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INFLUENCE OF MUSCLE LENGTH ON WORK FROM TRABECULAR MUSCLE OF FROG ATRIUM AND VENTRICLE

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Summary

The work capacity of segments of atrial and ventricular muscle from the frog *Rana pipiens* was measured as a function of muscle length using the work loop technique. Both the work done during shortening and the work required to re-lengthen the muscle after shortening increased with muscle length. Net work increased with length up to a maximum, beyond which work declined. The optimum sarcomere length for work output was 2.5–2.6 μm for both atrial and ventricular muscle.

Isometric force increased with muscle length to lengths well beyond the optimum for work output. Thus, the decline in work at long lengths is not simply a consequence of a reduction in the capacity of heart muscle to generate force. It is proposed that it is the non-linear increase in work required to re-lengthen muscle with increasing muscle length which limits net work output and leads to a maximum in the relationship between net work and muscle length.

Extension of the results from muscle strips to intact hearts suggests that the work required to fill the ventricle exceeds that available from atrial muscle at all but rather short ventricular muscle lengths.

Key words: heart, muscle, work, length, volume, frog, *Rana pipiens*.

Introduction

Greater filling of the chamber of a heart generally results in an increase in the work done ejecting blood during the following systole. The positive relationship between chamber volume and stroke work is known as the Frank–Starling law of the heart. The increase in work with increasing chamber volume is attributed to (1) an increase in the length of heart muscle fibers such that they operate on more favorable portions of their length–tension curve (e.g. Jewell, 1977; ter Keurs *et al.* 1980) and (2) greater muscle activation during systole (e.g. Jewell, 1977; Stephenson and Wendt, 1984; Babu *et al.* 1988; Reiser and Lindley, 1990).

In vertebrate hearts, there is a complex relationship between chamber volume and the length of the constituent muscle fibers of the chamber wall. The relative change in fiber length with change in chamber volume depends on the orientation of the fibers and on their position across the width of the chamber wall. It is unlikely that all the fibers in the chamber wall experience similar strains and forces. It is difficult, therefore, to quantify the consequences of changing muscle length on the capacity of the muscle to do work using measurements made from intact hearts. The following study investigated the relationship between muscle length and work capacity using small bundles of muscle with relatively parallel fibers. The bundles were trabeculae obtained from frog hearts. Work output was measured using the work loop technique, in which a muscle is subjected to cyclic length change (strain) and stimulated phasically in the length cycle. The work output per cycle was obtained as the product of muscle force and the change in length integrated over a full cycle (Josephson, 1985). With the muscle bundles used, it was possible to control and measure the muscle's length precisely and to measure directly both the work done by the muscle during shortening and the work required to re-lengthen the muscle after shortening. Measurements made from both atrial and ventricular muscle allow comparison of the work required to lengthen ventricular muscle with that available from atrial muscle during shortening.

Materials and methods

Muscle preparation and techniques

Muscle from hearts of the frog *Rana pipiens* (L.) were used. Animals were maintained and killed according to institutional guidelines for animal care and use. Details on the preparation and use of trabecular bundles have been described previously (Syme, 1993). Briefly, a bundle of ventricular or atrial trabeculae with as few side branches as possible was isolated from the heart of a frog and attached at one end to a force

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transducer and at the other end to the arm of a servomotor. Attachments were made *via* stainless-steel pins (76 μm diameter) to which the muscle was tied using a single strand of silk from a section of 6-0 gauge braided suture. The muscle was then mounted in a chamber filled with circulating, aerated, physiological saline, maintained at ambient temperature (22 °C). Bundles of ventricular muscle averaged 3.3 mm in length and 0.22 mg wet mass; atrial muscle averaged 4.1 mm and 0.31 mg wet mass. The length of the bundle was cycled in a sinusoidal fashion by the servomotor, and the muscle was activated with an electric shock (1 ms duration) during the length cycle. The timing of the stimulus in the length cycle was adjusted so that the muscle did maximum net work. Work was calculated by computer integration of the force and length values. Lengthening work (the work done on the muscle to stretch it) and shortening work (the work done by the muscle while shortening) were evaluated separately. Lengthening work is comparable to the work required to fill the chamber during diastole; shortening work is analogous to the gross stroke work done by a heart's chamber during systole. Net work is the difference between shortening and lengthening work over a complete cycle. Note that all strains (muscle length changes) are reported as peak-to-peak values as a percentage of the muscle's resting length.

In order to maintain stable work or force output, it was necessary to pace (stimulate) the muscles continually throughout the experiment. The stimulation/cycle frequency was maintained at 0.5 Hz for ventricular preparations and 0.9 Hz for atrial preparations. These frequencies are those that yield maximum work per cycle from frog heart muscle at 22 °C (Syme, 1993).

