and Cavagna, 1987) and of insect flight muscle undergoing sinusoidal cyclic length changes (Josephson and Stevenson, 1991) have been determined. Heart muscle undergoes cycles of extension and contraction as it pumps blood around the body. However, only a few studies have examined the metabolic energy cost of work production in cardiac muscle during cyclic length changes; for example, frog ventricular trabeculae (Syme, 1994) and rat papillary muscle (Baxi et al., 2000; Mellors et al., 2001; Mellors and Barclay, 2001).

In the present study, mechanical power output was measured in a trout ventricular preparation under conditions that simulate those *in vivo* using the work-loop technique. The effect of cycle frequency on rates of oxygen consumption and efficiency was investigated using cycle frequencies within the physiological range, including those shown to give maximum work and power output (Harwood et al., 1998). Energy use was estimated from the rate of oxygen consumption above the resting level associated with the muscle doing work, and efficiency was calculated. The rate of oxygen consumption reflects the total cost of muscle activation and crossbridge processes; it therefore provides a good measure of the total energy consumption during work (Josephson and Stevenson, 1991).

Materials and methods

Muscle preparation

Female rainbow trout [*Oncorhynchus mykiss* (Walbaum)] (287±13 g, N=20) purchased from Washburn Valley Trout Farm, North Yorkshire, UK, were maintained for up to 12 weeks in 2 m diameter tanks at between 12 and 15 °C on a 16 h:8 h light:dark photoperiod and fed commercial trout pellets *ad libitum*. Preparation of the ventricular muscle has been described by Harwood et al. (1998). Briefly, trout were killed by a sharp blow to the head and double-pithed in accordance with local and Home-Office-approved protocols. The heart was removed quickly and rinsed with oxygenated Ringer's solution (composition in $mmol^{-1}$: NaCl, 124; KCl, 3.1; $CaCl_2$, 2.5; $MgSO_4$, 0.9; Tes sodium salt 11.8; Tes free acid, 8.2; sodium pyruvate, 5.0; pH 7.8±0.5 at 15 °C) at 10 °C. Ventricular strips (mean length 7.5±0.1 mm, mean wet mass 12.7±0.7 mg; N=27) were dissected from the ventral vertex of the heart. The Ringer's solution was changed frequently and allowed to warm up gradually to approximately 15 °C.

Each end of the preparation was tied to a link of gold chain with 5-0 gauge suture thread, and the free tissue outside the tied ends was trimmed close to the knots. One end of the preparation was attached to a force transducer (AE801, SensoNor, Horten, Norway) and the other to a servomotor. Muscle length was increased until the passive force rose to just above zero (<0.1 mN) to take up the slack in the preparation. The preparation was allowed to recover for at least 1 h after mounting in the chamber, which was circulated with Ringer's solution saturated with 100 % oxygen at 15 °C.

Oxygen chamber

The oxygen chamber (Fig. 1) was constructed from Kel-F

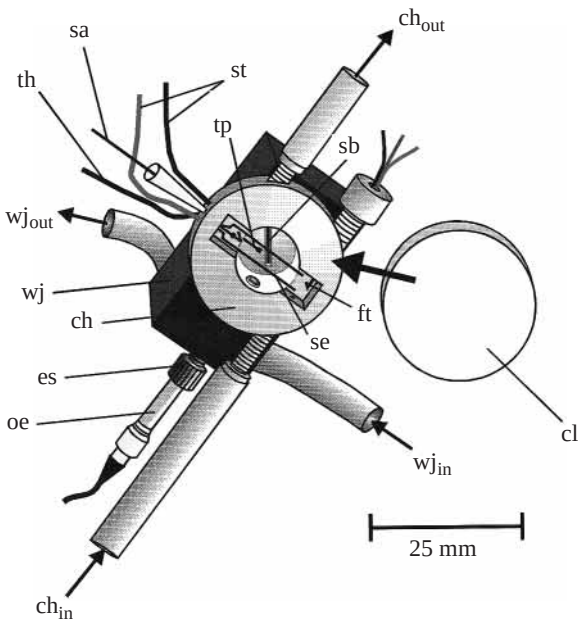

Fig. 1. The chamber assembly. The oxygen electrode (oe) was screwed into the chamber body by means of a membrane sleeve (es). Ringer's solution flowed through the chamber *via* inlet (ch_{in}) and outlet (ch_{out}) studs made of Kel-F. A water jacket (wj) surrounded the chamber (ch) and oxygen electrode. Water entered the jacket through a side port (wj_{in}) and exited at the rear of the jacket (wj_{out}). The chloride-coated stimulating electrodes (se) were connected *via* wires (st) to a Grass S48 stimulator. A temperature probe (tp) was located in the chamber cup and connected to a K-type thermometer (th). The muscle was attached at one end to a servo arm (sa) and at the other end to a force transducer (ft) mounted in a Kel-F stud. A glass-encapsulated stir bar (sb) lay in the cup of the chamber and was used to mix the Ringer's solution. The glass lid (cl) was sealed using silicon high vacuum grease.

(Polychlorotrifluoroethylene, 3M). Kel-F has near-zero moisture absorption (<0.01 % in 24 h) so its absorption and desorption of oxygen is minimal. Ringer's solution was circulated through the chamber at 30 ml min^{-1}. Chamber temperature was maintained at 15 °C. The chamber assembly (chamber body and water jacket) was orientated (Fig. 1) so that the outlet port was at the highest point to ensure that all air bubbles were flushed out of the chamber upon filling. Supramaximal (2 ms) square-wave pulses were delivered by two parallel, chloride-coated, silver stimulating electrodes. Stimulation artefacts (e.g. Baskin, 1961; Kushmerick and Paul, 1976; Heglund and Cavagna, 1987) were not apparent unless the electrode membrane was leaky or the integrity of the chloride layer on the oxygen electrode or the stimulus wires was compromised. In experiments without the muscle in the chamber, stimulation had no effect on the oxygen baseline.

The servomotor arm passed though a narrow tube filled with silicone grease to give a gas-tight seal. The chamber temperature was controlled by an aluminium water jacket (Diamond General Development Corporation, Ann Arbor, MI, USA). The temperature of the water circulating through the jacket was controlled using a refrigerated water circulator (Grant, UK) and monitored using a K-type thermocouple inside the chamber. The water jacket and chamber were insulated with foam to provide further thermal stability.

The volume of the fully assembled chamber without the muscle was determined by two methods: injecting water into the chamber using a syringe graduated in 0.01 ml increments until the chamber was full, and as the increase in chamber mass when filled with water. These gave chamber volumes of 1.31 and 1.3055 ml, respectively; an average of 1.308 ml was used in efficiency calculations.

To minimise bacterial oxygen consumption, each day the chamber was first flushed through with 100 % alcohol and then with approximately 500 ml of filtered, deionised water that had been sterilised by exposure to ultraviolet light.

