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THE EFFECT OF CYCLE FREQUENCY ON THE POWER OUTPUT OF RAT PAPILLARY MUSCLES *IN VITRO*

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Summary

Papillary muscles were isolated from the right ventricles of rats and the length for maximum active force generation (L_{max}) was determined isometrically. The work loop technique was used to derive the length for maximum work production (L_{opt}) at the cycle frequency, strain amplitude and stimulation phase shift found to be optimal for power output. L_{opt} was typically 7 % shorter than L_{max} and within the physiological length range (87.5 % L_{max} to L_{max}). Net work and power output were measured during sinusoidal strain cycles around L_{opt}, over the cycle frequency range 1–9 Hz, strain amplitude and phase shift being optimised for work and power at each frequency. Experiments were performed at 37 °C.

Distinct optima were found in both the work–frequency and the power–frequency relationships. The optimum cycle frequency for net work production was lower than the frequency for maximum power output. The mean maximum power output at 37 °C was 8.62±0.50 W kg^{-1} (mean ± S.E.M., N=9) and was achieved at a cycle frequency of approximately 6 Hz, close to the estimated resting heart rate of 5.8 Hz for the rats used (mean mass 223±25 g). The cycle frequency, strain amplitude and stimulation phase shift found to be optimal for power output produced an *in vitro* contraction closely simulating the basal *in vivo* contraction.

Key words: papillary muscles, power output, work loops, optimal design, rat.

Introduction

A. V. Hill (1950) and others since have suggested that natural selection has resulted in the optimisation of muscle contractile properties to suit their function *in vivo*. He proposed that each muscle was ‘designed’ for maximum power and efficiency in its important range of operating speeds.

Until comparatively recently, muscle power output *in vitro* was assessed using the product of developed force and shortening velocity derived from isotonic measurements. This technique has been applied to isolated cardiac muscle preparations (e.g. Sonnenblick, 1962). Isotonic experiments assess the power-generating capacity of a muscle from the maximum power developed during shortening at constant load and velocity, with maximal activation throughout the entire contraction. These conditions are rarely satisfied *in vivo* and isotonic studies do not therefore provide a realistic estimate of physiological power output (Josephson, 1993).

More recently, the work loop technique (Josephson, 1985) has been developed to study the work and power output of muscles *in vitro*. This technique allows the *in vivo* situation to be simulated. Both the work done by the muscle and the work required to re-extend it are measured during a realistic muscle length change (strain) cycle. Electrical stimulation is applied to the muscle at a given phase shift in a sinusoidal strain cycle. By plotting force against muscle strain over the whole cycle, a work loop is generated, the area of which represents the net work performed. Mean power output (W) is calculated as the product of net work (J) and cycle frequency (Hz). The similarity between the work loops generated from isolated muscle preparations and the pressure–volume loops derived from the whole heart is striking. This analogy was noted by Syme (1993), who used the work loop technique for the investigation of work and power output of frog cardiac trabeculae.

Several studies on skeletal muscles using the work loop technique have demonstrated that muscles *in vitro* produce maximum power output at cycle frequencies corresponding to their normal operating frequencies *in vivo* (see, for example, Stokes and Josephson, 1988; Josephson and Stokes, 1989; Altringham and Young, 1991; James *et al.* 1995). These studies support some of the ideas proposed by Hill (1950) on the evolution of an optimal design for muscles. Hill included the heart in his review and, since cardiac muscle has fundamental similarities to skeletal muscle, it is not unreasonable to propose that it too will be optimised for power production at an operating frequency similar to the *in vivo* situation. The present investigation examines this hypothesis by studying the relationship between power output and operating frequency in mammalian cardiac muscle, using rat papillary muscles.

Papillary muscles have been shown to be physiologically similar to trabeculae carneae taken from the ventricular wall, showing no significant difference in force-generating capacity and twitch kinetics (Wiggins, 1980). Contraction of papillary muscles in the heart prevents the prolapse of the valves during ventricular contraction (Cronin *et al.* 1969). *In situ* recordings of papillary muscle force, length and electrical activation patterns show that papillary muscles undergo cyclical length changes (Fisher *et al.* 1965; Semafuko and Bowie, 1975), achieve peak force at maximum length (Armour and Randall, 1970) and continue to produce force during shortening (Semafuko and Bowie, 1975). Therefore, the papillary muscles perform positive work during the ventricular ejection phase of the cardiac cycle and the rate at which work is done (i.e. the power output) is proportional to the heart-beat frequency.

In this study, we used the work loop technique to examine *in vitro* cardiac muscle performance in conditions chosen to resemble the physiological situation. The net work and power produced by the muscle were measured at different cycle frequencies at a temperature of 37 °C. The results are discussed with reference to the physiological situation.

