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INFLUENCE OF CYCLE FREQUENCY, MUSCLE STRAIN AND MUSCLE LENGTH ON WORK AND POWER PRODUCTION OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) VENTRICULAR MUSCLE

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Summary

This study investigates the effects of cycle frequency, strain and length on work and power output of isolated rainbow trout (*Oncorhynchus mykiss*) ventricular preparations using the work loop technique. These effects are discussed in the context of the whole heart using analogies with heart rate, stroke volume and end-diastolic volume.

Power output was dependent on cycle frequency, increasing threefold beween 0.3 and 1.1 Hz. The frequency for maximum power output was approximately 1.1 Hz, corresponding to the frequency for maximum power in perfused heart experiments.

The length for maximum work production (L_{opt}) was found to be the same as the length for maximum isometric force production (L_{max}). The decline in net work at lengths greater than L_{opt}/L_{max} was attributed to an increase in passive work (the work done on an unstimulated muscle) or to hysteresis and to a large increase in lengthening work.

The strain yielding maximum work decreased with increasing frequency. This is discussed in the context of the decline in stroke volume observed at increased heart rates *in vivo*.

Muscle strain in intact hearts paced at 0.3 Hz was ±11.9 % (23.8 % peak to peak), a value similar to the optimum strain at 0.3 Hz *in vitro* (±12 %).

Key words: muscle, heart, rainbow trout, *Oncorhynchus mykiss*, power, work, work loop, strain, frequency, length.

Introduction

There are several distinct differences between the hearts of fish and mammals in those factors determining cardiac output. Fish increase their cardiac output primarily by increasing stroke volume rather than heart rate. The highest heart rates achieved by fish are approximately 2.0 Hz, excluding tunas which have an exceptionally modified myocardium and unusually high heart rates (Farrell, 1991). End-systolic volume in fish is much lower than in mammals: normal contraction appears to empty the ventricle almost completely. In rainbow trout, for instance, the ejection fraction is usually close to 100 % (Franklin and Davie, 1992). However, at high aortic pressures, this falls, the heart does not empty completely and stroke volume decreases. Farrell *et al.* (1989) showed that stroke volume in isolated rainbow trout hearts declined with increased contraction frequency. This can be interpreted in two ways. Either ventricular filling is compromised at high heart rates because of a reduction in filling time or the decline in stroke volume is due to the myocardium failing to sustain the same cyclic length changes at higher frequencies.

The Frank–Starling law of the heart states that there is a positive relationship between chamber volume and stroke work. Myocardial power output is the product of stroke work and heart rate. Consequently, measurements of myocardial power output are particularly useful because they represent an index of integrated cardiac performance. Power output has previously been determined in intact fish heart preparations using echocardiography (Franklin and Davie, 1992) and pressure and flow measurements (Keen and Farrell, 1994; Farrell *et al*. 1996). While providing substantial information about the properties of the working intact heart, these studies yield only limited information about the mechanical properties of fish cardiac muscle. Recent studies (Layland *et al.* 1995*a*,*b*, 1997; Syme, 1993; Syme and Josephson, 1995) have used the work loop technique (Josephson, 1985) to investigate the relationships between myocardial power output, strain and frequency in mammals and amphibians. These studies made analogies between strain and stroke volume and between shortening work and stroke work to show how the mechanical properties of the myocardium influence the functional characteristics of the heart as a whole. We used the work loop technique to investigate the mechanical properties of isolated ventricular muscle preparations from rainbow trout *Oncorhynchus mykiss*. The effect of cycle frequency on work and power output was investigated by exercising the preparations over a physiological range of frequencies. Strain was varied systematically during these experiments to see how

it interacted with frequency in determining work and power output and how it might influence cardiac output.

We found that fish myocardium is unusually stiff at physiological lengths. This agrees with the observation of large amounts of connective tissue throughout the myocardium (Sanchez-Quintana *et al.* 1995, 1996). The elastic and viscoelastic properties of the myocardium will have a large effect on its mechanical properties. We examined the relative contributions of shortening, lengthening and passive (hysteresis) work to the net work output of the muscle. We aim to determine how the elastic elements of the heart influence its performance and whether they might function as a spring, storing elastic strain energy during the cardiac cycle. Unlike most skeletal muscle, the heart does not have a direct antagonistic muscle to re-extend it. In fish, atrial filling is attributed to both the residual venous pressure and the negative pericardial pressure (vacuum) developed during ventricular contraction, whereas ventricular filling is accomplished solely by the pressure developed by atrial contraction (Farrell and Jones, 1992). The high stiffness of the myocardium would appear to be a great disadvantage, resisting ventricular filling. However, the disadvantages of a stiff ventricular wall might be outweighed by the advantages of substantial elastic energy storage.

Marsh and Olson (1994) and James *et al.* (1995) found that maximum work output in skeletal muscle is attained in work loop studies using strains very close to those observed *in vivo*. Myocardial strain in the intact fish heart has not previously been measured. The trout ventricle is pyramidal in shape, with a triangular base rostrally and the apex situated caudally. Superficial muscle fibres run parallel along each of the vertices of this pyramid. We determined muscle strain from video recordings of markers attached to the epicardial surface along the ventral vertex. These strains were compared with optimum strains determined in our work loop study. One question we aim to address in this study is why the ejection fraction declines at higher heart rates in fish (Franklin and Davie, 1992). If this is caused by compromised myocardial muscle contraction and relaxation at higher heart rates, we would expect to see a decline in optimum strain with increasing frequency. In addition to addressing these specific questions, we aim to establish the basic mechanical properties of rainbow trout ventricular muscle prior to further studies, including the use of measured *in vivo* strain waveforms.

Materials and methods

Fish origin and maintenance

Female rainbow trout [*Oncorhynchus mykiss* (Walbaum)] (383±14 g, N=19; mean ± S.E.M.) were purchased from Glasshouses Trout Farm, North Yorkshire, UK. They were held for up to 12 weeks in indoor, 2 m diameter fibreglass tanks containing filtered, recirculated aerated water. The water temperature was maintained at 12–15 °C. The trout were exposed to a 16 h:8 h light:dark photoperiod and were fed commercial trout pellets *ad libitum*.