Electrode characteristics

A bipolar Electro-Tech 730 Clark-style electrode with a (25.4 μm thick) polyethylene membrane connected to a Chemical Microsensor II (Diamond General Development Corporation, Ann Arbor, MI, USA) was used to measure dissolved P_{O_2}. The electrode had a time constant of approximately 4 s and a temperature coefficient of 5 % $°C^{-1}$. The temperature was controlled using Grant refrigerated circulators accurate to 0.01 °C. A thermistor, accurate to 0.01 °C, showed temperature to fluctuate by less than 0.04 °C h^{-1}. Using a temperature coefficient of 5 % $°C^{-1}$ (50.65 kPa $°C^{-1}$), this fluctuation would produce an apparent change in partial pressure of oxygen of 2.026 kPa, approximately 10 % of the typically 20.26 kPa decline in oxygen partial pressure while the muscle is performing work. However, the error is probably much less than this as the temperature drift was assessed over the course of an hour whereas oxygen consumption was determined over 5–10 min. This is supported by the consistency and reproducibility of the recordings.

Measurement of mechanical work

The length for maximum isometric force production (L_{max}) was determined by increasing muscle length in steps of 0.2 mm until active force reached a maximum. The length for maximum work production (L_{opt}) in rainbow trout ventricular muscle peaks at 100 % L_{max} (Harwood et al., 1998). However, as Harwood et al. (1998) showed no significant difference between the work output at 95 and 100 % L_{max}, muscle length was reduced to 95 % L_{max} to avoid damage to the preparation from over-stretching during cyclic activity. Work-loop experiments (Josephson, 1985) were carried out over a range of cycle frequencies (0.6, 0.8, 1.0 and 1.2 Hz) within the physiological range of heart rates found in trout (0.3–1.5 Hz) (Priede, 1974). As in previous studies (Syme, 1994; Harwood et al., 1998; Baxi et al., 2000; Mellors et al., 2001), a sinusoidal wave pattern was chosen as a model for the cardiac waveform. Data from another study in this laboratory suggest that this is a good approximation, particularly at frequencies close to those yielding maximum power and efficiency (C. L. Harwood, I. S. Young and J. D. Altringham, in preparation). In addition, Mellors and Barclay (2001) noted that the energy cost per

twitch varied little with strain pattern in rat papillary muscle. Strain and phase parameters were taken from Harwood et al. (1998) to give maximum work output at each frequency employed.

Oxygen consumption

At the onset of each experiment, the flow of Ringer's solution through the chamber was turned off. This produced a large initial fall in the electrode current, which stabilised after 5 min. The remaining steady decline in P_{O_2} was due to the resting oxygen consumption of the muscle, the chamber and the electrode oxygen. After this stable level had been maintained for 10 min, the muscle was subjected to work-loop cycles. Three hundred cycles (5 min at 1.0 Hz) produced a sufficient change in P_{O_2} for accurate measurement. Therefore, work loops were performed for 5 min at 0.6 and 0.8 Hz; however, because of limitations of the A/D card, only 300 loops could be performed. Consequently, at 1.2 Hz, the muscle was oscillated for 4.17 min (300 cycles). It is unlikely that the shorter period affects efficiency (Syme, 1994; Josephson and Stevenson, 1991). After performing work, the resting rate of oxygen consumption was recorded for a further 5–10 min.

Throughout the experiment, force, length and chamber P_{O_2} were captured on-line. Each preparation was subjected to a single procedure to minimise errors in the calculation of efficiency under the different experimental regimes because it is uncertain how a decline in the mechanical performance of the muscle with increasing cumulative work may alter its efficiency. On completion, the muscle was weighed, and the cross-sectional area was calculated gravimetrically (Harwood et al., 1998). Mass-specific work, power and oxygen consumption were calculated.

Calculations

Total net work and power

Total net work output was calculated as the sum of the work for all the individual work loops during a trial. Total power output (E_p) was calculated and expressed as $J\,min^{-1}\,kg^{-1}$.

Oxygen solubility

The salinity of the Ringer's solution was determined using a conductivity meter (Hanna, HI 9033 Multirange) to be 7.06 ‰ using a linear regression equation fitted to a plot of conductivity *versus* salinity. The molar solubility of oxygen in the Ringer's solution as a function of salinity and temperature (15 °C) was 15.41 $mol\,O_2\,ml^{-1}\,kPa^{-1}$ at STPD (Cameron, 1986).

Correction for chamber leak

As in previous studies (e.g. Syme, 1994), the net rate of oxygen consumption was determined as the change in P_{O_2} above the resting rate of oxygen consumption of the muscle, while the muscle was performing work, minus other sources of oxygen consumption (i.e. the external oxygen leak, oxygen consumption by the chamber and oxygen electrode). These were estimated by measuring the decline in P_{O_2} in the absence of a muscle. A sixth-order polynomial regression provided an excellent fit to the data (r^2=0.92, P<0.001, N=8), and this was used to calculate a correction for this decline. This correction resulted in a decrease in the final calculated rate of oxygen consumption both during work and at rest. However, because the 'at rest' rate decreased by a larger amount, the rate of oxygen consumption above the resting level increased, which had the overall effect of decreasing the mean net efficiency of the muscle by 2.6±0.28 % (mean ± S.E.M., N=26).

Oxygen consumption and efficiency

Linear regressions were fitted to the pre-stimulus (resting) and stimulated (working) regions of the corrected plot of P_{O_2} *versus* time. The difference between the slopes yields the rate of change of P_{O_2} above the resting rate. This was multiplied by the molar solubility of oxygen (15.41 $nmol\,O_2\,ml^{-1}\,kPa^{-1}$) and the chamber volume (1.308 ml) to obtain the rate of molar oxygen consumption ($nmol\,O_2\,min^{-1}$). The rate of muscle oxygen consumption ($\dot{V}_{O_2}$, $ml\,O_2\,min^{-1}\,kg^{-1}$) was calculated. Pyruvate was the only exogenous source of fuel, so a respiratory quotient of 1 was assumed (Syme, 1994) and an energy equivalent of oxygen consumption was calculated (using 20.1 $J\,ml^{-1}\,O_2$ or $4.75\times10^{-4}\,J\,nmol^{-1}\,O_2$) (Heglund and Cavagna, 1987; Barclay, 1999). The joule equivalent was multiplied by the rate of molar oxygen consumption to give the rate of energy utilisation ($\dot{E}_{O_2}$) expressed as $J\,min^{-1}\,kg^{-1}$. Mechanical net efficiency (%) or active efficiency (Gibbs et al., 1967) was calculated by dividing E_p by $\dot{E}_{O_2}$.

Anaerobic metabolism

Muscle lactate production during isometric contractions was measured to determine its importance as source of energy in trout ventricular muscle The muscle preparations were mounted on a force transducer in a Perspex flow-through chamber circulated with oxygenated Ringer's solution at 15 °C. L_{max} was determined as above. Lactate production was measured under three conditions: 'experimental', 'anoxic' and 'control'. Under the 'experimental' condition, muscles were subjected to a full force/frequency trial over 20 min. Stimulation frequency was increased, in steps lasting 2 min, from 0.3 to 1.3 Hz. Under the 'anoxic' condition, muscles were stimulated at 1.0 Hz for 2 min then stimulated in Ringer's solution saturated with 100 % nitrogen. Lactate production was measured after isometric force had fallen to less than 10 % of that before nitrogen was added. 'Control' muscles were pinned out in a Petri dish (for 20 min) containing Ringer's solution bubbled with oxygen.