Materials and methods

Muscle preparation

Papillary muscles were dissected from the hearts of Wistar laboratory rats of both sexes (mass 223±25 g, mean ± S.E.M., N=9). Rats were killed by cervical dislocation and the heart was rapidly removed and rinsed in oxygenated mammalian cardiac Ringer at room temperature (composition in mmol l^{-1}: NaCl, 144; sodium pyruvate, 10; KCl, 6; $MgCl_2$, 1; $CaCl_2$, 2; NaH_2PO_4, 1; $MgSO_4$, 1; Hepes, 10; pH 7.4 at room temperature: after Daut and Elzinga, 1989). The heart was then mounted in a Petri dish on a Sylgard 184 base (Dow Corning) and immersed in fresh Ringer.

An incision was made through the wall of the right ventricle and the muscle was pinned back to expose the interior of the chamber. It was then possible to locate two or three papillary muscles. The papillary muscles were removed by cutting through the chordae tendineae at the valvular end of the muscle and isolating them from the rest of the myocardium by careful dissection, leaving a small piece of ventricular wall still attached to the papillary muscles at the non-tendinous end. The Ringer was replaced at frequent intervals during dissection and, once excised, the muscles were placed in separate Petri dishes and immersed in fresh oxygenated Ringer.

Small aluminium foil clips were attached to both ends of the papillary muscles, clamping the tendon at one end and the ventricular wall at the other. Clips were attached as close as possible to the papillary muscle fibres without causing damage. The muscle preparation was then mounted in a flow-through chamber containing oxygenated Ringer, attached to a force transducer (AME 801, SensoNor, Horten, Norway) at one end and a servomotor at the other. The temperature was maintained at 37 °C, and the Ringer was continually circulated by a peristaltic pump. Two parallel platinum wire stimulating electrodes were positioned within the chamber on either side of the muscle preparation. At least 30 min elapsed before experimentation began, to allow for stabilization of the preparation after dissection.

Optimisation of muscle length

The muscle length for maximum active force generation was established under isometric conditions. Active (developed) force was determined as the difference between total force and passive force. Single, supramaximal electrical stimuli of 2 ms duration were applied to elicit twitches. Twitch force was displayed on a digital storage oscilloscope (Gould DSO 1604), from which the active, passive and total force could be measured.

Muscle length was measured using a graticule eyepiece under 20× magnification. Beginning at an initial slack length (zero passive force), muscle length was increased in 0.1 mm increments. Twitches were elicited at each length and the resulting active and passive forces measured. This procedure was continued until active force was seen to decline. Active and passive forces were plotted against muscle length to derive a length–force relationship. From this, the muscle length for maximum active force generation (L_{max}) was determined.

Following these preliminary isometric experiments, the work loop technique was used to determine the length for maximum net work production. The physiological length range for cardiac muscle extends from 12.5 % below L_{max} to L_{max} (Brutsaert and Paulus, 1977), so initial starting length, about which muscle length was cycled, was varied in discrete 0.1 mm intervals within this physiological range. From each initial starting length, the muscle was subjected to four sinusoidal length change (strain) cycles and stimulated to contract at a particular phase (phase shift) in the sine wave. Stimulation was timed such that the muscle developed force during shortening and therefore did positive work. The cycle frequency (frequency of the sinusoidal oscillation), strain (extent of length change) and phase shift (start of stimulation) used were those determined to be optimal for maximum power production in preliminary experiments. Work loops were plotted for each cycle and muscles were allowed to recover for 5 min between each experimental run. In each case, net work was recorded for the first cycle, which gave the highest value, and was taken to represent the maximum work performed in that experimental run. Using these maximum values, it was possible to compare the work produced at different starting lengths within the physiological length range. A plot of starting length against work allowed the optimal length (L_{opt}) for work/power production to be derived; that is, the length that yields maximum power output. For the remainder of the investigation, the starting muscle length for all experiments was set at L_{opt}.

Deriving the power–frequency relationship using the work loop technique

Papillary muscle preparations (starting length set at L_{opt})

were subjected to sinusoidal length change cycles (strains), symmetrical about L_{opt}, over a range of physiologically relevant cycle frequencies (1–9 Hz). The magnitude of the length change (strain) is expressed as $\pm\%L_{opt}$, so that a strain of $\pm 5\,\%L_{opt}$ is a sinusoidal strain with a peak-to-peak amplitude of $10\,\%L_{opt}$.

At a given phase shift in the strain cycle an electrical stimulus is applied, causing the muscle to develop force. Stimulation phase shift is expressed in degrees (0 °/360 ° is L_{opt} in lengthening muscle; 90 ° is maximum length; 180 ° is L_{opt} during shortening; 270 ° is minimum length).

For a single cycle, a plot of muscle force against length produces a work loop. A work loop following an anti-clockwise direction indicates that the muscle is doing positive work (see Fig. 3). A clockwise work loop would indicate that work was being done in stretching the muscle (negative work). The area under the shortening limb of the anti-clockwise work loop represents the contractile work done by the muscle (shortening work). The area under the lengthening limb of this loop represents the work done by the apparatus to lengthen the muscle (lengthening work). The net work done by the muscle is calculated as the difference between shortening work and lengthening work and is represented by the area enclosed within the work loop (see Josephson, 1985, 1993).

Strain amplitude and stimulation phase shift were manipulated, for each cycle frequency, to maximise the area of the work loop and therefore the net work produced.