Determination of epicardial deformation

Trout were killed by a sharp blow to the head, and the spinal cord and brain were destroyed. The heart was removed and placed in a Petri dish filled with oxygenated Ringer's solution (composition in mmol^{-1}: NaCl, 124.1; 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±0.05 at 15 °C) chilled to 10 °C. A stainless-steel cannula was inserted through the sinus venosus into the atrium and secured using silk surgical suture thread. The cannula was then attached by silicone tubing to the open barrel of a 50 ml syringe that acted as a header reservoir. The syringe was secured to a clamp stand so that its height could be adjusted. Ringer's solution was pumped into the reservoir, where it was bubbled with 100 % oxygen before entering the heart at a constant flow rate. The temperature of the Ringer was maintained at 15±0.5 °C. The position of the heart was such that the intact bulbus arteriosus pointed upwards and so, even though the output was not cannulated, there was an inertial afterload. The coronary circulation was not cannulated, but as the Ringer was pumped out of the bulbus arteriosus it flowed back over the perfused heart and kept the compact layer irrigated and oxygenated. Farrell *et al.* (1996) found that, in fish weighing less than 750 g, the thickness of the compact layer was less than 1 mm. Since oxygenated perfusate has 20 times the oxygen partial pressure of venous blood, oxygen diffusion into the compact layer should be adequate.

The heart was paced at 0.3 Hz using a Grass S48 stimulator, which delivered 2 ms duration supramaximal stimuli *via* two stainless-steel electrodes in contact with the epicardial surface. Two small strips of white paper (approximately 0.4 mm wide × 7–8 mm long) were attached to the ventral vertex of the heart (the region between the bulbus arteriosus and the apex) by viscous forces. The markers were separated by 12–20 mm. A video camera was positioned so that the centre of its field of view corresponded to a point midway between and perpendicular to the two markers to avoid measurement errors due to parallax. The motion of the markers was recorded at 50 frames s^{-1}. The height of the syringe (filling pressure) was adjusted (Farrell *et al.* 1989) until there was no further increase in stroke volume, as observed by the distance between the two markers. The heart did not increase in size during the experiment, and the strain amplitude remained constant indicating that the stroke volume was stable throughout. The position of each marker was determined on every fifth frame for five consecutive heart beats chosen at random in the middle of a series.

Isolated muscle preparation and apparatus

Rainbow trout were killed as described above and their mass was recorded. The heart was quickly excised and rinsed in oxygenated Ringer's solution at 10 °C. It was then transferred to a shallow dissecting tray with a transparent, elastomer base (Sylgard 184, Dow Corning) and immersed in oxygenated Ringer's solution. The heart was pinned out, using small (25 gauge) hypodermic needles, through the bulbous arteriosus and each of the lateral walls, avoiding the region from which

preparations were taken. The atrium was then removed. To reduce inter-preparation variability, strips of muscle (<1 mm wide) were always taken from the ventral vertex of the heart (the same region that was filmed). Preparation thickness was reduced by removing as much of the spongy layer as possible, leaving mainly the compact layer (for preparation dimensions, see Table 1). Each end of the preparation was tied to a figure-of-eight-shaped platinum link by 5-0 gauge braided silk suture thread (Davis and Geck). During dissection, the Ringer's solution was changed frequently and allowed to warm up gradually to approximately 15 °C. The muscle preparations were mounted in a Perspex flow-through chamber, circulated with oxygenated Ringer's solution by a peristaltic pump. The temperature was maintained at 15±0.5 °C throughout the experiment, since peak cardiac performance has been found to occur at approximately 15 °C in rainbow trout (Farrell *et al.* 1996). The platinum link at one end of the muscle was attached to a fixed hook and the other end was attached to a force transducer (AME 801, SensoNor, Horten, Norway). The preparation was stimulated to produce a twitch, using a 2 ms supramaximal stimulus delivered by two parallel platinum wire electrodes lying either side of it. The output from the force transducer was amplified and the twitch force was displayed on a digital storage oscilloscope (Gould DSO 1604). The muscle length was increased until the passive force rose to just above zero (less than 0.1 mN) in order to take up the slack in the preparation. The preparation was then left for 1 h before experimentation to allow recovery from dissection.

Determination of the length for maximum isometric force

The muscle length was adjusted to an initial slack length where zero passive force was measured. Length was measured at 20× magnification using a stereo-microscope with an eyepiece graticule. Muscle length was increased in steps of 0.2 mm using a micromanipulator. When the length was increased, passive force increased; 5 min was allowed at each new length for passive force to decline to a stable value. A twitch was elicited and the peak twitch force and passive forces were recorded at each new length. Active force was calculated as the peak twitch force (total force) minus the passive force. The length was increased until active force had reached a peak and then started to decline. Active force was plotted against muscle length to determine the length for maximum force production (L_{max}). Cardiac muscle does not usually work on the descending limb of the force–length curve (Allen and Kentish, 1985) and is prone to damage at longer lengths. To avoid overstretching, the muscle length was only increased until L_{max} could be determined unambiguously. Isometric measurements were made using on-screen cursors on the oscilloscope. Measurements of maximum twitch force (P_{tw}), the times from the stimulus to half-peak twitch force ($t_{0.5a}$) and peak twitch force (t_p) and the times from peak force to 50 % ($t_{0.5r}$) and 90 % ($t_{0.9r}$) of peak force during relaxation were made from the twitch records.

Determination of the frequency for maximum work and power production

In work loop experiments, one end of the preparation was attached to a force transducer and the other to a servomotor. After L_{max} had been determined, muscle length was reduced to 95 %L_{max} (L_0). The physiological length range for mammalian cardiac muscle is 87.5–100 %L_{max} (Brusaert and Paulus, 1977). On the assumption that fish cardiac muscle operates within the same range, experiments were performed at 95 %L_{max} to avoid damage to the preparation. The preparations were subjected to sinusoidal length changes about the starting length (L_0), and the amplitude of the length change was expressed as ±%L_0. Stimulation was applied at selected phases of the strain cycle. Phase was expressed in degrees, where 0 ° denotes L_0 during lengthening and a complete strain cycle is 360 °. Experiments were carried out over a range of cycle frequencies (0.3–1.3 Hz) within the physiological range of heart rates found in trout (0.3–1.5 Hz; Priede, 1974). At each frequency, the length change (strain) and stimulus phase were manipulated systematically to achieve maximum net work. Net work was calculated as the difference between the work needed to lengthen the muscle and the work performed by the muscle during shortening. Force was plotted against length to give a series of loops, and net work was calculated as the area of the loop formed between the lengthening and the shortening work. Power output is the rate at which work is done and was calculated by multiplying the net work done during one cycle by the cycle frequency. At frequencies of 1.1 Hz and above, the muscle failed to relax completely during the second cycle; therefore, five strain cycles were performed in each experimental run and work was measured as the average of cycles 3 and 4. For consistency, this protocol was performed at all frequencies in this part of the study (for a full explanation, see Fig. 3 and associated text). A 5 min recovery period was allowed between runs. Contraction duration measurements were made from force records during work loop cycles using on-screen cursors on the oscilloscope. Contraction duration was measured as the time from the stimulus until the force fell to its initial baseline value.

