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THE LENGTH DEPENDENCE OF WORK PRODUCTION IN RAT PAPILLARY MUSCLES *IN VITRO*

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Summary

The influence of length on work production was investigated for rat papillary muscles using the work loop technique. Active and passive length–force relationships were first determined under isometric conditions and the length for maximum force production (L_{max}) was derived.

Starting from different lengths within the physiological range, a series of work loops was generated using the stimulation phase shift, strain amplitude and cycle frequency previously found to be optimal for power output at 37 °C. The relationship between muscle length and net work was used to determine the length at which work output was maximal (L_{opt}).

In order to examine the dynamic passive properties of the muscles, unstimulated muscles were subjected to the same regime of sinusoidal oscillation as used for the active loops. From the hysteresis loops, lengthening work (work done to extend the passive muscle), passive shortening work (work returned during shortening) and net energy loss (hysteresis) could be measured.

The decline in net work production at lengths greater than 95 % L_{max} could largely be attributed to the rapid and non-linear increase in muscle stiffness and the increase in net energy loss over this range of lengths.

The physiological significance of the length–work relationship is considered and the mechanical properties of active and passive papillary muscles are discussed with reference to sarcomere length and cardiac muscle ultrastructure.

Key words: papillary muscle, work loops, work, passive mechanical properties, sarcomere length, cardiac muscle, rat, muscle.

Introduction

Frank (1895) observed that the pressure developed during contraction of the frog ventricle was a function of end-diastolic volume. Later, using the mammalian heart and lung preparation, Starling and colleagues demonstrated that cardiac output was a function of filling pressure (reviewed by Allen and Kentish, 1985). In the isolated cardiac muscle preparation, an increase in length enhances the force of the subsequent contraction. Although, the Frank–Starling principle is not directly comparable with the length–force relationship of isolated cardiac muscle (reviewed by Allen and Kentish, 1985), muscle length undoubtedly has a profound influence on cardiac muscle performance.

The ascending limb of the cardiac length–force relationship is too steep to be accounted for by myofilament overlap and crossbridge formation alone (Jewell, 1977; ter Keurs *et al.* 1980). The length–force relationship is considered to be the consequence of both 'physical' and 'activation' factors, which are affected by muscle length (e.g. Jewell, 1977; Allen and Kentish, 1985). Physical factors are defined as those that are dependent on the extent of myofilament overlap at different lengths (Gordon *et al.* 1966). These include changes in the maximum number of active crossbridges and internal loads associated with sarcomere deformation, compression of crossbridges and thin filament buckling (for reviews, see Allen and Kentish, 1985; Brady, 1991). Muscle length is also known to influence activation processes such as the action potential and underlying currents, calcium movements and the binding of calcium to troponin. Length-dependent activation is attributed largely to increased calcium release and increased sensitivity of the contractile proteins to calcium with increasing length (Allen *et al.* 1974; Fabiato and Fabiato, 1975; Allen and Kentish, 1985). Length-dependent activation is said to be the major factor responsible for the steeper ascending limb of the length–force relationship observed in cardiac muscle compared with skeletal muscle (reviewed by Jewell, 1977; Allen and Kentish, 1985). In a recent study, it was proposed that the length–force relationship may also be explained by cooperativity between the conformational state of the crossbridges and the calcium affinity of the regulatory protein troponin (Landesberg and Sideman, 1994). The cooperativity theory states that an increase in muscle length increases the number of available cycling crossbridges which, in turn, enhances the calcium affinity of troponin. The resulting increase in levels of bound calcium leads to a further increase

in the number of crossbridges and so on, forming a positive feedback loop (Landesberg and Sideman, 1994).