As for the length–work determination, each experimental run consisted of four strain cycles with a 5 min rest between each run. For each cycle frequency, the first cycle was used to evaluate maximum work. The maximum net work (strain and phase shift optimised) was multiplied by the cycle frequency to give power output and used to derive the power–frequency relationship.

Monitoring the decline in muscle performance

Every third experimental run was followed by a control run at the cycle frequency, strain and phase shift previously found to produce maximum power. A plot of the maximum work in each control run against time allowed the decline in performance of the preparation to be monitored. The deterioration of muscle performance with time between control runs was approximately linear and, from the plot of work done in the control runs against time, correction factors were derived for each experimental run (see Stevenson and Josephson, 1990; Altringham and Young, 1991). Multiplication of the appropriate correction factor by the value of net work obtained for each run allowed the prediction of net work were muscle force to have been maintained. Comparison of muscle activation and relaxation times between the first and last control runs of a preparation showed no significant difference. Hence, muscle kinetics remained unaltered and the decline in net work was attributed to a decline in force alone. This was probably due to a progressive decrease in functional fibre volume resulting from a degree of damage. Muscle preparations were abandoned when the work done during the control run declined to less than 80 % of that in the initial control run. In many cases, the decline over the course of an experiment was very small or even absent. There was no indication that the moderate decline in force observed in some preparations influenced the relationships under study. The same results were found for muscles to which correction factors had been applied and those which showed no significant deterioration.

Data collection

At the end of each experiment, the papillary muscle was removed from the bathing chamber, and the aluminium clips, together with any remaining tendon or ventricular wall, were dissected away. The muscle was then weighed (to the nearest 0.01 mg) and power output calculated in $W\,kg^{-1}$. Muscle mass and length were used to calculate mean cross-sectional area, assuming a muscle density of $1.06\,g\,cm^{-3}$. Isometric stress ($kN\,m^{-2}$) was then calculated from the values of force and cross-sectional area.

The experiments were set up and controlled through a microcomputer and the data collected and analysed on line using in-house software. A statistical package (Graphpad Instat version 2.02) was used to compare variables measured at L_{opt} and L_{max} using paired Student's *t*-tests, significance being attributed at a probability of $P<0.05$.

Table 1. *Papillary muscle dimensions and results from isometric and work loop experiments at 37 °C*

Variable	
Muscle wet mass (mg)	0.53±0.07 (9)
Cross-sectional area at L_{opt} (mm^2)	0.17±0.02 (9)
L_{max} (mm)	3.19±0.20 (9)
L_{opt} (mm)	2.97±0.20 (9)
Comparison of lengths at L_{max} and L_{opt}	$P<0.0001$ ($L_{max}>L_{opt}$)
Active force at L_{max} (mN)	8.13±1.02 (8)
Active force at L_{opt} (mN)	6.56±1.11 (8)
Comparison of active forces at L_{max} and L_{opt}	$P<0.0005$ ($L_{max}>L_{opt}$)
Stress at L_{max} ($kN\,m^{-2}$)	44.4±3.37 (7)
Stress at L_{opt} ($kN\,m^{-2}$)	34.3±3.93 (7)
Passive:active force ratio at L_{max}	0.48±0.06 (6)
Activation half-time (ms)	27.4±0.87 (5)
Relaxation half-time (ms)	39.0±2.34 (5)
Time to 90 % relaxation (ms)	163.2±23.05 (5)
Maximum net work ($J\,kg^{-1}$) (at 3 or 4 Hz)	1.91±0.20 (7)
Maximum power output ($W\,kg^{-1}$) (at 6 Hz)	8.62±0.50 (9)

A paired Student's *t*-test was used to compare muscle lengths and forces measured at L_{opt} and L_{max}.

Values are means ± S.E.M. (*N*).

L_{opt}, muscle length for maximum work; L_{max}, muscle length at which isometric active force is maximal.

Results

Optimisation of muscle length for work production

The results of isometric and work loop experiments, together with results from statistical analyses, are summarised in Table 1.

Preliminary isometric experiments established the length at which maximum active force was developed (L_{max}). The initial work loop experiments used a standard set of variables (cycle frequency, strain and stimulus phase shift optimised to produce maximum power) applied to different starting lengths, to establish the length giving maximum work (L_{opt}). The relationship between L_{opt} and L_{max} for a typical muscle preparation is shown in Fig. 1A. Fig. 1B summarises the length–force and length–work relationships for all the preparations used. Since L_{opt} and L_{max} were slightly different for each preparation and muscle length was adjusted in discrete 0.1 mm steps, expressing muscle length as a percentage of L_{max} resulted in an unmatched series of length measurements. Therefore, for graphical presentation, force and work recordings for similar muscle lengths were grouped and averaged (see Syme, 1993). Groupings were of the order of 2 %L_{max} (e.g. ⩾85 % but <87 %L_{max}; ⩾87 % but <89 %L_{max}; ⩾89 % but <91 %L_{max}, etc.). The mean ± S.E.M. was plotted against the middle value of the grouping (e.g. for the grouping including values greater than or equal to 85 % but less than 87 %L_{max}, the mean ± S.E.M. was plotted at 86 %L_{max}).