Work was measured over a series of muscle lengths, varied in steps of approximately 0.15 mm (4–5 % resting length). Work recordings were made both during strain cycles in which the muscle was stimulated and during cycles when the muscle was not stimulated. The imposed cyclic strain was interrupted intermittently during cycles with stimulation to measure isometric force. Length was decreased until work was near zero, and increased until net work reached or closely approached a plateau. The muscle was prone to damage at long lengths (see below) so, rather than risk overstretching the muscle, the length at which there was only a small (less than 5 %) increase in work when muscle length was increased further was designated as the optimum length, L_{opt}. Near the end of the experiment, work measurements were made when the muscle was stretched beyond L_{opt}. In all cases, the work output at L_{opt} was found to be at least 95 % of the maximum obtainable. The amplitude of the sinusoidal length change during work measurements was set at 0.05, 0.1 or 0.3 mm, the order being varied between experiments; since the length of the fiber bundles was about 3.3 mm, these length changes correspond to peak-to-peak strains of approximately 1.5, 3 and 9 % respectively.

Frequent checks on the preparation's stability were made by recording the work done at a fixed reference length. The reference length was usually the first length tested during an experiment and was always shorter than L_{opt}. As long as the muscle was not stretched beyond L_{opt}, the work output at the reference length remained essentially constant over hours of experimentation. However, when length was increased much beyond L_{opt} there were often changes in the preparation, as shown by a decline in work output at the reference length. Measurements on a preparation were terminated when a marked reduction in performance at the reference length was noted (work tended to deteriorate precipitously when the muscle was over-stretched, often by up to 45 %). Because of the frequent decline in muscle performance after being stretched to lengths greater than L_{opt}, measurements in this length range were limited. The decreased work capacity of stretched muscle may be due to slippage of the tied ends or tearing of the muscle. It was not due to fatigue, as the work done at any given length, even the longest, was stable for long periods.

In a second set of experiments, the muscle length was set at L_{opt} as determined above, at 0.15 mm longer (approximately 105 %L_{opt}) or at 0.3 mm shorter (approximately 91 %L_{opt}), and net work was measured over a range of strain amplitudes. The strain was varied between 0.1 mm and 1–1.5 mm, a range within which net work reached a maximum. Work output at a reference strain (0.3 mm) was checked several times during the experiment to monitor the preparation's stability. Work at the reference strain at the end of the experiments averaged 98.5±7.3 % (S.D.; N=7) of that at the beginning.

Upon completion of the experiment, the muscle mass was determined as described previously (Syme, 1993). Isometric force was standardized to muscle cross-sectional area and work to muscle wet mass. Area was recalculated at each new muscle length.

Sarcomere length

Six ventricular and six atrial preparations were prepared for measurement of sarcomere lengths. L_{opt} at each of the three strains studied (1.5, 3 and 9 %) and the optimal length for isometric twitch force had been determined for these preparations. The muscles were held and fixed at the length that was optimal for work at 9 % strain. The initial fixation was in cold 3.5 % glutaraldehyde and 3 % sucrose in 0.1 mol l^{-1} phosphate buffer, pH adjusted to 7.2 (at 5 °C). The muscles were post-fixed in 2 % osmium tetroxide in 0.1 mol l^{-1} phosphate buffer and embedded in Epon. Five longitudinal sections, 1–2 μm thick, were cut at different levels across the width of each muscle. The sections were stained with Toluidene Blue. Sarcomere lengths were measured using a light microscope. Measurements were made from 5–18 adjacent sarcomeres along the lengths of 3–29 different myofibrils from each muscle. All sections with clearly visible sarcomeres were used.

Results

Optimal lengths for work and force

Net work increased to a maximum with increasing muscle length, while isometric force continued to increase with