Effect of work loop number

This protocol was used to determine the number of work loops to which a muscle must be subjected to give a representation of sustainable work. At the end of each work–frequency and power–frequency experiment, the muscle was subjected to 300 length change cycles at either 1.0 or 1.3 Hz using the strain and phase parameters yielding maximum work output for these frequencies.

Determination of length for maximum work output

L_{max} was determined using the protocol described above. Work was measured over a series of muscle starting lengths that were varied in small increments within the range 80–105 %L_{max}. The preparations were subjected to a series of work loop cycles at 1.0 Hz. Power production at 1.0 Hz was not significantly different from that at 1.1 Hz, the frequency for maximum power output. The strain (±8 % of starting length) and stimulus phase (30 °) parameters that were found to be optimal at 1.0 Hz were used throughout the experiment.

Because the strain was held at a constant percentage of L_0 (±8 %), absolute strain increased with increasing starting length. Lengthening work (the work done on the muscle to stretch it) and shortening work (the work output from the muscle during shortening) were determined and recorded separately. At 1.0 Hz, the muscle relaxes fully during loop 2; all experiments to determine the effect of length on work were carried out at 1.0 Hz. Therefore, four cycles were performed in each experimental run with a 5 min rest between runs, and work was measured as the average work of cycles 2 and 3 (see Results for a full explanation).

Determination of the dynamic passive properties, dynamic stiffness and resilience

Work cycles were also performed in which the muscle was not stimulated. The work recorded during these cycles is referred to as passive work. These loops generated by the unstimulated muscle are clockwise, showing that the work done on the muscle to stretch it is greater than the work returned during shortening. Passive work (or hysteresis) was calculated as the area between the shortening and lengthening limbs of the passive loop. Resilience was determined as the amount of energy returned during relaxation as a proportion of the amount of energy put into the system during extension. An ideal elastic element would have a resilience of 100 %. We calculated dynamic stiffness in both active and passive loops by calculating the difference between maximum and minimum force and between maximum and minimum length in each work loop cycle and then dividing the change in force by the change in length (Josephson, 1997). This was performed at each increment in muscle length during the determination of the length for maximum work.

Controlling for muscle deterioration

All muscles were monitored for any deterioration or increase in net work by performing a series of control cycles after every third experimental run. To avoid a bias being introduced into the results, the order in which the muscles were subjected to different frequencies was varied from experiment to experiment. Control runs were carried out at the first cycle frequency used during the work–frequency, power–frequency experiments. Similarly, the order in which the muscle length was changed was varied during work–length experiments (all at 1.0 Hz), and control runs were carried out at the first starting length tested. Correction of the net work per cycle was made by assuming that it declined or increased linearly between consecutive control runs. Similar corrections have been applied in other work loop studies (e.g. Marsh and Olson, 1994; Layland *et al.* 1995*a*,*b*). Muscle activation and relaxation times between the first and last control runs were not significantly different (P<0.05) and the shapes of the loops were consistent over time. This suggests that any deterioration was due to a decline in force rather than to a change in muscle kinetic properties (Altringham and Young, 1991). Preparations were discarded if the force declined to less than 75 % of its initial value.

Conclusion of experiment

Upon completion of the experiment, the preparation was removed from the chamber and the platinum links were cut away with the aid of a stereo-microscope. The muscle was blotted on laboratory tissue and weighed. The volume of the muscle was determined gravimetrically (i.e. volume = mass/density) assuming a muscle density of 1060 kg m^{-3}. Isometric stress was calculated as muscle force divided by muscle cross-sectional area, and mass-specific work and power were calculated.

Data acquisition and statistics

The stimulation frequency and magnitude of the length change imposed on the preparation together with the phase of stimulation were controlled using a PC microcomputer. Force and length data were captured and analysed on-line using in-house software. A Student's *t*-test (Sigmastat statistical software, SPSS) was used to compare the work output from different loops within a series of 300 strain cycles and the muscle activation and relaxation times between the first and last control runs.

Results

Epicardial deformation

Five consecutive hearts beats from each of three hearts were analysed. The mean strain was found to be 23.8±0.86 % of the maximum length (mean ± S.E.M., N=3). Fig. 1 illustrates epicardial strain as a function of time for a typical heart. Shortening was more rapid than lengthening, and there was a broad plateau at maximum length. There was no apparent variation in strain amplitude with time.

Isometric experiments

Results from isometric experiments are summarised in Table 1. The maximum isometric stress of rainbow trout ventricular muscle was found to be 22.00±0.83 kN m^{-2} (mean ± S.E.M.,

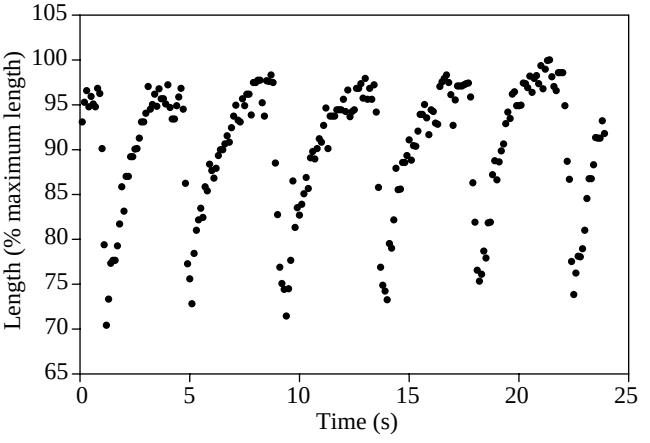

Fig. 1. A typical recording of muscle length from the anterior vertex of a rainbow trout heart. The frequency of contraction is approximately 0.3 Hz. The change in length has been normalised to the maximum excursion, which has been assigned a value of 100 %. Strain is measured as the difference between the maximum and minimum excursion for each contraction.

Table 1. *Muscle mechanical (isometric and dynamic) properties, at* L_{max}*, of rainbow trout ventricular preparations*

Muscle wet mass (mg)	4.27±0.20 (19)
Muscle length at L_{max} (mm)	7.65±0.20 (19)
Muscle diameter at L_{max} (mm)	0.82±0.02 (19)
t_p at L_{max} (ms)	437.4±7.4 (19)
$t_{0.5a}$ at L_{max} (ms)	136.5±3.9 (19)
$t_{0.5r}$ at L_{max} (ms)	400.4±10.7 (19)
$t_{0.9r}$ at L_{max} (ms)	586.3±14.1 (19)
Peak active force at L_{max} (mN)	11.61±0.49 (19)
Peak stress at L_{max} (kN m^{-2})	22.00±0.83 (19)
Passive force:active force ratio at L_{max}	0.92±0.03 (19)
Maximum net work (J kg^{-1}) (at 0.6 Hz)	1.42±0.13 (9)
Maximum net power (W kg^{-1}) (at 1.1 Hz)	1.27±0.08 (9)

Values are means ± S.E.M. (*N*).