Lactate production was determined using a diagnostics kit for measuring blood lactate (Sigma catalogue no. 826-A). Lactate and NAD are converted to pyruvate and NADH, respectively, by the action of lactate dehydrogenase (LDH). Lactate was measured spectrophotometrically as an increase in absorbance at 340 nm (A_{340}) due to the generation of NADH.

Immediately after each procedure, the muscles were placed into liquid nitrogen. The muscles were homogenised for 1 min

on ice in 0.2 ml of 8 % perchloric acid (PCA), left for 1 min and then homogenised for a further 1 min. The samples were centrifuged at 1300 ***g*** for 10 min in a cooled centrifuge (below –5 °C). The diagnostic kit technique was modified as follows: a 10 mg pre-weighed vial of NAD was reconstituted with 1.0 ml of 0.6 mol l^{-1} glycine buffer containing hydrazine, 2.0 ml of distilled water and 0.05 ml of LDH. A sample (0.85 ml) of this solution was pipetted into blank and test cuvettes; 0.15 ml of 8 % PCA was then added to the blank cuvette and 0.15 ml of the PCA supernatant was added to the test cuvette. Both were incubated for 15 min at 37 °C, and the absorbance was measured and converted into lactate concentration using a calibration measured using a series of lactate standards.

Statistical analyses

The statistical significance of variations in muscle lactate concentration under the three conditions and in muscle power output, rates of oxygen consumption and efficiency with cycle frequency was determined using a one-way analysis of variance (ANOVA; SigmaStat Statistical Software, SPSS). The Student–Newman–Keuls multiple-comparison *a posteriori* test was carried out to compare each condition. Significance levels were set to $P<0.05$. All data are expressed as means ± S.E.M.

Results

Anaerobic metabolism

The assay was linear between 0 and 150 µmol l^{-1} lactate ($r^2=0.99$, $P<0.001$). Lactate concentration in the samples tested (length 8.04±0.17 mm, $N=12$, mass 5.7±0.5 mg, $N=18$; where N is the number of replicates) ranged from 7 to 50 µmol l^{-1}. Therefore, the test was linear over the range being measured. Lactate content in the experimental muscle at the end of 20 min of stimulation (3.25±0.36 nmol mg^{-1}, $N=6$) was not significantly different from that of the control, resting muscle (2.74±0.23 nmol mg^{-1}, $N=6$). The mean lactate concentration in muscle stimulated to anoxia was approximately three times higher (8.26±0.69 nmol mg^{-1}, $N=6$) and significantly different from that of both control and experimental muscles ($P<0.05$).

Resting rates of oxygen consumption

After correction for chamber effects, the remaining decline in P_{O_2} before stimulation was due to oxygen consumption by the resting muscle. The mean resting $\dot{V}_{O_2}$ of trout ventricular preparations at 15 °C was 3.04±0.38 ml O_2 min^{-1} kg^{-1} ($N=26$). The energetic equivalent was 61±8 J min^{-1} kg^{-1}. The resting metabolic rate of each preparation was used in our calculation of total mechanical efficiency.

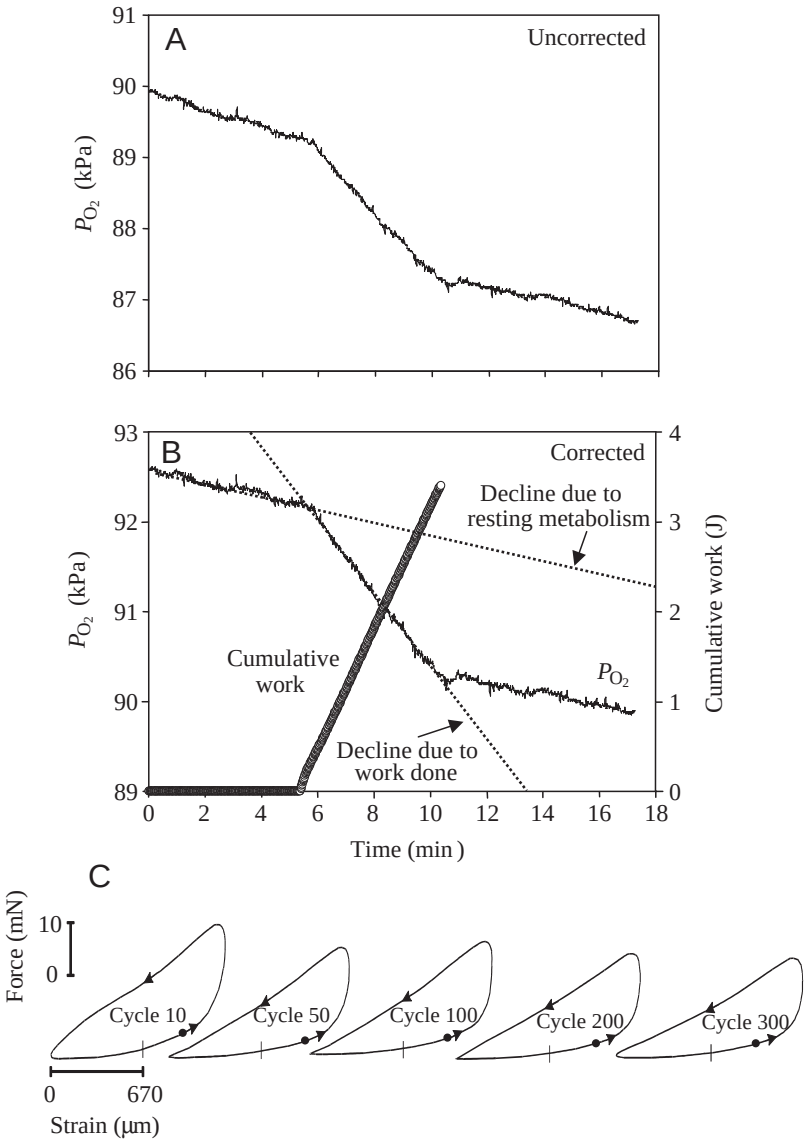

Fig. 2. A sample recording of uncorrected (A) and corrected (B) P_{O_2} (solid line) plotted against time. The dotted lines are linear regressions applied to the decline in P_{O_2} before and during stimulation (B). The cumulative work (open circles) plotted against time for a rainbow trout ventricular preparation is also shown in B. This muscle was subjected to 300 work loops (5 min) at 1.0 Hz. Starting length (L_0) was 95 % L_{max}, where L_{max} is the length for maximum isometric force production, strain was ±8 % L_0 (16 % peak-to-peak) and phase of stimulation (circles on work loops) was 30 °. Representative work loops from cycles 10, 50, 100, 200 and 300 are also illustrated (C). The loops are counterclockwise, with their area representing net work. Arrows indicate the direction of travel and the stimulus timing is indicated by a filled circle.