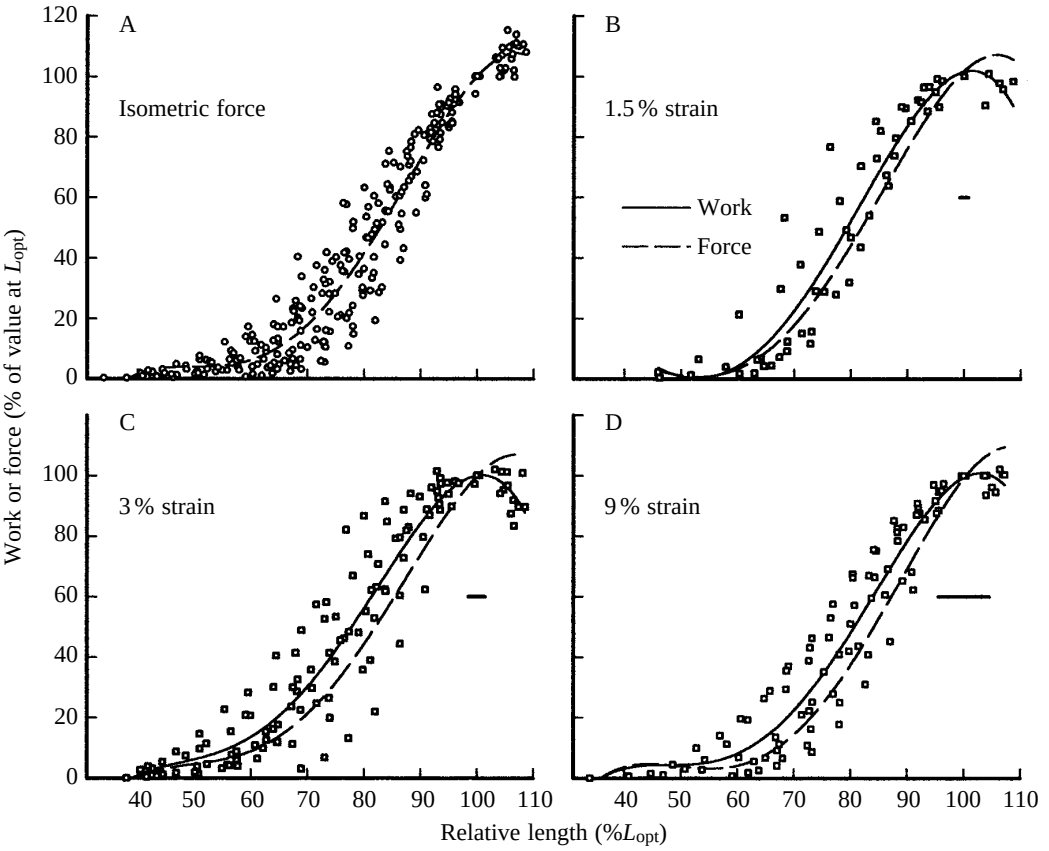

Fig. 1. Net work and isometric force from frog ventricular muscle at different muscle lengths and strains. Values are standardized to the value at L_{opt} (the muscle length giving 95–100 % maximum net work). L_{opt} was determined separately at each strain. Strain is expressed as a percentage of L_{opt}. L_{opt} (measured from the raw data, not the curve fits) was not significantly different (P=0.099) at different strains, so the isometric force data from all studies (B, C and D) were pooled in A. (B,C,D) Net work (solid line) at the indicated strain and isometric force (broken line, data points are not shown for clarity). The data sets used for the isometric force curves in B, C and D have not been pooled; the isometric force data for each curve were collected during the work experiments at the indicated strain. The horizontal bars on B, C and D show the strain amplitude; the bars are placed so as to show the muscle lengths encountered during a cycle about L_{opt}. The cycle frequency was 0.5 Hz. Curves were fitted by fourth-order polynomial regressions.

increasing muscle length beyond the length at which work was maximal (Figs 1, 2). For example, the length at which isometric force was maximal was 113.6±8.0 % (S.D.; N=7) of the optimal length for work with 9 % strain in ventricular muscle, and 111±6.8 % (S.D.; N=7) of the optimal length for work with 7.4 % strain in atrial muscle [both differences are significantly different from 100 %, one-way analysis of variance (ANOVA) using paired comparisons then least squares difference (LSD) comparisons, P<0.0001]. The sarcomere lengths in ventricular muscle at the optimal muscle length for net work (9 % strain) were 2.53±0.20 μm (S.D.; N=6), and those in atrial muscle at the optimal length for net work (7.4 % strain) were 2.63±0.16 μm (S.D.; N=6). No attempt was made to correct for shrinkage during fixation or errors in sectioning angle. However, these potential errors are small and, in both cases, will lead to an underestimate of the actual sarcomere length. Thus, the measured sarcomere lengths may be underestimates of the actual lengths by a few per cent.

Peak isometric forces, measured at the optimal muscle length for force production, were 62±17.1 kN m^{-2} (S.D.; N=8) for ventricular muscle at 0.5 Hz twitch frequency and 16±4.3 kN m^{-2} (S.D.; N=7) for atrial muscle at 0.9 Hz. Accounting for stimulus frequency, these are similar to other values reported for frog heart muscle (Niedergerke, 1956; Reiser and Lindley, 1990). It is not known why the force-generating capacity of frog atrial muscle is less than that of ventricular muscle (for discussion see Syme, 1993, and Grant *et al.* 1964).