A major difference between mammalian cardiac and skeletal muscle is the dramatic increase in passive force observed in cardiac muscle as length is increased towards the length for maximum force production (e.g. Sonnenblick, 1962; Spiro and Sonnenblick, 1964; Julian and Sollins, 1975; Brutsaert and Paulus, 1977). In the physiological situation, this large resting stiffness probably confines cardiac muscle to working at lengths on the ascending limb of the length–active force curve (Allen *et al.* 1974). Passive forces can be attributed largely to the network of connective tissue which forms a supporting structure for the cardiac cells and defines the range of lengths over which they operate (Winegrad, 1980). Collagen is the major connective tissue protein in muscle and, therefore, tensile properties of passive muscles are largely attributed to the amount, type and structure of collagen (Heerkens *et al.* 1987). However, even in skinned cardiac myocytes, passive stresses are greater than for skinned skeletal preparations at comparable length. This difference has been related to the higher cytoskeletal protein content (especially of titin) in cardiac compared with skeletal cells (reviewed by Brady, 1991).

Passive forces affecting the resting length, the amount of shortening and re-extension of cardiac cells influence diastolic size, change of shape, ejection fraction and diastolic filling in the intact heart (Winegrad, 1980; Nikolic *et al.* 1988). Therefore, the effects of muscle length on passive properties should also be considered when examining cardiac muscle in its physiological context.

Muscle length also influences the time course of mechanical activity (Jewell, 1977; ter Keurs *et al.* 1980; Reiser and Lindley, 1990; reviewed by Syme, 1993) and active shortening reduces the muscle's capacity to produce tension (Edman and Nilsson, 1971). Length influences mechanical activity in all types of contraction (reviewed by Cooper, 1990) and, in the intact heart, it modulates shortening velocity, total shortening and work output in addition to force production (Allen and Kentish, 1985). Cardiac muscle *in vivo* operates under complex mechanical constraints: the normal cardiac cycle consists of isovolumetric contraction and relaxation phases (analogous to isometric) and ejection and refilling phases (shortening and lengthening respectively). Therefore, length-dependent processes will operate at all stages of the cardiac cycle to determine muscle performance and, ultimately, work and power production.

The aim of this study was to investigate the influence of initial muscle length on the work done by isolated papillary muscle *in vitro*. Net work was assessed using the work loop technique, in which muscles are stimulated to generate force while undergoing sinusoidal changes in length. The function of papillary muscles *in vivo* is to prevent the prolapse of the atrioventricular valves during ventricular contraction. *In situ* recordings of papillary muscle force, length and electrical activation patterns during the cardiac cycle show that muscle length changes are approximately sinusoidal (Semafuko and Bowie, 1975); maximum force is produced around peak muscle length (Armour and Randall, 1970; Semafuko and Bowie, 1975) and the muscles perform work during the ejection phase, i.e. continue to produce force while undergoing shortening (Semafuko and Bowie, 1975). Therefore, by using the work loop technique, net work is measured under conditions which resemble the physiological contraction. Recently, the *in situ* strain waveform of ventricular subepicardial fibres has been observed using surface markers and was shown to be approximately sinusoidal (Delhaas *et al.* 1993*a*,*b*). Therefore, results obtained using the work loop technique in the present study may also be applicable to fibres in the ventricular wall.

Work loops (Josephson, 1985) were obtained from papillary muscles at different starting lengths within the physiological length range. The work loop technique assesses both the contractile work done by the muscle and the work required to re-extend it during a realistic pattern of cyclical contractions. Hysteresis loops were also produced to relate the passive properties of the muscle to the active relationship.

Materials and methods

Muscle preparation

Wistar laboratory rats of both sexes (mass 297±19 g, mean ± S.E.M., N=10) were killed by cervical dislocation and the heart removed as quickly as possible and rinsed in oxygenated mammalian cardiac Ringer (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; after Daut and Elzinga, 1989). The heart was then mounted on a Sylgard 184 base (Dow Corning) and immersed in fresh oxygenated 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 and to locate the papillary muscles.

The papillary muscles were dissected by cutting through the chordae tendineae at one end and isolating the muscle from the rest of the myocardium, finally removing the papillary muscle together with a small piece of ventricular wall at the non-tendinous end. Dissection was carried out at room temperature and the Ringer was replaced at frequent intervals throughout dissection.