It was found that L_{opt} was, on average, 7 % shorter than L_{max} (paired Student's *t*-test, $P<0.01$). Force measured at L_{opt} was on average 19 % lower than the force at L_{max} (paired *t*-test, $P<0.01$). L_{opt} fell within the physiological length range (87.5 %L_{max} to L_{max}, after Brutsaert and Paulus, 1977) and corresponded to the muscle length showing the maximum difference between active and passive forces, as measured isometrically. Note that twitch kinetics were all measured isometrically at L_{max}.

Optimisation of strain and phase shift for net work and power production

Fig. 2 shows the optimal stimulation phase shifts and strain amplitudes for work and power output at each cycle frequency. From this figure, it is seen that larger strains of ±8 %L_{opt} were optimal for work and power output at the lowest cycle frequencies. However, at frequencies of 5 Hz and above, the optimum strain was reduced to ±5 %L_{opt}. Optimum phase shift decreased substantially as the cycle frequency was increased, occurring at 130 ° at 1 Hz and decreasing to 10 ° at the higher frequencies

Fig. 3 illustrates the shapes of work loops produced at each cycle frequency. Strain and phase shift are optimised

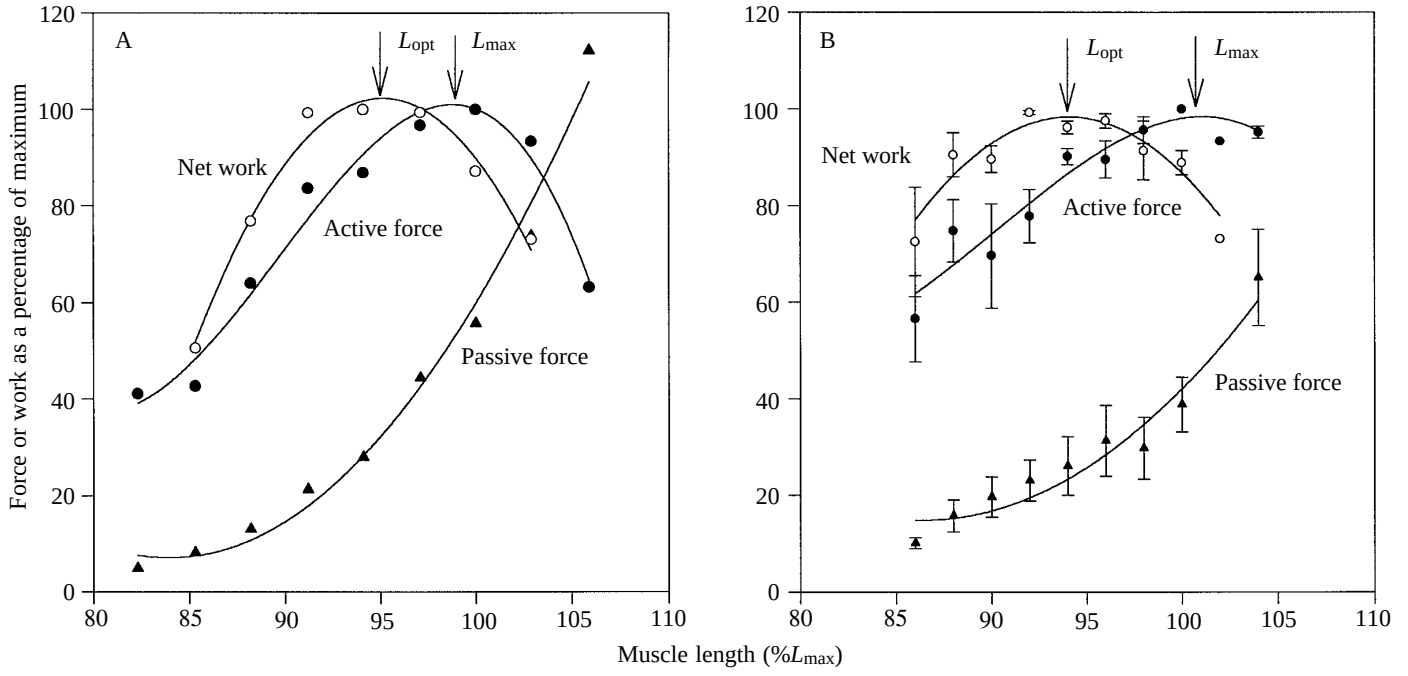

Fig. 1. (A) The relationship between active force, passive force and work for a typical papillary muscle. L_{opt} refers to the muscle length for maximum work and L_{max} to the length at which isometric active force is maximal. Curves were fitted using second- or third-order regressions. The discrepancy between the arrow indicating L_{max} and 100 %L_{max} on the length axis is an artefact of the curve-fitting process. (B) The relationship between active force, passive force and work for all the preparations used. Symbols and error bars represent mean ± S.E.M. Details of how the data were grouped are given in the Results. For clarity, the numbers of replicates are not plotted. The absence of an error bar indicates that there is only one data point. In all other cases, the number of replicates lies between 3 and 8. At 100 %L_{max}, the error bar is very small. Curves were fitted using second- or third-order regressions. Again there is a discrepancy between the arrow indicating L_{max} and 100 %L_{max} on the length axis, which is an artefact of the curve-fitting process and the use of discrete points in analysis.