Effect of cycle frequency on oxygen consumption

Experimental recordings of rate of oxygen consumption from a rainbow trout ventricular preparation before, during and after stimulation are presented in Fig. 2. Fig. 2A shows

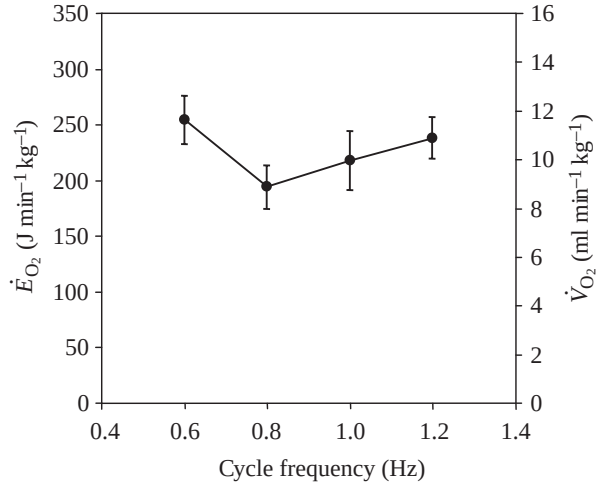

Fig. 3. The effects of cycle frequency on net oxygen consumption ($\dot{V}_{O_2}$) and its energetic equivalent ($\dot{E}_{O_2}$). Values are means ± S.E.M., N=5–8.

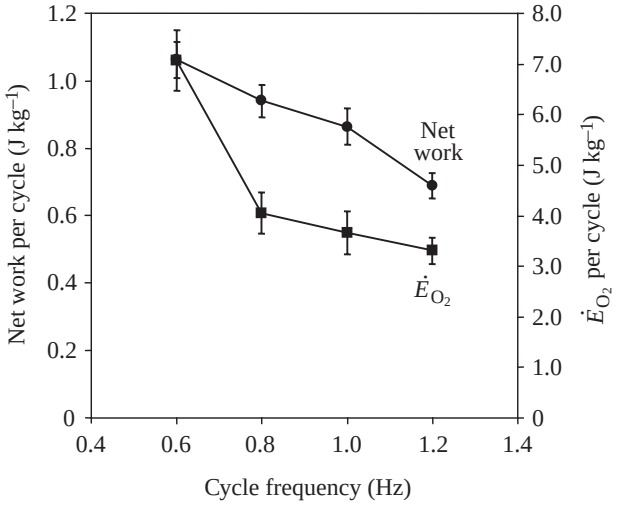

Fig. 4. The effects of cycle frequency on net work (circles) and the rate of energy utilisation, $\dot{E}_{O_2}$, per cycle (squares). Values are means ± S.E.M., N=5–8.

uncorrected results from the chamber after the recorded voltage had been converted into partial pressure of oxygen (kPa). P_{O_2} declined gradually before stimulation because of resting oxygen consumption and more rapidly when the muscle was stimulated and produced work. Fig. 2B shows the same results after the correction for chamber effects (using the polynomial to predict chamber decline) had been applied. Fig. 2B also shows the cumulative work per cycle performed by the muscle over the 5 min work-loop trial. Linear regressions (dotted lines, Fig. 2B) applied to the resting and active rates of O_2 consumption components of the P_{O_2} trace after correction for chamber effects are also shown. For this preparation, the resting and active declines in chamber P_{O_2} were 0.07 and 0.41 kPa min^{-1}, respectively. Representative work loops are shown in Fig. 2C.

Fig. 3 shows the effect of cycle frequency on the net rate of oxygen consumption ($\dot{V}_{O_2}$) and its energetic equivalent ($\dot{E}_{O_2}$). Net oxygen consumption appeared to decline between 0.6 and 0.8 Hz, after which it increased. However, there were no significant differences in $\dot{E}_{O_2}$ and $\dot{V}_{O_2}$ between 0.6 and 1.2 Hz. The values of $\dot{E}_{O_2}$ and $\dot{V}_{O_2}$ ranged from to 254±22 J min^{-1} kg^{-1} and 12.65±1.08 ml O_2 min^{-1} kg^{-1}, respectively, at 0.6 Hz to 194±20 J min^{-1} kg^{-1} and 9.66±0.98 ml O_2 min^{-1} kg^{-1}, respectively, at 0.8 Hz.

Constant $\dot{V}_{O_2}$ with increasing cycle frequency

In contrast to studies on mammalian heart (e.g. Van Beek and Westerhof, 1991), we found no significant variation in the rate of oxygen consumption over the range of cycle frequencies studied (Fig. 3). While the rate of oxygen consumption is constant, more work loops are performed per unit time and less oxygen is consumed per cycle as frequency is increased. In addition, work per cycle decreases with increasing cycle frequency. Fig. 4 shows how both oxygen consumption per cycle and work per cycle decrease with frequency.

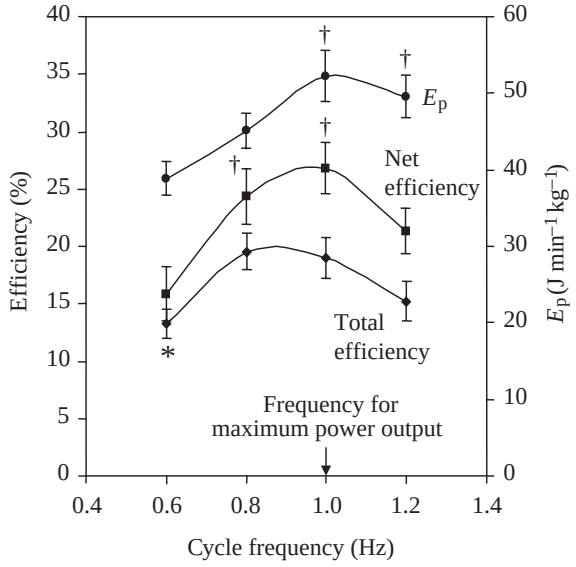

Fig. 5. The effects of cycle frequency on total efficiency (total power output divided by the sum of resting and active rates of energy consumption) (diamonds), net efficiency (squares) and total power output, E_p (circles). Values are means ± S.E.M., N=5–8. † and * indicate statistically significant differences (P<0.05, ANOVA) from 0.6 Hz and 1.0 Hz, respectively.

Effects of cycle frequency on net and total efficiency

Fig. 5 illustrates the net efficiency/cycle frequency relationship. Maximum net efficiency was 26.8±2.3 % (N=8) at a cycle frequency of 1.0 Hz. Net efficiency showed a marked dependence on cycle frequency; it increased between 0.6 and 1.0 Hz and then declined as frequency was increased further. Net efficiency at 0.6 Hz was significantly different (P<0.05, ANOVA) from that at 0.8 and 1.0 Hz.