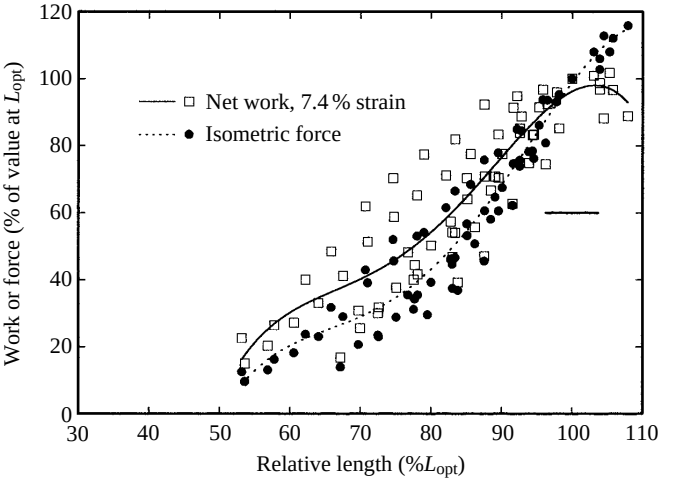

Fig. 2. Net work and isometric force from atrial muscle at different muscle lengths. Work and force are standardized to the value at L_{opt}. The strain was approximately 7.4 %L_{opt}. The horizontal bar shows the amplitude of a 7.4 % strain cycle; the bar is placed so as to show the muscle lengths encountered during a cycle about L_{opt}. The cycle frequency was 0.9 Hz.

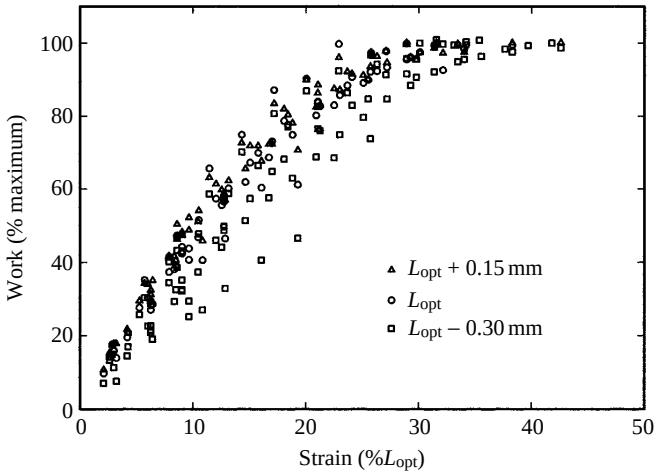

Fig. 3. Net work done by ventricular muscle as a function of strain amplitude at three different muscle lengths. The fiber bundle lengths were approximately 3.3 mm at L_{opt}, so +0.15 mm and −0.3 mm represent muscle lengths of approximately 105 % and 91 % of L_{opt}. Work is standardized to the largest value at each muscle length. Strain is expressed as a percentage of L_{opt}.

Optimal length and strain

L_{opt} for work output from ventricular muscle was not obviously dependent on strain. Using L_{opt} with 9 % strain as a reference length (i.e. L_{opt} with 9 % strain=1.00), L_{opt} with 1.5 % strain was 1.04±0.065 (S.D.; N=7) and L_{opt} with 3 % strain was 1.01±0.060 (S.D.; N=7) (paired comparisons, differences not significant, P=0.099, one-way ANOVA, LSD comparison). Correspondingly, over the range studied, net work was maximum at the same strain (32–34 %L_{opt}) regardless of whether the muscle was at an intermediate, short or long length (Fig. 3).

Work and muscle length

The patterns of increase in shortening, lengthening and net work with increasing muscle length were similar at the three strains (1.5 %, 3 % and 9 %) in which this was examined in detail (Fig. 4, only results at 3 % and 9 % strain are shown for clarity; results at 1.5 % strain were qualitatively the same). Both shortening and lengthening work in the ventricle increased with increasing muscle length (Fig. 4). At short muscle lengths, shortening work increased more rapidly with increasing muscle length than did lengthening work, but at long lengths shortening work and lengthening work increased in parallel, giving a plateau in net work as a function of muscle length (Fig. 4). Below about 80 %L_{opt} lengthening work was near zero, so net work equalled shortening work. Similar trends were observed for atrial muscle (Fig. 5).

The mass-specific shortening, lengthening and net work of atrial muscle was about 20 % of that from the ventricle (compare Fig. 4B with Fig. 5), reflecting lower force-generating capacity and lower resting stiffness in atrial muscle.