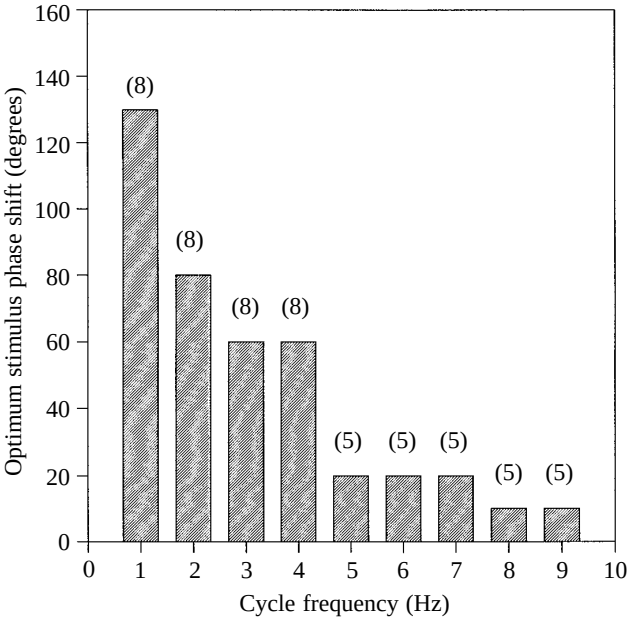

Fig. 2. The relationship between optimum stimulus phase shift and cycle frequency at 37 ˚C. The bars represent modal values for optimum phase shifts. The numbers in parentheses represent the optimum strain (± %L_{opt}) at each cycle frequency, again derived from the mode.

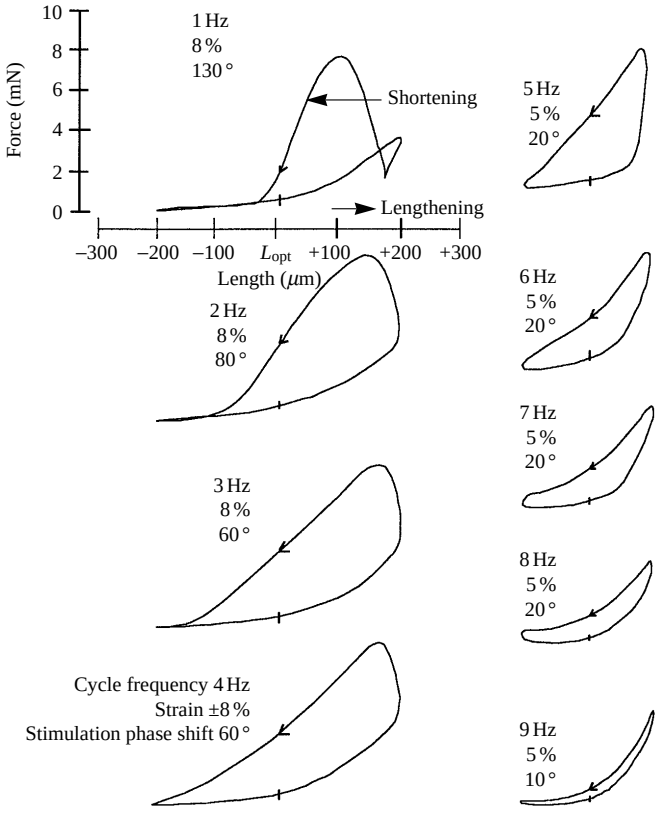

Fig. 3. A typical work loop for each cycle frequency, with optimum strains and phase shifts given alongside the corresponding loops.

in each case and are indicated next to the appropriate loop.

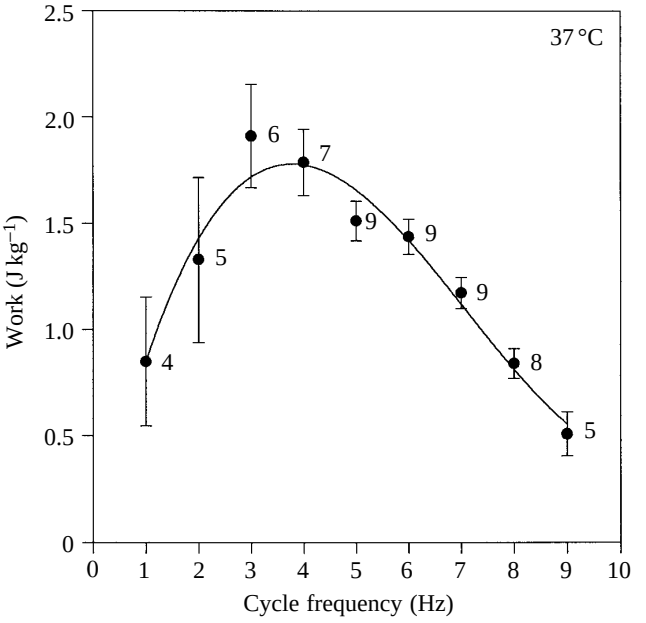

Fig. 4. The relationship between maximum net work and cycle frequency. Numbers of replicates are indicated against symbols; all values are mean ± S.E.M. The curve was fitted using a third-order regression.