L_{max}, muscle length for maximum isometric force production; t_p, time from stimulus to peak twitch force; $t_{0.5a}$, time from stimulus to half-peak twitch force; $t_{0.5r}$, time from peak force to 50 % peak force; $t_{0.9r}$, time from peak force to 90 % peak force.

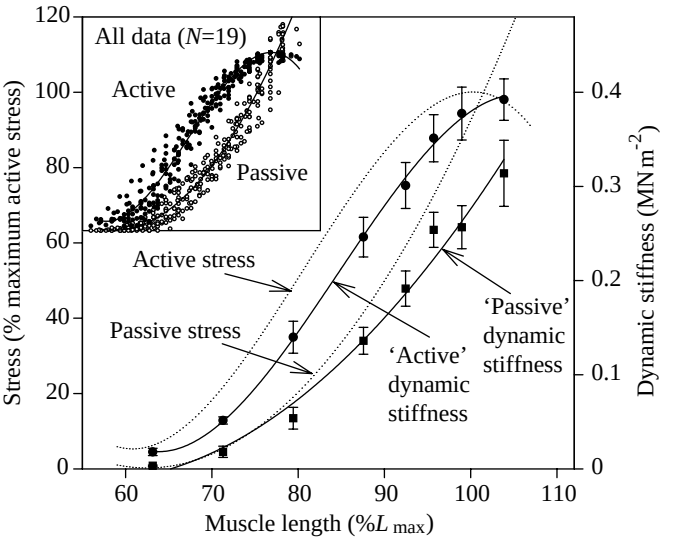

Fig. 2. Active (peak twitch stress or the total stress minus the passive stress) and passive isometric stress at different muscle lengths for the combined data set (*N*=19). Data points for the same data set are shown in the inset. Isometric data were collected both during preliminary experiments and during work and power experiments. Combined values of stress are standardised to the stress obtained at the muscle length for maximum isometric force production (L_{max}). The lines are second- and third-order polynomial regressions fitted to the data using least-squares regression. Dynamic stiffness is defined as the ratio of the change in force to the change in length. Active dynamic stiffness was derived from active work loops and passive dynamic stress from passive (hysteresis) loops. All values are means ± S.E.M., *N*>6.

N=19). Fig. 2 shows the active and passive force–length relationship for 19 ventricular preparations. Active isometric force rose rapidly with increasing length to an optimum length and then started to decline. Passive force rose steeply with length, increasing beyond active force at approximately L_{max}. The curves and the data points for the same combined data set are shown in the inset in Fig. 2.

Fig. 2 also shows the relationship between dynamic stiffness (active and passive) and length. Like stress, dynamic stiffness increases with increasing length. Active dynamic stiffness increases more rapidly with length than does passive dynamic stiffness up to approximately 100 %L_{max}, at which point it appears to begin to plateau. This corresponds to the length at which the muscle begins to develop progressively less force during contraction on the descending limb of the force–length relationship.

Work loop experiments

Changes in work with work loop number

Fig. 3 illustrates the effect of work loop number on net work production. At 1.0 Hz, net work declined after the first cycle and continued to decline until cycle 23, when work started to recover (Fig. 3). By cycle 299, net work had recovered to a plateau at a value close to the mean work for cycles 2 and 3. A *t*-test showed no significant difference between the mean work of cycles 2 and 3 and the work of cycle 299. The mean work of cycle 299 was always lower than the initial value for cycle 1. Similar results were obtained at all frequencies below 1.0 Hz. When the muscle was subjected to 300 cycles of work at frequencies of 1.1 Hz and above, the results were a little different. At 1.3 Hz, the force during the first cycle was high, but the muscle did not relax before the second cycle; consequently, net work output in cycle 2 was very low (Fig. 3). The mean work of cycles 3 and 4 was not significantly different from the work of cycle 299.

In work–frequency experiments, which cover the whole frequency range from 0.3 to 1.3 Hz, the muscle was subjected to five cycles and sustainable work output was estimated as the mean work from cycles 3 and 4. Work–length experiments were all carried out at 1.0 Hz. Because the muscle relaxed fully during loop 2 at 1.0 Hz and below, there was no need to subject the muscle to more than four work loop cycles, and work was calculated as the mean of loops 2 and 3.

Cycle frequency, work and power

Fig. 4 illustrates the work–frequency and power–frequency relationships. Maximum net work was 1.42±0.13 J kg^{-1} at a cycle frequency of 0.6 Hz. Net work output first increased between 0.3 and 0.4 Hz, then remained almost constant between 0.4 and 0.8 Hz, after which it declined. Mechanical power output also showed a marked dependence on cycle frequency, increasing with increasing cycle frequency up to a maximum of 1.27±0.08 W kg^{-1} at 1.1 Hz. The frequency for maximum power output occurred at higher frequencies than for maximum work.

Stimulus phase and muscle strain

Stimulus phase and strain were varied with cycle frequency to achieve maximum mechanical work output. Optimum stimulus phase decreased with increasing cycle frequency:

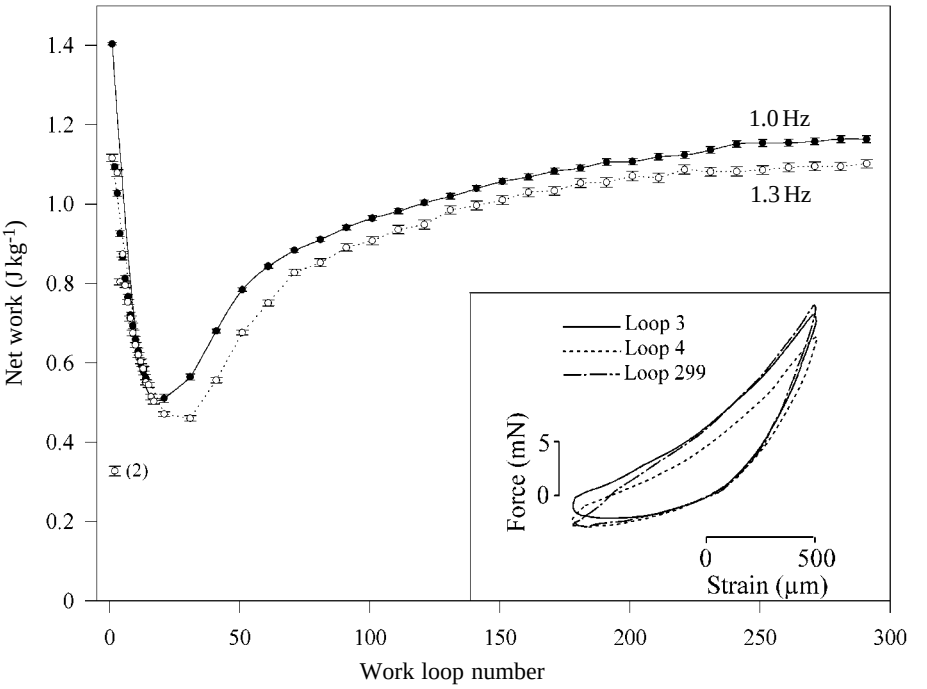

Fig. 3. The net work production (means ± S.E.M., *N*=4) over a series of 299 cycles at 1.0 Hz (filled circles) and 1.3 Hz (open circles). Work loop cycles were carried out at the cycle frequency, strain and stimulus phase yielding maximum power output determined during preliminary experiments. Mean net work from every cycle between cycles 1 and 14 is shown for 1.0 Hz and between cycles 1 and 17 for 1.3 Hz; after this, every fifth cycle is plotted. Cycle 2 at 1.3 Hz, which produces particularly low net work, is labelled (2). The inset illustrates the similarity in work loop shape at 1.3 Hz between cycles 3, 4 and 299.

from 100 ° at 0.3 Hz to 1 ° at 1.3 Hz. To maximise net work production, it was necessary to decrease strain with increasing cycle frequency; from ±12 %L_0 at 0.3 Hz to ±8 %L_0 at 0.9 Hz and above. Fig. 5 shows records of force and strain plotted against time. Increasing values of strain correspond to muscle lengthening, decreasing values correspond to contraction. The time from the onset of the twitch until the force declined to zero was taken to be the duration of contraction.

Contraction duration decreased with increasing cycle frequency from 0.82±0.01 s at 0.3 Hz to 0.58±0.01 s at 1.2 Hz. However, because shortening duration decreased with increasing frequency proportionately more than contraction duration, the contraction period became a progressively larger proportion of the total cycle period. Maximum work output was obtained when the phase of stimulation was altered so that force was generated predominantly during shortening. The maximum work achievable at 0.3 Hz was obtained using a stimulation phase of 100 °. Force was developed over only approximately half the shortening period and work was consequently relatively low. The contraction duration and

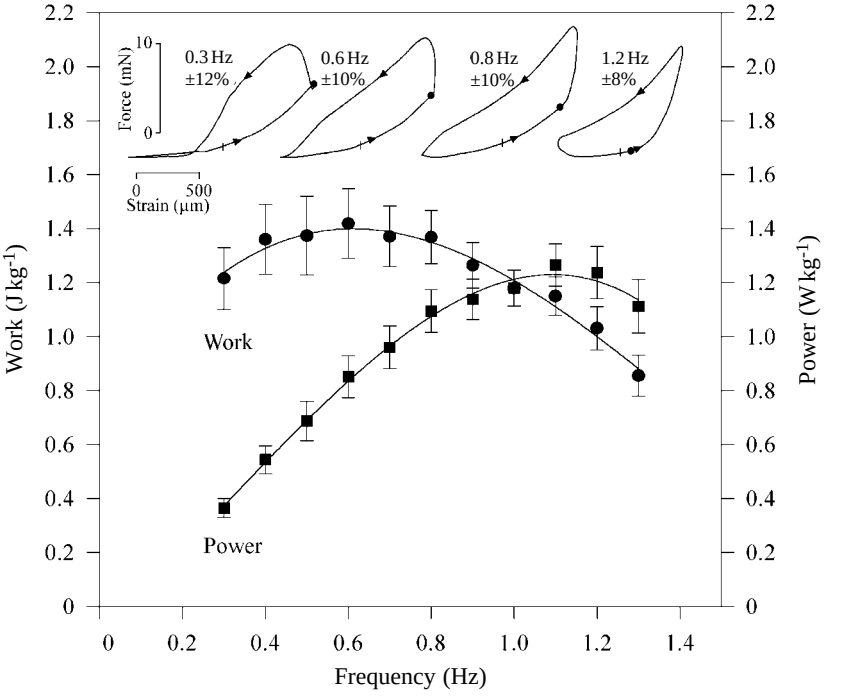

Fig. 4. The effect of cycle frequency on work and power. All values are means ± S.E.M., *N*=5–9). The lines are third-order polynomials fitted to the data using a least-squares regression. Representative work loops are illustrated above, arrows indicate the direction of travel and the stimulus timing is indicated by a black dot.

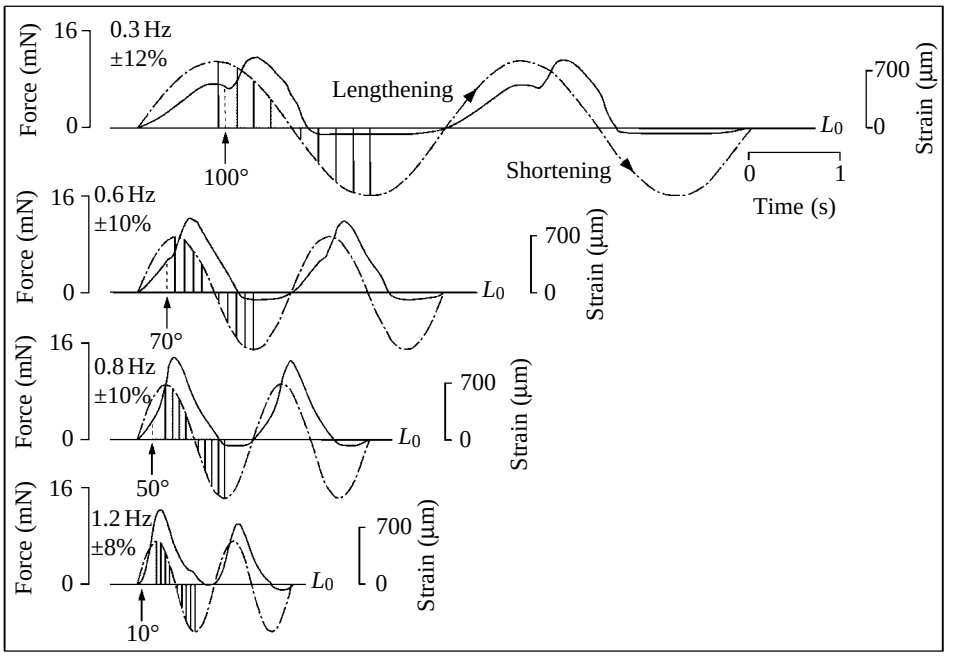

Fig. 5. Muscle strain (dashed-dotted line) and force (solid line) records from the third and fourth cycles at four frequencies covering the physiological range. The arrows indicate stimulus timing (given in degrees). The solid vertical lines between the strain curve and the horizontal axis delineate the shortening phase of the cycle. The shaded region indicates the duration of contraction by the muscle. L_0, muscle starting length.