The increase in lengthening work with increasing muscle length is seen clearly in work loops (plots of muscle force *versus* length, Fig. 6). The large, upper loop of each pair is from muscle that was stimulated near the onset of shortening (maximizing net work). In these loops, for which force is higher during shortening than during lengthening, the loop is traversed counterclockwise and the muscle does net positive work. The narrow, lower loop of each pair is from the same muscle, subjected to the same strain and at the same length and cycle frequency, but without stimulation. Force during lengthening is greater than that during shortening. The loops are narrow and clockwise, and the muscle does net negative work (i.e. work is done on the muscle).

From the loops in Fig. 6 it can also be seen that the work required to lengthen a muscle that was stimulated while shortening was greater than the work required to lengthen a muscle that was not stimulated at all during the length cycle; the lower arms (lengthening force) of the upper, counterclockwise, stimulated loops are higher on the force

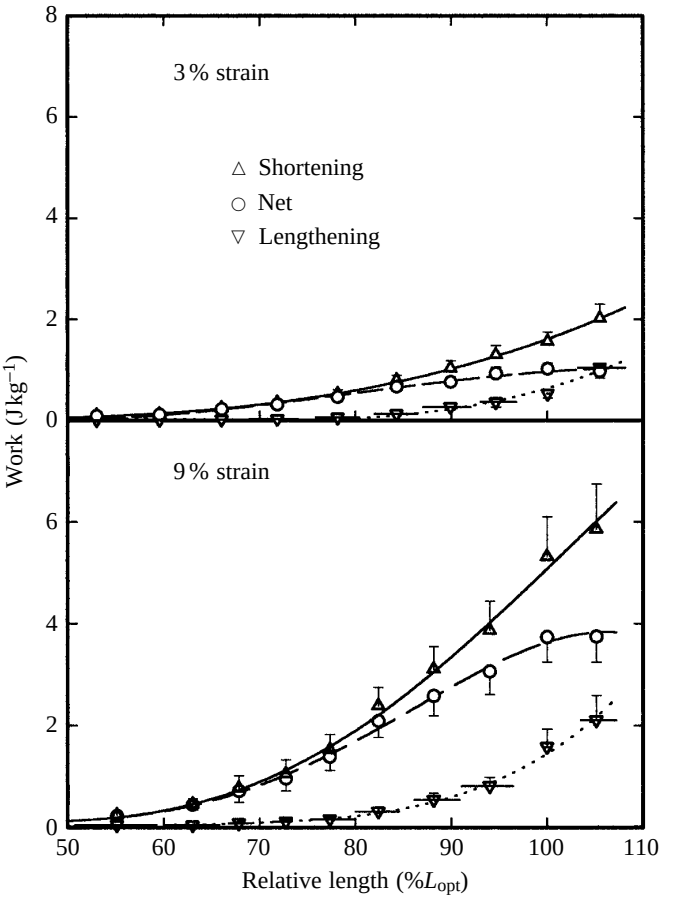

Fig. 4. Shortening work, lengthening work and net work from ventricular muscle at different muscle lengths (mean ± S.E.M., N=9). L_{opt} was determined separately for each strain amplitude. Vertical error bars are shown in only one direction for clarity. Data have been grouped into 3–5 % length bins based on clustering patterns; horizontal bars shown on the lengthening work curves show the range of lengths used in each length bin and are the same for the shortening and net work curves. Cycle frequency was 0.5 Hz.

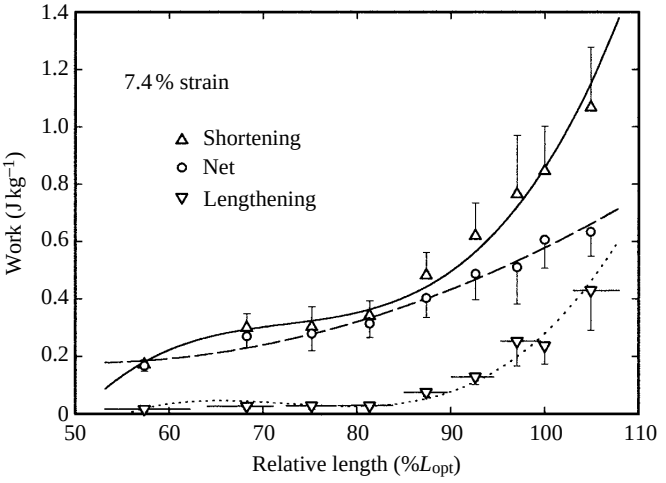

Fig. 5. Shortening work, lengthening work and net work from atrial muscle at different muscle lengths (mean ± S.E.M., N=8). Cycle frequency was 0.9 Hz. See Fig. 4 for further details.

scale than are the upper arms (lengthening force) of the clockwise, unstimulated loops. The extra work required to lengthen muscle that was cyclically stimulated (relative to that of an unstimulated muscle) was length-dependent, ranging from zero at short muscle lengths (no difference between stimulated and unstimulated muscle) to 0.3–0.4 J kg^{-1} at L_{opt} in ventricular muscle and 0.05–0.1 J kg^{-1} at L_{opt} in atrial muscle. This extra lengthening work corresponds to 5–24 % of the shortening work done by ventricular muscle and 6–12 % of the shortening work done by atrial muscle.