Total efficiency is the work output expressed as a proportion of the sum of resting and net energy output (Gibbs et al., 1967)

and was always less than net efficiency. The dependence of total efficiency on cycle frequency was similar to that of net efficiency (Fig. 5). Total efficiency increased as cycle frequency was increased from 0.6 to 0.8 Hz. It remained almost constant between 0.8 and 1.0 Hz, after which it declined. Maximum total efficiency was 19.6±1.6 % and occurred at 0.8 Hz, a lower cycle frequency than that for maximum power output (N=6). There was a significant difference between total efficiency at 1.0 Hz and that at 0.6 Hz (P<0.05, ANOVA). Mechanical power output increased to a maximum of 52.3±3.4 J min^{-1} kg^{-1} (N=8) at 1.0 Hz and then decreased. There was a significant difference between mechanical power output at 0.6 Hz and that at 1.0 and 1.2 Hz (P<0.05, ANOVA). This power/cycle frequency relationship is consistent with previous observations in trout ventricular preparations (Harwood et al., 1998).

Discussion

The contribution of anaerobic metabolism to the total energy budget

Our estimates of mechanical efficiency assume that the myocardium generates ATP aerobically and not by glycolysis (Davie and Franklin, 1992). Several studies on a variety of teleost species have measured oxygen consumption and lactate production simultaneously. They demonstrate that the anaerobic contribution to metabolism is minimal when oxygen is available (Forster, 1991; Arthur et al., 1992; Driedzic, 1983; Driedzic et al., 1983). In our study, the Ringer was saturated with 100 % oxygen and P_{O_2} fell by 10 % during the course of an experiment, from close to 100 % to approximately 90 %. Further, we found no significant difference between lactate content in resting preparations and after 20 min of isometric contractions in oxygen-saturated Ringer's solution. Both these values were significantly lower than for muscle preparations stimulated in Ringer saturated with nitrogen ('anoxic' state in Materials and methods). Assuming that one ATP is generated per lactate produced by fermentation and that 29.3 kJ of energy is produced per mole of ATP consumed (Syme, 1994), then lactate would contribute 4.8 J min^{-1} kg^{-1} [calculated as 3.25 nmol lactate mg^{-1} 20 min^{-1} × 1 mol ATP mol^{-1} lactate × 29.3 kJ mol^{-1} ATP]. This is 2.5 % of the mean $\dot{E}_{O_2}$ in trout cardiac muscle (200 J min^{-1} kg^{-1}) and is comparable with the value calculated by Driedzic et al. (1983), who found that anaerobic metabolism accounted for, at most, 5 % of total ATP consumption in isolated sea raven hearts.

Resting rates of oxygen consumption

Basal metabolic rate is generally higher in cardiac muscle than in skeletal muscle (Gibbs et al., 1967). It has been found to vary 20-fold among species, techniques and investigators (Suga, 1990; Loiselle and Gibbs, 1979) (for a review, see Gibbs, 1995). However, in mammals, it is approximately 20–30 % of the active $\dot{V}_{O_2}$ (Gibbs et al., 1967; Ebus and Stienen, 1996). The basal metabolic rates of the intact rat (Lochner et al., 1968) and dog (Nozawa et al., 1988) heart are approximately 15 and 10 ml O_2 min^{-1} kg^{-1}, respectively. We used a similar technique to Syme (1994) to measure resting $\dot{V}_{O_2}$. He found the resting metabolic rate of unstimulated, isolated frog ventricular trabeculae was 6.9 ml O_2 min^{-1} kg^{-1} at 20 °C with a Q_{10} of 2.5, which equates to 4.8 ml O_2 min^{-1} kg^{-1} at 15 °C, the temperature used in our study.

We found that mean resting $\dot{V}_{O_2}$ was 3.04 ml O_2 min^{-1} kg^{-1} at 15 °C, approximately one-quarter of the active rate of oxygen consumption. This is at the low end of the range of 'basal metabolic rate' in beating, perfused teleost hearts, estimated by extrapolation of a plot of $\dot{V}_{O_2}$ *versus* power output to zero power output (3–9 ml O_2 min^{-1} kg^{-1}) (Farrell et al., 1985; Graham and Farrell, 1990; Davie and Franklin, 1992). When the total energy utilised by an intact beating heart is plotted against pressure/volume area (an index of external work) (Suga, 1990), the $\dot{V}_{O_2}$ intercept represents the sum of basal metabolic rate and activation costs (Gibbs, 1995). Gibbs (1995) notes that this value of basal metabolic rate may overestimate basal metabolic energy consumption. This could explain why our direct measurement of mean resting oxygen consumption in trout myocardium was lower than values calculated by extrapolation of the power *versus* $\dot{V}_{O_2}$ relationship.

The energy-consuming processes that constitute basal metabolic rate are open to debate, but we know they are dominated by the energy cost of maintaining the intracellular ionic environment (Suga, 1990). Mammalian cardiac muscle basal metabolic rate depends upon SL Na^+/K^+-ATPases and the Ca^{2+}-ATPase activity of the SR (Gibbs, 1995). Ebus and Stienen (1996) found that several processes contribute to the large basal metabolic rate in rat myocardium: myosin ATPase accounts for approximately 40 %, the Na^+/K^+-ATPase approximately 15 % and the SR Ca^{2+}-ATPase approximately 8 % of the basal ATPase activity. They suggested that the remaining activity (approximately 35 %) could be due to a Ca^{2+}-independent Mg^{2+}-ATPase located in the T-tubules and/or the SL. Fish cardiac muscle does not possess T-tubules (Santer, 1985) and has a sparse SR, which might explain why the basal metabolic rate of trout cardiac muscle constitutes a lower percentage of the total metabolic rate than does that of mammalian cardiac muscle (25 % in fish compared with 33 % in mammals). Clearly, further experiments are needed to explain the processes involved in basal metabolic rate in fish cardiac muscle.

The metabolic cost of muscle activation in fish

Amphibian cardiac muscle, like fish cardiac muscle, has a sparse SR. The activation energy in cane toads was found to be higher than in mammalian cardiac muscle (Holroyd and Gibbs, 1993). It was suggested this was due to Ca^{2+} being extruded from the cell primarily by the Ca^{2+}/Na^+ exchanger with a minor contribution from the SL Ca^{2+} pumps. These mechanisms use one ATP for each Ca^{2+} transported (Ponce-Hornos, 1989; Caroni and Carafoli, 1980), whereas the SR Ca^{2+}-ATPase, which dominates mammalian cardiac Ca^{2+} regulation, is twice as economical, removing two Ca^{2+} per ATP (Hasselbach and Oetliker, 1983). It is likely that Na^+/Ca^{2+} exchange and SL Ca^{2+}-ATPase are the most important mechanisms for removal

of Ca^{2+} in teleost cardiac muscle (Tibbits et al., 1991, 1992). Therefore, as in amphibians, the energy cost of activation will constitute a larger proportion of the total energy consumed, and any effect of cycle frequency on activation energy would have a large effect on muscle efficiency.

In the present study, the rate of oxygen consumption remained almost constant over the whole range of cycle frequencies, whereas in mammalian cardiac muscle the rate of oxygen consumption increases with heart rate (Boerth et al., 1969; Shaddy et al., 1989; Van Beek and Westerhof, 1991). Activation metabolism constitutes 10–30 % of the total energy cost of contraction in mammalian heart preparations. If a fixed amount of Ca^{2+} is released, then resequestered, each time the muscle is activated, activation cost should increase with frequency. However, if the amount of activator Ca^{2+} decreases with increasing cycle frequency, then a constant rate of myocardial oxygen consumption might be observed. This is consistent with the decrease in the systolic $[Ca^{2+}]_i$ transient with increasing stimulation frequency demonstrated in trout ventricular myocytes (Harwood et al., 2000).