shortening period were approximately equal at 0.8 Hz. However, work output varied little between 0.4 and 0.8 Hz because optimisation of the stimulation phase ensured that the muscle generated force during most of the shortening period. At high cycle frequencies, contraction duration was found to be longer than the shortening period such that force was developed during both the lengthening and shortening phases of the strain cycle. Force development during lengthening increases the work done to re-extend the muscle, decreasing net work. At 1.2 Hz, maximum work was achieved when the muscle was stimulated at 10 ° and generated force during most of the lengthening (doing negative work and resisting lengthening) and all the shortening period.

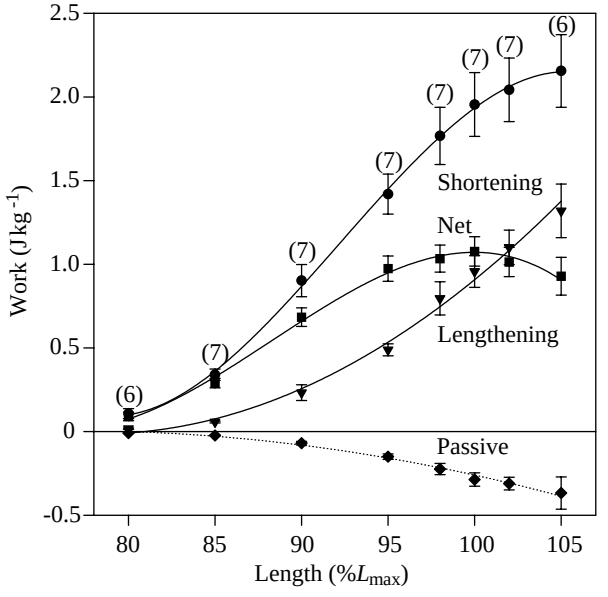

Fig. 6. Shortening work, lengthening work, net work and passive work at different muscle starting lengths. The numbers of replicates are indicated in parentheses; all values are means ± S.E.M. The lines are second- or third-order polynomials fitted to the data using a least-squares regression. L_{max}, muscle length for maximum isometric force production.

Work–length relationship

The length about which the muscle was oscillated had a marked effect on the shape of the work loop produced and the net work output. Fig. 6 illustrates the effect of length on the components of work. The length for maximum net work output (L_{opt}) was the same as the optimum length for isometric force production. Shortening work, lengthening work and passive work all increased with increasing muscle starting length. At 102 %L_{opt}, lengthening work increased beyond net work and net work started to decline.

Fig. 7 shows the effect of length on net work and resilience (the amount of energy returned during relaxation as a percentage of the work done during the lengthening of a passive muscle). Representative active and passive work loops are also shown. The resilience of trout myocardium increases with length, from less than 40 % at 80 %L_{max} to approximately 75 % at 95–105 %L_{max}. This shows an increase in the amount of the work done in lengthening the myocardium being returned during shortening at lengths producing close to maximum net work.

Discussion

Maximum isometric stress

The maximum isometric stress for most vertebrate and invertebrate skeletal muscles lies between 150 and 400 kN m^{-2} (Josephson, 1993). In contrast, cardiac muscle is highly aerobic and has a lower fractional myofibrillar volume than skeletal

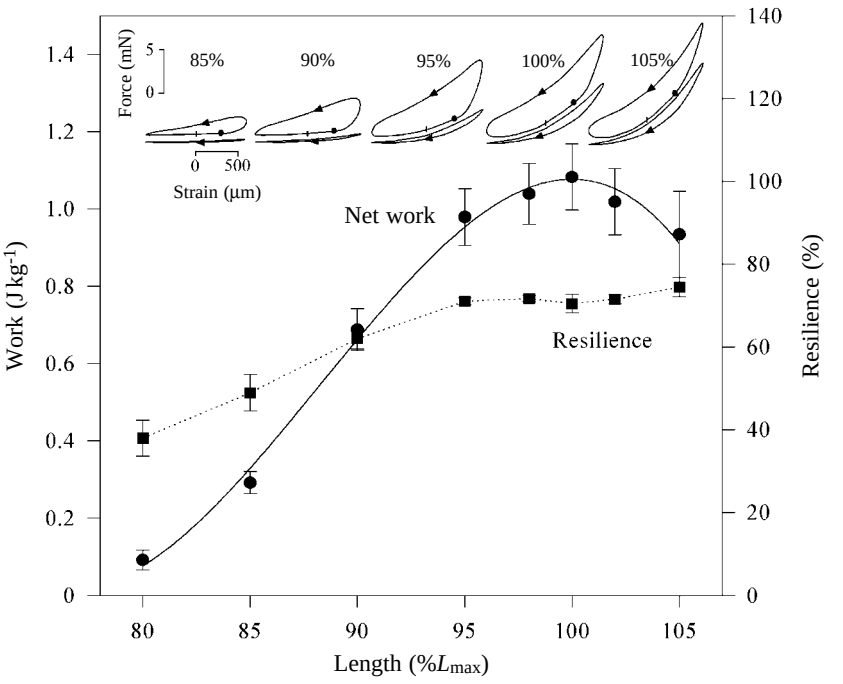

Fig. 7. Resilience and net work plotted against muscle starting length L_0. All values are means ± S.E.M., N>6. L_{max}, muscle length for maximum isometric force production. Typical work loops are illustrated at the top. Cycle frequency is 1.0 Hz; strain is ±8 % and stimulus phase is 30 ° (indicated by a black dot on the work loop). The upper, active loop is counterclockwise, and the area represents net positive work. The lower passive loop is from a cycle under the same conditions but without stimulation. This loop is clockwise, and the area represents net energy loss or hysteresis. Arrows indicate the direction of travel.