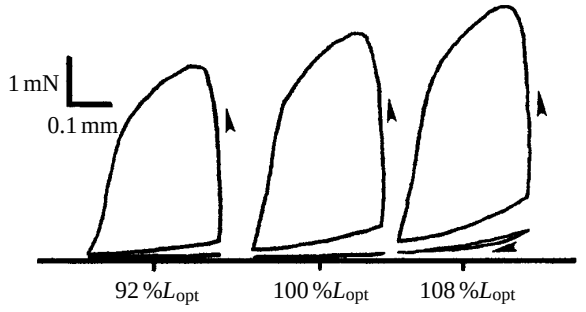

Fig. 6. Work loops, formed by plotting muscle force against length for a full length cycle, from a frog ventricular muscle bundle. The cycle frequency was 0.5 Hz. Strain was 0.3 mm. Loops are shown at each of three different muscle lengths: L_{opt} (the length, measured at mid-cycle, at which net work was 95–100 % maximal, =4.4 mm), 108 %L_{opt} and 92 %L_{opt}. A pair of loops is shown for each muscle length. The upper, larger loop of each pair is from a cycle during which the muscle was stimulated near the beginning of the shortening portion of the length cycle. The loop is counterclockwise; the muscle shortened while it developed active force and did net positive work. The narrow loop immediately below is from a cycle at the same length, strain and cycle frequency, but without stimulation. The loop is clockwise; more force was required to lengthen the muscle than was produced during shortening, and the muscle did net negative work.

Discussion

Relationship between chamber geometry, fiber length and work

In the intact heart, an increase in end-diastolic volume, over much of its possible range, leads to an increase in stroke work (the Frank–Starling law). The increased stroke work is the result of an increase in stroke volume and/or an increase in ejection pressure. In isolated fibers, an increase in fiber length led to an increase in shortening work (Figs 4, 5). The strain (stroke volume) was held constant in these measurements, so the increase in work must have been due to an increase in force.

A geometrical transformation allows a more exact comparison between changes in fiber length in isolated muscle strips and equivalent changes in heart volume and work. If it is assumed that the frog ventricle has a spherical morphology, which is reasonable for these hearts, the length of fibers changes in proportion to the cube root of the chamber volume V (i.e. $V=(4/3)\pi R^3$, where R is ventricular radius). Using data from Fig. 4, but substituting L^3 (i.e. volume) for muscle length (L or R), yields work as a function of relative cavity volume (Fig. 7). At a constant strain (constant stroke volume), shortening work (stroke work) was a linear function of L^3 (cavity volume) over most of the range of lengths studied (Fig. 7). Over the linear range (r=0.95), the slope of the relationship between percentage maximal work and percentage relative volume was 1.23, meaning that an increase of 10 % in relative volume resulted in an increase in work output of 12.3 % of the maximal value.

There are two components to shortening work: (1) work generated by cycling cross-bridges and (2) work due to elastic

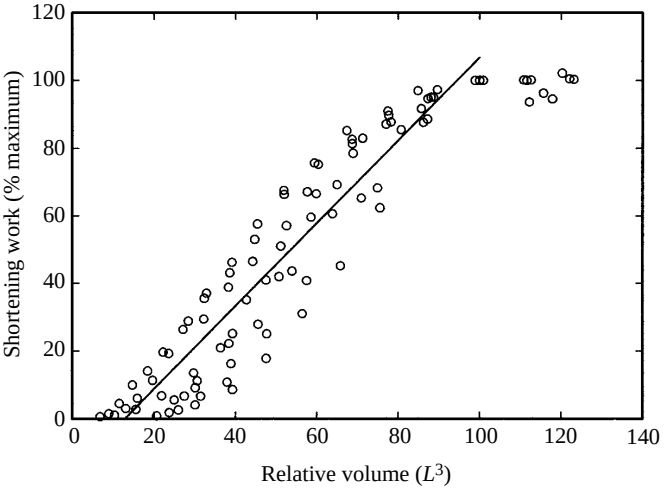

Fig. 7. Shortening work done by ventricular muscle from Fig. 4 plotted against muscle length L raised to the third power. L^3 is proportional to cavity volume in an intact heart (see text). Thus, the plot is analogous to a plot of stroke work against average cavity volume for an intact heart. Work is standardized to the value at L_{opt}, where 100 %L_{opt}=100 % relative volume, 80 %L_{opt}=51 % relative volume (80^3), etc. The strain (stroke volume) was held constant at 9 %L_{opt}. The cycle frequency was 0.5 Hz. Regression slope=1.23, r=0.95, P<0.05.