Small aluminium foil clips were attached to both ends of the papillary muscle, clamping the tendon at one end and the ventricular wall at the other. Care was taken to attach the clips as close to the papillary muscle as possible 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 of the Ringer was gradually increased to 37 °C and maintained at this level throughout the experiment. The muscle preparation was continuously irrigated with oxygenated Ringer at 37 °C, circulated using a peristaltic pump. Two platinum wire

stimulating electrodes were positioned within the chamber to lie alongside the muscle preparation. 30 min was then allowed before experimentation, to allow the muscle to stabilise following dissection.

Determination of L_{max}

The length for maximum active force (L_{max}) was determined under isometric conditions. Supramaximal electrical stimuli of 2 ms duration were applied to elicit twitches with the muscle held at a fixed length. Twitch force was displayed on a digital storage oscilloscope (Gould 1604) from which total, passive and active force (defined as the difference between total and passive force) could be measured. Muscle length was measured using a graticule eyepiece. Starting from an initial slack length (zero passive force), muscle length was increased in 0.1 mm increments using a micromanipulator. Twitches were elicited at each length and the resulting active and passive forces recorded. This procedure was repeated until active force was seen to decline. Active force and passive force were plotted against muscle length to derive a length–force relationship and L_{max}.

Determining the length–net work relationship in active muscle

Starting length was varied within the physiological length range for cardiac muscle (87.5–100 %L_{max}, Brutsaert and Paulus, 1977) and the muscle was cycled around this starting length. For the first experimental run, the starting length was set at approximately 95 %L_{max} (to the nearest 0.1 mm). The muscle was then subjected to a series of sinusoidal length change cycles at the cycle frequency, strain amplitude and stimulus phase found to be optimal for maximum power production at 37 °C in previous experiments (Layland *et al.* 1995): 6 Hz cycle frequency, 5 % strain (10 % peak-to-peak) and 10 ° phase shift. Phase shift is expressed in degrees into the sine wave, i.e. 0 °/360 ° equals lengthening through initial length; 90 ° equals maximum strain; 180 ° equals shortening through initial length; 270 ° equals minimum strain. The force and length records produced by this regime of stimulation closely simulate the physiological contraction (see Semafuko and Bowie, 1975, Figs 2, 3). Having calculated the strain as ±5 % of muscle length at 95 %L_{max}, the absolute value of the strain was kept constant for the remainder of the experiment.

Muscle force was plotted against strain to produce anticlockwise work loops (Fig. 1A). To avoid confusion between variables measured from active and passive loops, the following terminology was used. The area under the shortening limb of the loop represents the work done (μJ) during active shortening (active shortening work, SW_a). The area enclosed by the lengthening limb of the loop and the zero force axis represents the work required to lengthen the muscle (LW_a, lengthening work for the active loop). The net work (W_{net}) done by the muscle is calculated from the area of the work loop itself and represents the difference between SW_a and LW_a (SW_a=W_{net}+LW_a). The same regime of sinusoidal oscillation was applied to the muscle from different starting lengths at 0.1 mm increments within the 85–105 %L_{max} range. At each starting length, the muscle was subjected to four sinusoidal cycles. The area of the first and largest loop in the series was taken to represent the maximum work that could be produced from that length (Layland *et al.* 1995) and was used to derive the length–work relationship. Preliminary experiments using larger numbers of loops established that work declined after the first loop but then recovered, such that the same work was produced from loop 20 as from the initial loop and this was maintained for a further 20 loops or more. Approximately 5 min was allowed between each experimental run. A plot of muscle length (%L_{max}) against net work allowed the length for