muscle, and stresses are low (Huxley, 1961). However, the lower isometric stresses cannot be explained simply by differences in myofibrillar volume and must lie in part in differences in the cross-bridges themselves (Huxley, 1961). The maximum isometric stress found in this study was 22.00±0.83 kN m^{-2}. This is substantially higher than values found in previous studies: 1.6±0.33 kN m^{-2} at 12 °C and 0.2 Hz stimulation frequency (Shiels and Farrell, 1997) and 4.1±0.2 kN m^{-2} (at 75 % of peak length) at 10 °C (Gesser, 1996). Frog ventricular muscle has a reported stress of 51 kN m^{-2} (21–23 °C) (Layland *et al.* 1995) and rat papillary muscle has a reported stress of 46.7 kN m^{-2} (37 °C) (Syme, 1993), approximately twice that found for rainbow trout in this study. Myofibrillar densities of 47 % have been recorded in the myocardial cells of brown trout (*Salmo trutta*) ventricles (Yamauchi and Burnstock, 1968) compared with approximately 60 % in mammalian species (Barth *et al.* 1992). This could only partly explain the twofold difference between the stresses we found for rainbow trout cardiac muscle and mammalian and amphibian myocardium. The remaining inequality must be due to differences in the contractile proteins between these classes of animal.

The effect of repeated work cycles

The protocol used was chosen primarily to establish whether a small number of work cycles could provide useful information about the steady-state operation of the heart. We have established that the mean work from cycles 2 and 3 at frequencies below 1.0 Hz and the mean work from cycles 3 and 4 at higher frequencies provide a good index of the sustainable work that a muscle can produce. The close similarity in loop shape between these cycles supports this supposition and also suggests further investigation of myocardial dynamics using this protocol. The effect of repeated work cycles in itself has raised further questions. The reduction in work from cycles 1 to 25 was due largely to a reduction in force. Maximum force declined with work to approximately cycle 25 and then recovered towards a plateau. The muscle was always fully relaxed before the next cycle (with the exception of cycle 2 at higher frequencies), indicating that impairment of relaxation rate was not the main reason for the decline in work. The initial high work output may be due to post-rest potentiation, which is commonly seen in cardiac muscle and has been attributed to increased Ca^{2+} release from the sarcoplasmic reticulum (Bers, 1985). Ventricular strips from rainbow trout demonstrate pronounced post-rest potentiation after 5 min of quiescence at 15 °C (El-Sayed and Gesser, 1989). The magnitude of the potentiation effect increases at higher frequencies, as seen in ventricular strips from the skate (Driedzic and Gesser, 1988). The sarcoplasmic reticulum in fish heart is particularly sparse (Santer, 1985), which is taken as evidence that it plays an insignificant role in the activation of contraction. This would seem contrary to our observations under these conditions and would merit further investigation.

The work–frequency relationship

The maximum work output of 1.42 J kg^{-1} is close to that for mammalian papillary muscle at 37 °C (1.91 J kg^{-1}; Layland *et al.* 1995*a*). Interestingly, the ratio of myofibrillar fractional volume between fish and mammalian cardiac muscle (47 %/60 %=0.78) is very similar to the ratio of work output (1.42 J kg^{-1}/1.91 J kg^{-1}=0.74). This seems at odds with the observation that myofibrillar fractional volume cannot explain the difference in stress between mammalian and fish myocardium.

The similarity in work output, despite the large difference in

operating temperatures, suggests significant temperature compensation; this compensation being for stress-determined rather than for rate-determined processes as shown in previous studies (Johnston and Altringham, 1989). The power output of rainbow trout ventricle (1.27 W kg^{-1}) is much lower than that of rat papillary muscle (8.62 W kg^{-1}; Layland *et al.* 1995*a*). This difference can be reconciled by the lower frequency at which maximum power output is obtained. We shall consider this below.

Maximum work output varies with cycle frequency, but not in a linear manner. We found that the highest values for maximum work were obtained between 0.4 and 0.8 Hz. At 0.3 Hz, force is developed for only approximately half the shortening period, compared with almost all the shortening period between 0.4 and 0.8 Hz. Consequently, at 0.3 Hz, the distance that the muscle shortens while it is developing force is only half of the imposed ±12 % strain (i.e. half of 24 % total strain, or 12 %). In Fig. 5, we can see that the muscle develops a similar force at 0.6 Hz to that at 0.3 Hz but throughout the entire shortening period. The optimum strain in this case is ±10 %, so that the muscle develops force over a greater distance (20 % of total strain) and more work is performed.

At frequencies higher than 0.8 Hz, the contraction duration becomes progressively longer than the shortening period, and force is developed during lengthening. This increases lengthening work. Because net work is shortening work minus lengthening work, any increase in lengthening work results in a decrease in net work. These results agree with previous studies on rats and frogs (Layland *et al.* 1995*a*; Syme, 1993) in which net work production was found to be maximal at a frequency where the contraction duration was the same as the shortening period. There are many similarities in the properties of vertebrate cardiac muscle, not least that the duration of contraction cannot normally be increased by increasing the number of stimuli. It is not surprising, therefore, to find that the frequency for maximum work output across these classes of animals is largely determined by contraction duration.

Optimum strain

The pyramidal shape and complex fibre orientation in the rainbow trout ventricle make it difficult to relate preparation strains directly to changes in ventricular volume. However, it is useful to consider this relationship in general terms. Stroke volume and muscle strain are related. The ejection fraction in rainbow trout is normally close to 100 % (Franklin and Davie, 1992), but falls at high aortic pressures as end-systolic volume increases. Farrell *et al.* (1989) also found that stroke volume declined at high heart rates. We report that the strain for maximum work output decreases with increasing frequency. If strain is related to stroke volume (Layland *et al.* 1995*a*,*b*, 1997; Syme, 1993; Syme and Josephson, 1995), then this decline in optimal strain could explain the observed decline in stroke volume at high heart rates (Farrell *et al.* 1989; Franklin and Davie, 1992).

Previous work loop studies have found that optimised muscle strains correspond very closely to those observed *in vivo* (Marsh and Olson, 1994; James *et al.* 1995). In our perfused heart experiments, the ventral vertex of the rainbow trout heart was observed to be shortening and lengthening along its axis by 23.8 % at a frequency of 0.3 Hz, giving a strain of ±11.9 %L_0. This corresponds extremely well to the optimum strain of ±12 % found in our work loop study at 0.3 Hz.