recoil, for which the energy input was work done on the muscle during lengthening. At lengths up to 80 %L_{opt} (51 % relative volume, Fig. 7), lengthening work was very small (Fig. 4) and most of the increase in work with increasing volume can be attributed to increased cross-bridge work alone. At lengths above 80 %L_{opt} (51 % relative volume, Fig. 7), a significant amount of work was absorbed by the muscle during lengthening (Fig. 4). If the load against which this work is done is elastic, much of this work could be released subsequently during shortening. Therefore, both cross-bridge work and work from elastic recoil may contribute to the linear increase in work with increasing chamber volume.

What limits the increase in work with increasing length?

At all strains studied, isometric force continued to increase with increasing muscle length well beyond the length found to be optimal for work output (Figs 1, 2). Josephson and Stokes (1989) also found that work output in a crab muscle reaches a maximum before the muscle length reaches the peak of the isometric length–tension curve. For the strain amplitudes used here with frog heart muscle, net work reached a maximum and began to decline even though the muscle did not cross or even reach the peak of the isometric length–tension curve at any time during the length cycle (see strain bars on Figs 1 and 2). The sarcomere lengths at the optimal muscle length for work were 2.5 μm with 9 % strain in ventricular muscle and 2.6 μm with 7.4 % strain in atrial muscle. In frog myocardium, the thick filaments are approximately 1.5 μm long and the thin filaments 2.05 μm long (Winegrad, 1974). Thus, at the optimal lengths for both work and isometric force, the sarcomere lengths are longer than those expected to yield maximum myofilament overlap and maximal force. This suggests that perhaps (1) an increased level of activation associated with increased muscle length can more than compensate for the decline in force associated with decreased myofilament overlap in both working muscle and muscle contracting isometrically and (2) isometric force and work have different dependencies on the elevated level of activation. Shortening deactivation in working muscle may defeat some of the potentiation gained from working at long muscle lengths, leading to a shorter optimal length for work than for isometric force. Phillips and Woledge (1992) suggest that sarcomere length inhomogeneity during isometric contractions (creep) may lead to the average measured sarcomere length being longer than that expected to yield maximum force, while during shortening contractions the associated high velocities of shortening prevent significant sarcomere length inhomogeneity from occurring, so power reflects more closely the degree of myofilament overlap.

A second observation appears to contribute to the disparity between optimal lengths for work and isometric force. More work was required to stretch a muscle that was being cyclically stimulated than was required to stretch a muscle that was not being stimulated (Fig. 6 and Results). This observation indicates that the phasically stimulated muscle did not relax fully before being re-lengthened, and so there was residual cross-bridge activity during lengthening. Some part of the negative work done to re-lengthen a shortened muscle may be stored elastically and recovered during subsequent shortening. How much, if any, of this negative work is recovered is unknown. The lost energy would not contribute to subsequent shortening work, and net work would thus be reduced.

The extra work required to lengthen stimulated muscle was greater at longer muscle lengths (see Results). Josephson and Stokes (1989), in an analysis of work loops from a crab muscle, also noted a substantial increase in the minimum force during the length cycle as average muscle length was increased. This progressively incomplete relaxation with increasing muscle length would result in progressively greater negative work required to re-lengthen the muscle and progressively greater reductions in net work. Thus, net work would reach a maximum at a shorter muscle length than the optimum length for cross-bridge work (and isometric force) during shortening.

Atrial contribution to ventricular work

The effects of tension or volume on the performance of atrial and ventricular muscle provide insight into the conditions necessary for ventricular filling during diastole. Brockman (1963) and Linden and Mitchell (1960) have shown that increasing the end-systolic ventricular volume results in a decrease in the part of ventricular fiber extension that is brought about by atrial contraction and a decline in the ability of the atrium to augment ventricular stroke work. At L_{opt}, the mass-specific work done by frog atrial muscle was less than the mass-specific work required to lengthen ventricular muscle (Figs 4, 5), and there is more ventricular muscle than atrial muscle in the frog heart (Brady, 1964). Thus, the atria cannot do enough work to account for ventricular filling at the ventricular volume corresponding to a fiber length of L_{opt}. By way of demonstration, the ventricle constitutes about 70 % of the frog heart by mass and the atria about 15 % (Brady, 1964). Using the data from Figs 4 and 5 (9 % and 7.4 % strain for ventricle and atrium respectively), at L_{opt} the work required to lengthen ventricular muscle is 1.0 J kg^{-1} ventricle, and with 0.7 kg ventricle kg^{-1} heart, this gives 0.7 J kg^{-1} heart. The shortening work of which the atria are capable is 0.6 J kg^{-1} atrium which, with 0.15 kg atrium kg^{-1} heart, gives 0.09 J kg^{-1} heart. If both muscles were operating at L_{opt}, the atria would clearly not do enough work to lengthen (fill) the ventricle. The above calculation is based on relatively small strains; nonetheless, the same conclusion is reached using work values at 30 % strain in both muscles (data from Syme, 1993). However, the work required to lengthen the ventricle declined to near zero at about 80 %L_{opt}, while the shortening work done by the atrium was finite even at 60 %L_{opt} (Figs 4, 5). Thus, if the ventricle operated at a relatively short muscle length, atrial shortening work could easily exceed ventricular lengthening work. Ventricular volume is thus critical in determining the ability of the atria to contribute to ventricular diastolic filling.