The effects of cycle frequency on net efficiency and total efficiency

Power output increased by 25 % while the rate of oxygen consumption remained constant between 0.6 and 1.0 Hz. The increase in net and total efficiency over this range reflects this increase in power output. Barclay (1994) obtained similar results for mouse soleus muscle which, like cardiac muscle, has slow contraction kinetics and relies principally on oxidative metabolism. An earlier study to measure the net efficiency of cardiac muscle (frog ventricular trabeculae) undergoing cyclic length changes (Syme, 1994) found a constant net efficiency of 11 % as cycle frequency was varied. Syme (1994) showed that efficiency remained constant, which he suggested was because $\dot{V}_{O_2}$ correlated with force production (Braunwald, 1971; Weber and Janicki, 1977; Cooper, 1990; Takaoka et al., 1993), which did not vary with frequency. He also proposed that efficiency might be independent of frequency if heart rate changes were achieved by decreasing the diastolic interval while the rate, duration and amplitude of force development remain constant.

This is not the case in the trout, in which contraction duration decreases with frequency (Harwood et al., 1998) and work per cycle declines as a result of changes in the rate, duration and amplitude of force development. Net efficiency in trout cardiac muscle was higher and increased with cycle frequency up to a maximum of 27 % at 1.0 Hz, after which it declined. Total efficiency incorporates the cost of resting metabolism, muscle activation and cycling crossbridges and represents the cost of operating and maintaining a muscle. The maximum total efficiency of 20 % found in this study agrees with those of perfused fish hearts measured in other studies; for example, 22 % in sea raven at 10 °C (Farrell et al., 1985), 20 % at 10 °C (Houlihan et al., 1988), 25 % at 15 °C (Graham and Farrell, 1990) and 21 % at 15 °C in dogfish (Davie and Franklin, 1992). Further, although the comparison is of arguable value on phylogenetic grounds, the total efficiency of mammalian heart lies between 10 and 30 % (for a review, see Suga, 1990).

We observed a maximum total efficiency at 0.8 Hz, whereas maximum power output was obtained at 1.0 Hz. Similarly, Curtin and Woledge (1993) found that maximum mechanical efficiency occurred at a lower cycle frequency than maximum power output in dogfish red (slow) myotomal muscle. Trout myocardium is most efficient (total efficiency is higher) at lower heart rates, corresponding to the more commonly used slower, sustainable swimming speeds. Efficiency declines at higher heart rates, corresponding to very fast swimming speeds. Generally, these high rates are attained only sporadically and for brief periods, and economy at these rates is probably not a dominant factor in determining muscle design.

This trade-off between efficiency and power may also be related to the decline in stroke volume at higher frequencies (Farrell et al., 1989; Harwood et al., 1998). Stroke volume is analogous to muscle strain (Syme, 1993). The strain yielding maximum power output declines from $\pm 10\,\% L_0$ at 0.6 and 0.8 Hz to $\pm 8\,\% L_0$ at 1.0 and 1.2 Hz (Harwood et al., 1998), where L_0 is muscle starting length. Higher heart rates require a higher rate of pressure generation (Farrell, 1991); in this case, at higher frequencies, a relatively small amount of blood is moved at high pressures. Rainbow trout achieve maximum stroke work primarily by increasing pressure (Tota and Gattuso, 1996). Higher efficiencies are achieved when work is performed at low stroke volumes and high output pressures compared with high stroke volumes and low output pressures (Davie and Franklin, 1992). Therefore, it seems likely that trout benefit from the favourable energetics of a high-pressure, low-volume cardiac cycle at high heart rates.

Importance of measuring myocardial performance during cyclic activity

Many studies have shown the importance of contraction protocol for mechanical efficiency (Barclay, 1994). Stretching a contracting muscle tends to enhance the force and increases the amount of work performed during a subsequent shortening (Heglund and Cavagna, 1987). Conversely, shortening during relaxation tends to abbreviate force generation and energy turnover (Lou et al., 1998).

Harwood et al. (1998), using identical work-loop parameters to those used in this study, demonstrated an increased rate of the decline in muscle force at higher cycle frequencies in trout ventricular preparations. The shortening period, and thus the time available for the muscle to develop force, decreases with increasing cycle frequency. Increasing the rate of relaxation allows a higher force to be developed for a larger proportion of the shortening phase (increasing positive work output) while still allowing the muscle to relax before re-lengthening (minimising negative work). The result is an increase in net work production. Woledge and Curtin (1993) described two conflicting energetic consequences of shortening in fish myotomal muscle: an increase in the rate of relaxation of the

muscle, which reduces energetic cost, and an increase in the rate of crossbridge turnover and therefore of energy consumption. The second of these, a shortening-induced increase in energy consumption (above that observed in isometric contractions), is not generally found in cardiac muscle (Gibbs and Barclay, 1998). It seems that cardiac muscle should gain all the benefits of shortening without suffering the penalties.

While shortening deactivation and the force/velocity relationship dictate that a muscle during shortening will produce less force than during an isometric contraction, stretching a contracting muscle tends to increase force. The magnitude of the increase in force depends on the velocity of the stretch: the faster the muscle is stretched, the higher the force (Edman et al., 1978). Therefore, force enhancement will have a larger effect at higher cycle frequencies. Under isometric conditions, the force of contraction in fish cardiac muscle decreases with increasing stimulation frequency (Driedzic and Gesser, 1985, 1988; Bailey and Driedzic, 1990; Hove-Madsen, 1992; Shiels and Farrell, 1997). In the present study and in previous work (Harwood et al., 1998), we found that force developed during work-loop cycles was preserved despite increasing cycle frequency. This is consistent with the negative force/frequency effects being offset by increasing force enhancement with increasing cycle frequency.

Curtin and Woledge (1993) found that the initial mechanical efficiency of dogfish red myotomal muscle was greater when stimulation started during stretch. The increase in force has been attributed to an increase in the Ca^{2+}-sensitivity of the myofilaments. This is particularly marked in cardiac muscle (for a review, see Calaghan and White, 1999). Further, an increase in myofilament sensitivity to Ca^{2+} in trout cardiac muscle was seen as an increase in twitch duration at longer muscle lengths (as a result of slower dissociation of Ca^{2+} from troponin) (Harwood et al., 1998). Therefore, while increasing myofilament sensitivity to increase force development does not use any extra energy, muscle efficiency would be increased because of the increase in power output.

In conclusion, the most important result of this study was that there was no significant variation in the rate of oxygen consumption over the range of cycle frequencies studied. This is very different from the findings of previous studies on mammalian cardiac muscle in which the rate of oxygen consumption was found to be more-or-less proportional to the increase in heart rate. This difference may be due to differences in Ca^{2+} handling in cardiac muscle of fish and mammals. The constant $\dot{V}_{O_2}$ and the concurrent increase in power output with cycle frequency result in efficiency increasing to a maximum before declining at high frequencies.