The strain for maximum work output in rainbow trout was ±8 %L_0, higher than the value of ±5 %L_0 for rat papillary muscle (Layland *et al.* 1995*a*), but lower than that in frog ventricular muscle (greater than ±13 %L_0; Syme, 1993). This may be related to the way in which cardiac output is regulated in different animals. Mammals tend to increase heart rate while maintaining the same stroke volume. Cardiac output will increase proportionately, i.e. a doubling of heart rate will result in a doubling of cardiac output. In contrast, fish cardiac muscle displays a 'negative staircase effect' where increases in contraction frequency cause a decrease in the duration and the force of contraction (Driedzic and Gesser, 1988). Perhaps constrained by this, fish control their cardiac output primarily by increasing stroke volume, which involves increasing myocardial strain. However, because volume increases in proportion to the cube of the increase in length, relatively small changes in muscle strain can produce relatively large changes in stroke volume and hence in cardiac output.

The effect of myocardial length on force and work

The force–length relationship for trout myocardium was found to be very steep. Reducing muscle length from 100 % to 70 %L_{max} reduced force development to less than 10 % of the maximum. However, it has been shown in mammalian cardiac muscle that changes in myofilament overlap have less influence on muscle force than activation effects, i.e. length-dependent changes in Ca^{2+} release or Ca^{2+} sensitivity of the myofibrils (Allen and Kentish, 1985). The steep force–length relationship in rainbow trout suggests that activation of the contractile elements is length-dependent. Furthermore, the time course of the twitch (both $t_{0.5r}$ and $t_{0.9r}$) increased with length. This may also indicate an increase in myofilament sensitivity to Ca^{2+}. Twitch duration may increase at longer lengths as a result of increased Ca^{2+} binding and slower Ca^{2+} dissociation from troponin (Allen and Kurihara, 1982).

At lengths above 100 %L_{max}, myofilament overlap and the number of active cross-bridges decrease, there is an increase in hysteresis and a massive increase in passive muscle force, as seen by the increase in lengthening and passive work (Fig. 6). Net work increased initially, reached a maximum and then started to decline as lengthening work approached shortening work. These results are similar to those obtained from rat papillary muscle (Layland *et al.* 1995*b*) and frog ventricular muscle (Syme and Josephson, 1995), where both dynamic active and dynamic passive force were shown to interact to determine net work output.

Rainbow trout ventricular muscle exhibited very high passive forces that increased with muscle length; from close to zero at 65 %L_{max} to more than 90 % of active force at L_{max}. Cardiac muscle contains more connective tissue, and non-

activated preparations are known to be much stiffer than skeletal muscle (Spiro and Sonnenblick, 1964). Rainbow trout ventricular muscle was found to be much stiffer than rat papillary muscle. The passive-to-active force ratio at L_{max} was double that found in rat (0.92 compared with 0.48). Why have a very stiff myocardium given that a stiffer muscle requires more force to extend it? Is some of the energy used to extend the muscle returned during shortening? To act as a useful energy store, a spring must be sufficiently stiff so that an appreciable amount of energy can be stored by its normal deformation and a large proportion of this energy must be recovered during recoil. It has been suggested that intramyocardial connective tissue prevents overstretching of the myocardial cells and confers viscoelastic properties on the heart (Robinson *et al.* 1988). Titin, connecting the thick filaments to the Z-line, represents a considerable parallel elastic element in cardiac muscle (Allen and Kentish, 1985). Further connective tissue, in the form of coiled perimysial fibres, is abundant in the myocardium of pyramidal-shaped hearts (Sanchez-Quintana *et al.* 1996).

Josephson (1997) used the indices of dynamic stiffness and resilience to examine the possible energy-saving elastic mechanisms in bumblebee (*Bombus terrestris*) flight muscle. Dynamic stiffness was defined as the ratio of the change in force to the change in length, and resilience was determined as the amount of energy returned during relaxation as a proportion of the amount of energy put into the system during extension. An ideal elastic element would have a resilience of 100 %. Josephson (1997) found that passive dynamic stiffness in bumblebee flight muscle became larger than active dynamic stiffness at long muscle lengths. He explained this apparent reduction in stiffness during muscle activation by arguing that the passive stiffness had an element that was associated with muscle activation, presumably in the cross-bridges. In cardiac muscle, we find that the active dynamic stiffness is higher than the passive dynamic stiffness between 60 and 105 %$L_{max.}$ This shows that dynamic stiffness is not decreased by muscle activation and could be accounted for by the connective tissue structures within the myocardium. The resilience of rainbow trout myocardium varies with length, from less than 40 % at 80 %L_{max} to approximately 75 % at 95–105 %$L_{max.}$ More energy is returned at muscle lengths producing close to maximum work. Without elastic storage, the energy expended by the atrium in refilling the heart would be lost. With elastic storage, over the length range yielding close to maximum work, up to 75 % of the energy used to refill the heart could be returned during ventricular contraction.

The power–frequency relationship

The frequency for maximum power output occurred at 1.1 Hz (15 °C), higher than the frequency for maximum work. This corresponds to a heart rate of 66 beats min^{-1}. Farrell *et al.* (1996), using an *in situ* heart with an intact pericardium, found 'peak cardiac performance' at approximately 70 beats min^{-1} (1.17 Hz) at 15 °C. In the present study, power output increased with frequency to a maximum and then decreased. However, isometric force declines with increasing stimulation frequency in many teleost ventricles, including that of trout (Driedzic and Gesser, 1985). The dynamic properties of muscle are apparent only when it is allowed to shorten or has a strain cycle imposed upon it. Active lengthening can lead to enhancement of force (e.g. Edman *et al.* 1978), and shortening can cause muscle deactivation (Ekelund and Edman, 1982). These dynamic properties partially offset the effects of increasing stimulation frequency observed under isometric conditions (Altringham and Johnston, 1990).

We found an increase in the rate of decline in muscle force at higher frequencies. This could be explained by shortening deactivation and is important when we consider the dynamics of ventricular filling. Isometric studies show an increase in the rate of force development and of relaxation with frequency (Shiels and Farrell, 1997). However, the same study showed that force remains high for almost the entire interpulse interval. From isometric results alone, we would expect either that ventricular refilling is particularly rapid or that it occurs against a substantial ventricular pressure caused by maintained myocardial activity. Ventricular filling is believed to be driven only by atrial contraction (Farrell and Jones, 1992); the contraction of the atrium forces blood into the ventricle and increases its volume. Shortening deactivation would cause an increase in the rate of decline of ventricular activity and reduce the amount of work required during refilling. Shortening deactivation is seen as a more rapid decline in force during shortening at higher cycle frequencies. Therefore, there is a reduction in the subsequent lengthening work. This allows net work output to be maintained at high contraction frequencies and, in turn, an increase in myocardial power output.

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