Grant *et al.* (1964), in an analysis of pressure–volume work from human hearts, demonstrated that passive elastic recoil in the atrium accounted for about 97 % of the work done during atrial systole (i.e. active atrial contraction contributed only an

additional 3 % to gross atrial stroke work in normal hearts). In frog atrial muscle at L_{opt}, the contribution of active atrial contraction to shortening (stroke) work is nearer 75 % (Fig. 5, the ratio of net work to shortening work) and it approaches 90–100 % at lengths below 75 %L_{opt}. Atrial contraction in the frog appears to contribute significantly to gross atrial stroke work. However, the shortening (stroke) work done by frog atrium, which is potentially transferred to the ventricle, is small compared with the shortening (stroke) work done by the ventricle; only 2–4 % (at L_{opt}) using data from Figs 4 and 5 and assumed relative atrial and ventricular masses of 15 and 70 % of the heart. The results from frog heart support the conclusion of Grant *et al.* (1964) that the atrium's primary function is not to stretch a resisting ventricle and, in this way, to contribute substantial amounts of potential energy to the ventricle, but rather to transfer volumes of blood to a compliant ventricle.

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References

BABU, I., SONNENBLICK, E. AND GULATI, J. (1988). Molecular basis for the influence of muscle length on myocardial performance. *Science* **240**, 74–76.

BRADY, A. J. (1964). *Physiology of the Amphibia* (ed. J. A. Moore). New York: Academic Press.

BROCKMAN, S. K. (1963). Dynamic function of atrial contraction in regulation of cardiac performance. *Am. J. Physiol.* **204**, 597–603.

GRANT, C., BUNNELL, I. L. AND GREENE, D. G. (1964). The reservoir function of the left atrium during ventricular systole. *Am. J. Med.* **37**, 36–43.

JEWELL, B. R. (1977). A reexamination of the influence of muscle length on myocardial performance. *Circulation Res.* **40**, 221–230.

JOSEPHSON, R. K. (1985). Mechanical power output from striated muscle during cyclic contraction. *J. exp. Biol.* **114**, 493–512.

JOSEPHSON, R. K. AND STOKES, D. R. (1989). Strain, muscle length and work output in a crab muscle. *J. exp. Biol.* **145**, 45–61.

LINDEN, R. J. AND MITCHELL, J. H. (1960). Relation between left ventricular diastolic pressure and myocardial segment length and observations on the contribution of atrial systole. *Circulation Res.* **8**, 1092–1099.

NIEDERGERKE, R. (1956). The 'staircase' phenomenon and the action of calcium on the heart. *J. Physiol., Lond.* **134**, 569–583.

PHILLIPS, S. K. AND WOLEDGE, R. C. (1992). A comparison of isometric force, maximum power and isometric heat rate as a function of sarcomere length in mouse skeletal muscle. *Pflügers Arch.* **420**, 578–583.

REISER, P. J. AND LINDLEY, B. D. (1990). Activation in frog atrial trabeculae: dependence on temperature and length. *Am. J. Physiol.* **258**, H1087–H1096.

STEPHENSON, D. G. AND WENDT, I. R. (1984). Length dependence of changes in sarcoplasmic calcium concentration and myofibrillar calcium sensitivity in striated muscle fibres. *J. Muscle Res. Cell Motil.* **5**, 243–272.

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.

TER KEURS, H. E. D. J., RIJNSBURGER, W. H., VAN HEUNINGEN, R. AND NAGELSMIT, M. J. (1980). Tension development and sarcomere length in rat cardiac trabeculae: evidence of length-dependent activation. *Circulation Res.* **46**, 703–714.

WINEGRAD, S. (1974). Resting sarcomere length–tension relation in living frog heart. *J. gen. Physiol.* **64**, 343–355.