The authors would like to thank Dr Chris Barclay for his advice and comments on an earlier version of this manuscript, John Oughton for helping with electronics and Chris Smith helping with computing issues. Thanks also to Stuart Pickersgill for caring for the trout used in this study. C. L. H. was supported by a BBSRC Studentship.

References

Agnisola, C. and Houlihan, D. F. (1991). Oxygen supply and *in vitro* performance of the systemic heart of *Octopus vulgaris*: effects of haemocyanin. *J. Exp. Biol.* **157**, 523–541.

Aho, E. and Vornanen, M. (1999). Comparison of contractile properties of atrial and ventricular tissue of the rainbow trout (*Oncorhynchus mykiss*) heart: effect of thermal acclimation. *J. Exp. Biol*. **202**, 2663–2677.

Arthur, P. G., Keen, J. E., Hochachka, P. W. and Farrell, A. P. (1992). Metabolic state of the *in situ* perfused trout heart during severe hypoxia. *Am. J. Physiol.* **263**, R798–R804.

Bailey, J. R. and Driedzic, W. R. (1990). Enhanced maximum frequency and force development of fish hearts following temperature acclimation. *J. Exp. Biol*. **149**, 239–254.

Barclay, C. J. (1994). Efficiency of fast- and slow-twitch muscle of the mouse performing cyclic contractions. *J. Exp. Biol*. **193**, 65–78.

Barclay, C. J. (1999). Efficiency of energy conversion by muscles and its relation to efficiency during locomotion. In *Biochemistry of Exercise X* (ed. M. Hargeaves and M. Thompson), pp 115–123. Champaign, IL: Human Kinetics.

Baskin, R. J. (1961). The variation of muscle oxygen consumption with load. *J. Physiol., Lond.* **181**, 270–281.

Baxi, J., Barclay, C. J. and Gibbs, C. L. (2000). Energetics of rat papillary muscle during contractions with sinusoidal length changes. *Am J. Physiol*. **278**, H1545–H1554.

Bers, D. M. (1991). *Excitation–Contraction Coupling and Cardiac Contractile Force.* The Netherlands: Kluwer Academic Publishers.

Boerth, R. C., Covell, J. W., Pool, P. E. and Ross, J., Jr (1969). Increased myocardial oxygen consumption and contractile state associated with increased heart rate in dogs. *Circ. Res*. **24**, 725–734.

Braunwald, E. (1971). Control of myocardial oxygen consumption: Physiological and clinical considerations. *Am. J. Cardiol*. **27**, 416–432.

Calaghan, S. C. and White, E. (1999). The role of calcium in the response of cardiac muscle to stretch. *Prog. Biophys. Mol. Biol.* **71**, 59–90.

Cameron, J. N. (1986). *Principles of Physiological Measurement*. San Diego: Academic Press Inc. 257pp.

Caroni, P. and Carafoli, P. (1980). An ATP-dependent Ca^{2+}-pumping system in dog heart sarcolemma. *Nature* **283**, 765–767.

Chapman, J. B. and Gibbs, C. L. (1975). Fluorometric and myothermic studies of cardiac energetics. *Rec. Adv. Cardiac Struct. Meth.* **5**, 449–455.

Cooper, G. (1990). Load and length regulation of cardiac energetics. *Annu. Rev. Physiol.* **52**, 505–522.

Curtin, N. A. and Woledge, R. C. (1993). Efficiency of energy conversion during sinusoidal movement of white muscle fibres from the dogfish *Scyliorhinus canicula*. *J. Exp. Biol*. **183**, 137–147.

Davie, P. S. and Franklin, C. E. (1992). Myocardial oxygen consumption and mechanical efficiency of a perfused dogfish heart preparation. *J. Comp. Physiol. B* **162**, 256–262.

Driedzic, W. R. (1983). The fish heart as a model system for the study of myoglobin. *Comp. Biochem. Physiol*. **76A**, 487–493.

Driedzic, W. R. and Gesser, H. (1985). Ca^{2+} protection from the negative inotropic effect of contraction frequency on teleost hearts. *J. Comp. Physiol. B* **156**, 135–142.

Driedzic, W. R. and Gesser, H. (1988). Differences in force/frequency relationships and calcium dependency between elasmobranch and teleost hearts. *J. Exp. Biol.* **140**, 227–241.

Driedzic, W. R., Scott, D. L. and Farrell, A. P. (1983). Aerobic and anaerobic contributions to energy metabolism in perfused isolated sea raven (*Hemitripterus americanus*) hearts. *Can. J. Zool*. **61**, 1880–1883.

Ebus, J. P. and Stienen, G. J. M. (1996). Origin of concurrent ATPase activities in skinned cardiac trabeculae from rat. *J. Physiol., Lond.* **492**, 675–687.

Edman, K. A. P., Elzinga, G. and Noble, M. I. M. (1978). Enhancement of mechanical performance by stretch during tetanic contractions of vertebrate skeletal muscle fibres. *J. Physiol., Lond.* **281**, 139–155.

Farrell, A. P. (1991). From hagfish to tuna: A perspective on cardiac function in fish. *Physiol. Zool.* **64**, 1137–1164.

Farrell, A. P., Gamperl, A. K., Hicks, J. M., Shiels, H. A. and Jain, K. E. (1996). Maximum cardiac performance of rainbow trout (*Oncorhynchus mykiss*) at temperatures approaching their upper lethal limit. *J. Exp. Biol*. **199**, 663–672.

Farrell, A. P., Small, S. and Graham, M. S. (1989). Effect of heart rate and hypoxia on the performance of a perfused trout heart. *Can. J. Zool.* **67**, 274–280.

Farrell, A. P., Woods, S., Hart, T. and Driedzic, W. R. (1985). Myocardial oxygen consumption in the sea raven, *Hemitripterus americanus*: the effects of volume loading, pressure loading and progressive hypoxia. *J. Exp. Biol.* **117**, 237–250.

Forster, M. E. (1991). Myocardial oxygen consumption and lactate release by the hypoxic hagfish heart. *J. Exp. Biol.* **156**, 583–590.

Gibbs, C. L. (1978). Cardiac energetics. *Physiol. Rev.* **58**, 174–254.

Gibbs, C. L. (1995). Mechanical determinants of myocardial oxygen consumption. *Clin. Exp. Pharm. Physiol*. **22**, 1–9.

Gibbs, C. L. and Barclay, C. J. (1998). Efficiency of skeletal and cardiac muscle. *Adv. Exp. Med. Biol.* **453**, 527–535.

Gibbs, C. L., Mommaerts, W. F. H. M. and Ricchiuti, N. V. (1967). Energetics of cardiac contractions. *J. Physiol., Lond.* **191**, 25–46.

Graham, M. S. and Farrell, A. P. (1990). Myocardial oxygen consumption in trout acclimated to 5 °C and 15 °C. *Physiol. Zool*. **63**, 536–554.

Harwood, C. L., Howarth, F. C., Altringham, J. D. and White, E. (2000). Rate-dependent changes in cell shortening, intracellular Ca^{2+} levels and membrane potential in single, isolated rainbow trout (*Oncorhynchus mykiss*) ventricular myocytes. *J. Exp. Biol*. **203**, 493–504.