Work–frequency and power–frequency relationships

The work–frequency relationship is given in Fig. 4 and shows that maximum work is produced at a cycle frequency of 3–4 Hz.

Fig. 5 shows the relationship between the duration of the shortening period and the mean contraction duration calculated for papillary muscles operating over a range of cycle frequencies. Contraction duration is measured from oscilloscope tracings of the oscillatory work cycles and is the time from the application of the stimulus until force relaxes to its initial baseline value. The point of intersection of the curves (i.e. where contraction duration matches the duration of the shortening period) corresponds to the cycle frequency found to be optimal for net work production (Fig. 4).

The power–frequency relationship in Fig. 6 demonstrates that the optimum cycle frequency for power output is approximately 6 Hz (range 5–7 Hz). Power–frequency curves derived from loops other than the first loop show the same relationship, having the same optima, as that illustrated in Fig. 6, although the absolute values of power are different. Power declined after the first loop but then recovered, such that the same power was produced from loop 20 as from the initial loop and this was maintained for a further 20 loops or more. Experiments are currently in progress to investigate the steady-state power–frequency relationship at 37 ˚C.

Discussion

Isometric experiments

The average maximum isometric twitch stress produced by the papillary muscles was 44.4±3.37 kN m^{-2}, which is

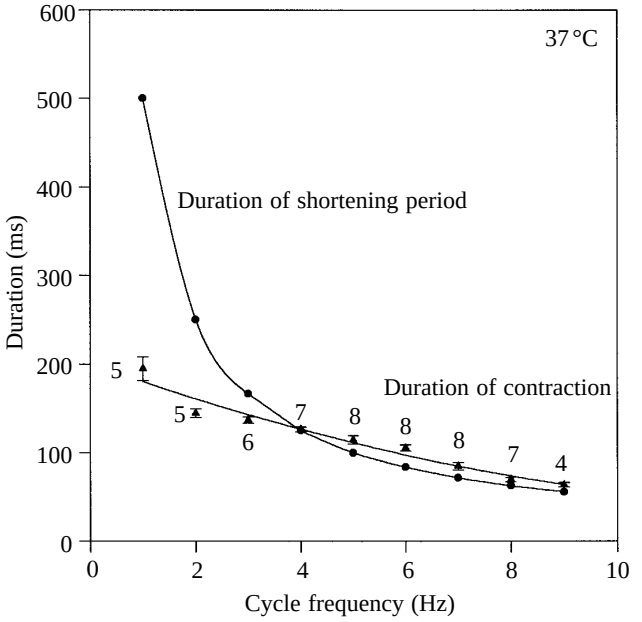

Fig. 5. The relationship between cycle frequency and the duration of the twitch (time from stimulus to complete relaxation) and the duration of the shortening period of the length change cycle. Numbers of replicates are indicated against symbols; all values are mean ± S.E.M. Curves were fitted to the points for contraction duration using a second-order regression and to the duration of the shortening period using a spline function.

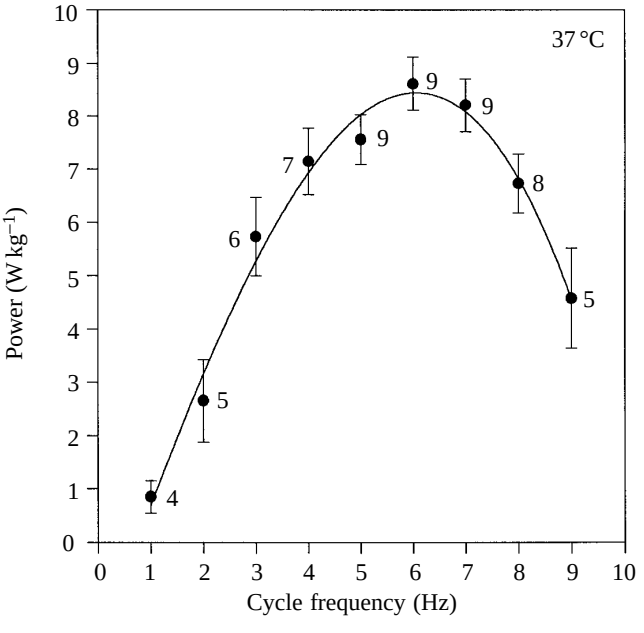

Fig. 6. The power–frequency relationship derived using the oscillatory work loop technique for papillary muscles operating at 37 °C. Numbers of replicates are indicated against symbols; all values are mean ± S.E.M. The curve was fitted using a third-order regression.

comparable to the maximum isometric twitch stress of 51 kN m^{-2} reported for frog ventricular trabeculae operating at 21–23 °C (Syme, 1993) and to the 50 kN m^{-2} reported for cat trabeculae operating at 37.5 °C (Elzinga and Westerhof, 1981).