Harwood, C. L., Young, I. S. and Altringham, J. D. (1998). Influence of cycle frequency, muscle strain and muscle length on work and power production of rainbow trout (*Oncorhynchus mykiss*) ventricular muscle. *J. Exp. Biol*. **201**, 2723–2733.

Hasselbach, W. and Oetliker, H. (1983). Energetics and electrogenicity of the sarcoplasmic reticulum calcium pump. *Annu. Rev. Physiol*. **45**, 325–339.

Heglund, N. C. and Cavagna, G. A. (1987). Mechanical work, oxygen consumption and efficiency in isolated frog and rat muscle. *Am. J. Physiol*. **253**, C22–C29.

Hisano, R. and Cooper, G. (1987). Correlation of force–length area with oxygen consumption in ferret papillary muscle. *Circ. Res.* **61**, 318–328.

Holroyd, S. M. and Gibbs, C. L. (1993). The energetics shortening amphibian cardiac muscle. *Pflügers Arch.* **424**, 84–90.

Houlihan, D. F., Agnisola, C., Lyndon, A. R., Gray, C. and Hamilton, N. M. (1988). Protein synthesis in a fish heart: responses to increased power output. *J. Exp. Biol*. **137**, 565–587.

Hove-Madsen, L. (1992). The influence of temperature on ryanodine sensitivity and the force–frequency relationship in the myocardium of rainbow trout. *J. Exp. Biol*. **167**, 47–60.

Josephson, R. K. (1985). Mechanical power output from striated muscle during cycle contraction. *J. Exp. Biol.* **114**, 493–512.

Josephson, R. K. and Stevenson, R. S. (1991). The efficiency of a flight muscle from the locust *Schistocerca americana*. *J. Physiol., Lond.* **442**, 413–429.

Kushmerick, M. J. and Paul, R. J. (1976). Aerobic recovery metabolism following a single isometric tetanus in frog sartorius muscle at 0 °C. *J. Physiol., Lond.* **254**, 693–709.

Layland, J., Young, I. S. and Altringham, J. D. (1995). The effect of cycle frequency on the power output of rat papillary muscles *in vitro*. *J. Exp Biol.* **198**, 1035–1043.

Lochner, W., Arnold, G. and Muller-Ruchholtz, E. R. (1968). Metabolism of the artificially arrested heart and of the gas perfused heart. *Am. J. Cardiol.* **22**, 299–311.

Loiselle, D. S. and Gibbs, C. L. (1979). Species differences in cardiac energetics. *Am. J. Physiol.* **237**, H90–H98.

Lou, F., Curtin, N. A. and Woledge, R. C. (1998). Shortening during stimulation vs. during relaxation: How do the costs compare? *Adv. Exp. Med. Biol.* **453**, 545–553.

Mellors, L. J. and Barclay, C. J. (2001). The energetics of rat papillary muscles undergoing realistic strain patterns. *J. Exp. Biol*. **204**, 3765–3777.

Mellors, L. J., Gibbs, C. L. and Barclay, C. J. (2001). Comparison of the efficiency of rat papillary muscles during afterloaded isotonic contractions and contractions with sinusoidal length changes. *J. Exp. Biol*. **204**, 1765–1774.

Neely, J. R., Liebermeister, H., Battersby, E. J. and Morgan, H. E. (1967). Effect of pressure development on oxygen consumption by isolated rat heart. *Am. J. Physiol*. **212**, 804–814.

Nozawa, T., Yasumura, Y., Futaki, S., Tanaka, N., Uenishi, M. and Suga, H. (1988). Efficiencies from consumed O_2 and pressure–volume area to work of *in situ* dog heart. *Jap. J. Physiol*. **38**, 713–728.

Ponce-Hornos, J. E. (1989). Heart energetics: A framework for ion flux analysis. *J. Mol. Cell. Cardiol.* **21**, 115–117.

Priede, I. G. (1974). The effect of swimming activity and section of the vagus nerves on heart rate in rainbow trout. *J. Exp. Biol*. **60**, 305–318.

Santer, R. M. (1985). Morphology and innervation of the fish heart. *Adv. Anat. Embryol. Cell Biol.* **89**, 1–102.

Shaddy, R. E., Tyndall, M., Teital, D., Carlos, L. and Mills, A. (1989). The effect of changes in heart rate and aortic systolic pressure on left ventricular myocardial oxygen consumption in lambs. *J. Dev. Physiol*. **11**, 213–217.

Shiels, H. A. and Farrell, A. P. (1997). The effect of temperature and adrenaline on the relative importance of the sarcoplasmic reticulum in contributing Ca^{2+} to force development in isolated ventricular trabeculae from rainbow trout. *J. Exp. Biol.* **200**, 1067–1621.

Shiels, H. A., Stevens, E. D. and Farrell, A. P. (1998). Effects of temperature, adrenaline and ryanodine on power production in rainbow trout *Oncorhynchus mykiss* ventricular trabeculae. *J. Exp. Biol*. **201**, 2701–2710.

Suga, H. (1990). Ventricular energetics. *Physiol. Rev.* **70**, 247–277.

Syme, D. A. (1993). Influence of extent of muscle shortening and heart rate on work from frog heart trabeculae. *Am. J. Physiol*. **265**, R310–R319.

Syme, D. A. (1994). The efficiency of frog ventricular muscle. *J. Exp. Biol.* **197**, 143–164.

Takaoka, H., Takeuchi, M., Odake, M., Hayashi, Y., Hata, K., Mori, M. and Yokoyama, M. (1993). Comparison of haemodynamic determinants for myocardial oxygen consumption under different contractile states in human ventricle. *Circulation* **87**, 59–69.

Tibbits, G. F., Moyes, C. D. and Hove-Madsen, L. (1992). Excitation–contraction coupling in the teleost heart. In *Fish Physiology*, vol. 12A, *The Cardiovascular System* (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 1–88. San Diego: Academic Press.

Tibbits, G. F., Hove-Madsen, L. and Bers, D. M. (1991). Ca^{2+} transport and the regulation of cardiac contractility in teleosts: a comparison with higher vertebrates. *Can. J. Zool.* **69**, 2014–2019.

Tota, B. and Gattuso, A. (1996). Heart ventricle pumps in teleosts and elasmobranchs: a morphodynamic approach. *J. Exp. Zool.* **275**, 162–171.

Van Beek, J. H. G. M. and Westerhof, N. (1991). Response time of cardiac mitochondrial oxygen consumption to heart rate steps. *Am. J. Physiol*. **260**, H613–H625.

Weber, K. T. and Janicki, J. S. (1977). Myocardial oxygen consumption: The role of wall force and shortening. *Am. J. Physiol*. **233**, H421–H430.

Whalen, W. J. (1961). The relation of work and oxygen consumption in isolated strips of cat and rat myocardium. *J. Physiol., Lond.* **157**, 1–17.

Woledge, R. C. and Curtin, N. A. (1993). The efficiency of energy conversion by swimming muscle of fish. *Adv. Exp. Med. Biol*. **332**, 735–743.