Work-loop experiments

The effect of muscle length on work production

The influence of muscle length on myocardial performance is well recognised (e.g. Jewell, 1977; ter Keurs *et al.* 1980; Cooper, 1990) and is evident in this study. It was found that L_{opt} was on average 7 % shorter than L_{max}, which agrees with work on frog ventricular trabeculae, where L_{opt} was approximately 10 % shorter than L_{max} (Syme, 1993). At a mean length of L_{opt}, a strain of ±5 %L_{opt} gives an operating range of 88–98 %L_{max}, comparable to the physiological length range of 87.5–100 %L_{max} quoted by Brutsaert and Paulus (1977). Elzinga and Westerhof (1981) used cardiac trabeculae to investigate the linear analogue of the 'pressure–volume' relationship and tried to simulate the *in vivo* situation as closely as possible. They set the working length of the muscle to a length at which peak isometric stress was similar to that in the left ventricular wall during the isovolumic contraction. This length also proved to be below L_{max} (approximately 87 %L_{max}), where the passive force had not yet started to rise rapidly. These results conform to the observation that cardiac muscle always operates on the ascending limb of its length–force curve (e.g. Katz, 1992).

The optimal strain for power output

For rat papillary muscle preparations, the strain amplitude producing maximum power output is 5 %L_{opt} (i.e. 10 % peak-to-peak). Measurements of papillary muscle length changes *in situ* in the equine heart suggested that the difference in length between end diastole and peak systole was approximately 10 % (Semafuko and Bowie, 1975). The strain waveforms for papillary muscles *in situ* were virtually sinusoidal (Semafuko and Bowie, 1975), indicating that our *in vitro* experiments closely simulate *in vivo* conditions.

Mathematical modelling of left ventricular mechanics has recently shown the possibility of homogeneity of muscle fibre stress and strain in the left ventricle during ejection (Arts *et al.* 1991). Evidence to support this hypothesis comes from Delhaas *et al.* (1993*a*,*b*), who measured *in situ* strain from subepicardial fibres of the dog left ventricle and found it to correspond to strain calculated using the model of homogeneous strain. They found that the subepicardial fibres shorten by approximately 10 % during ejection, corresponding to a strain of ±5 %. This agrees with the *in situ* measurements of papillary muscle strain amplitude (Semafuko and Bowie, 1975) and correlates well with the optimum strain amplitude for power output measured in the present study.

An optimum strain amplitude of 5 % is comparable to the strain amplitudes for maximum power output reported for many skeletal muscles studied using the work loop technique (e.g. Stevens, 1988; Stokes and Josephson, 1988; Josephson and Stokes, 1989; Syme and Stevens, 1989; Altringham and Johnston 1990*a*,*b*; Altringham and Young, 1991; James *et al.* 1995).

At the slower cycle frequencies studied here (up to 5 Hz), the optimum strain amplitude was greater than 5 % (see Fig. 2). This agrees with skeletal muscle studies which have demonstrated that larger strains are required to maximise the work done at cycle frequencies below the optimum for maximum power production (e.g. Josephson and Stokes, 1989; Rome and Swank, 1992; James *et al.* 1995).

There is a reciprocal relationship between cycle frequency and optimal strain, since muscles working at high frequencies must operate at relatively small strains to produce high power, as a result of force–velocity constraints. As cycle frequency increases, for a given strain there is an increase in shortening velocity and, consequently, a reduction in the force developed and the work done. A reduction in strain amplitude can compensate for this effect but will, in itself, decrease work. Since work is determined by both force and strain, it is evident that there must be a compromise at a point where the product of force and strain, i.e. work, is maximal, although force and strain may not themselves be at their highest values.

The optimum stimulation phase shift for power output

The optimum phase shift for stimulation is defined as that which maximises net work and, therefore, following multiplication by the appropriate cycle frequency, power output. In the present study, phase shift was manipulated in order to maximise net work and power production at each cycle frequency. At the cycle frequency for maximum power output (6 Hz), which corresponds to the resting heart rate of the animal, stimulation occurs during lengthening (i.e. at 20 °). The muscle begins to develop force while it is being lengthened, but peak force is achieved when the muscle is at its maximum length (Fig. 3). The time course of the twitch is such that force, although declining, is produced during most of the shortening period. Recordings of papillary muscle force and length measurements *in situ* show that the muscle is activated while the increase in ventricular pressure causes it to lengthen (Semafuko and Bowie, 1975). The muscle produces maximum force as it reaches maximum length (Armour and Randall, 1970), after which the muscle actively shortens and force declines. It is apparent that the patterns of length and force changes observed for the papillary muscle *in situ* are well matched by the regime of sinusoidal oscillation and stimulation used in this study.

Semafuko and Bowie (1975) suggested that the externally applied stretch as the muscle was activated resulted in a marked increase in force and designated this the ‘lengthening contraction force’. This observation implies that force enhancement by active pre-stretch was occurring in the papillary muscles *in situ*, as has been observed experimentally in both skeletal (Edman *et al.* 1978) and cardiac (Pollack and Kreuger, 1976) muscle. Active pre-stretch has been shown to be important in maximising power output from skeletal muscle (e.g. Altringham and Johnston, 1990*a*,*b*).

The work–frequency relationship

Maximum net work production occurs at 3–4 Hz (Fig. 4). Fig. 5 shows that this frequency corresponds to the frequency at which the duration of contraction (time from the stimulus to complete relaxation) matches the duration of the shortening period. This is similar to the observation in frog cardiac trabeculae (Syme, 1993) that net work was maximal when twitch duration at half-maximum amplitude (measured isometrically as the time from 50 % contraction to 50 % relaxation) equalled half the cycle duration.

Net work is calculated from the area of the work loop (Fig. 3) and represents the difference between the work done by the actively shortening muscle and the work required to lengthen it. For maximum net work, the force/time integral, during the shortening phase of each cycle, must be maximised (Altringham and Johnston, 1990*a*) and lengthening work minimised. It is not possible to increase the duration of a cardiac muscle contraction by increasing the number of stimuli, although this is a possibility for skeletal muscle. Cardiac muscle is maximally activated by a single stimulus under normal conditions, yielding a single twitch. The duration of this contraction will determine how long the force is maintained during the shortening period of the cycle, which in turn will influence the amount of work produced at a particular cycle frequency. At cycle frequencies below that for maximum work generation, active force is generated over a smaller fraction of the shortening period of the length change cycle (see Fig. 3) and net work declines (Syme, 1993). The duration of a single twitch has less importance in work loop studies for skeletal muscle, since the number of stimuli can be manipulated to maximise the force/time integral (Altringham and Johnston, 1990*a*).

Contraction duration declines as the cycle frequency increases (Fig. 5). In cardiac muscle, twitch duration is known to decrease as the interstimulus interval is reduced. This has been observed even in the isometric situation (Schouten, 1990). In addition, in the present study, it is likely that twitch duration will also be affected by the deactivating effect of shortening (e.g. Edman and Nilsson, 1971) as the muscle shortens during cycles of oscillatory work.

In spite of the decrease in twitch duration with increasing cycle frequency, at cycle frequencies above the optimum for work production, twitch duration is always longer than the duration of the shortening period. As a result, the muscle is still active during extension (re-lengthening) and therefore the work required to re-extend the muscle is increased. Since net work is the difference between the work done during shortening and the work required for extension (Josephson, 1985; Syme, 1993), the increase in lengthening work at the higher cycle frequencies reduces the net work done by the muscle (Fig. 3).

Note that, at the higher cycle frequencies studied, the contraction duration and the duration of the shortening period are very similar (the curves approaching a second intersection at 9 Hz in Fig. 5) but, nevertheless, net work declined with increasing cycle frequency. This can be attributed to a number of velocity-dependent factors since, for a given strain amplitude, the shortening velocity must be greater at high frequencies than at low frequencies. The force–velocity

relationship (Hill, 1938) dictates that the force achieved by a contracting muscle is reduced with increasing shortening velocity, resulting in a reduction in the work done. Enhanced shortening deactivation at higher cycle frequencies (Colomo *et al.* 1986; Altringham and Johnston, 1990*b*) would also reduce the work done by the muscle. Furthermore, increased viscous resistance at the higher cycle frequencies would increase the work required to re-lengthen the muscle (Josephson, 1985; Syme, 1990), thus reducing the net work done.

The power–frequency relationship

The optimum cycle frequency for power output was found to be approximately 6 Hz (Fig. 6). The speed at which papillary muscles operate *in vivo* (to develop force and undergo length changes), and therefore the rate at which mechanical work is done (power output), is determined by the heart rate of the animal. Using an allometric equation relating heart rate to body mass (Stahl, 1967), the resting heart rate of the rats used in this study (223 g) was calculated to be approximately 5.8 Hz. This corresponds to the frequency of 6 Hz (range 5–7 Hz) at which maximum power output was produced.

Physiological significance

The cycle frequency for maximum power output (6 Hz) corresponds to the estimated resting heart rate of the rats used. At this cycle frequency, the optimum strain amplitude and stimulation phase shift used resulted in a regime of contraction that closely simulated the physiological situation. Our results suggest, therefore, that papillary muscles have evolved to produce maximum power at the normal (resting) heart-beat frequency, as Hill (1950) suggested. It is interesting that Steiger (1971), working on stretch activation of isolated myocardial fibres, predicted that the frequencies for optimal oscillation in rabbit and guinea pig myocardial fibres at 37 °C correlated with their heart-beat frequencies. This suggests that the contractile machinery itself has evolved to operate at a particular frequency.

Under resting conditions (as simulated here), the heart is working at submaximal contractile potential (Katz, 1992). As a consequence, cardiac muscle possesses considerable contractile reserve and is able to enhance its performance to meet the increased circulatory demands of the body as they arise. In such circumstances, both inotropic and chronotropic processes are activated, increasing the force developed and the heart rate respectively (Katz, 1992). In the presence of inotropic and chronotropic agents, we would expect an increase in muscle power output together with an increase in the cycle frequency at which maximum power output was achieved. We are currently investigating the power–frequency relationship in the presence of such stimulation.

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