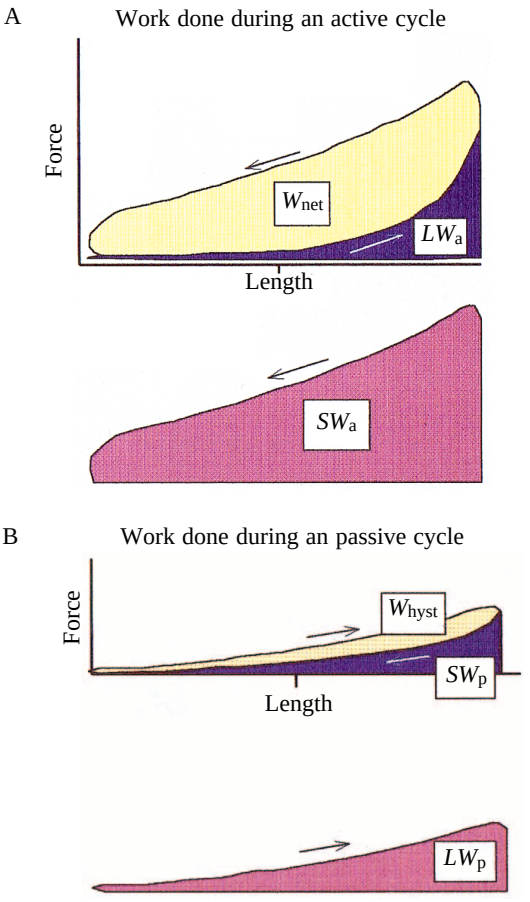

Fig. 1. Diagrams illustrating the derivation of active (A) and passive (B) work loops. To derive the active loops, twitch contractions (using a single stimulus) were elicited during the the imposed length change cycles. The plot of force against length for a single cycle generated an anticlockwise work loop (A), the area of which represents the net work done (W_{net}) during the active cycle. Length changes were also applied to the passive muscle and clockwise passive loops were generated (B). The area enclosed within the passive loop represents the net energy loss or hysteresis (W_{hyst}) during the passive cycle. The area enclosed between the shortening limb of the loops and the zero force axis represents the work during shortening, either active (SW_a) or passive (SW_p). The area underneath the lengthening limb of the loops, enclosed by the zero force axis, represents the work required to lengthen the active (LW_a) or passive (LW_p) muscle. Hence: W_{net}=SW_a−LW_a; W_{hyst}=LW_p−SW_p.

maximum work production (L_{opt}) to be derived. Julian and Sollins (1975) reported that the resting sarcomere length at L_{max} in living rat papillary muscles was 2.23 μm. Using this measurement to approximate the sarcomere length at L_{max} for the rat papillary muscles used in the present study, sarcomere lengths at L_{opt} and other points on the length–work curve were estimated, assuming a direct relationship between muscle length and resting sarcomere length above 85 %L_{max} (Grimm *et al.* 1970).

Monitoring the decline in muscle performance

Every third experimental run was followed by a control run using the same work loop parameters and starting length as used for the first run (i.e. at 95 %L_{max}). A plot of the maximum work in each control run against time allowed the decline in muscle performance to be monitored and corrected for, assuming a linear deterioration in muscle performance with time. Comparable methods of correcting for the decline in muscle performance are in common use (e.g. Kentish *et al.* 1986; Marsh, 1990; Stevenson and Josephson, 1990). Isometric twitch force at each length was also monitored throughout the experiment and compared with the length–force curve derived at the beginning. Twitch activation and relaxation times, together with the consistent shapes of the work loops, indicated that muscle kinetics did not change with time. Therefore, the deterioration in muscle performance was attributed to a decline in force alone, probably due to a progressive decrease in functional fibre volume resulting from a degree of damage. Muscle preparations were abandoned if the net work done during a control run declined to less than 80 % of its initial value.

Investigating the influence of length on the dynamic passive properties

Passive loops were generated in order to examine the energy loss (hysteresis) during sinusoidal strain cycles applied at different initial muscle lengths. The muscle was subjected to the same regime as for active loops but no stimulation was applied. A plot of the passive force record against the strain record produced a clockwise hysteresis loop (Fig. 1B). As for the active loop, the area enclosed by the lengthening limb and the zero force axis represents the work required to extend the passive muscle (LW_p, lengthening work for a passive loop). The area enclosed by the shortening limb of the loop and the zero force axis represents the work returned during shortening, passive shortening work (SW_p). The area enclosed within the passive loop represents the energy lost or hysteresis (W_{hyst}) during the cycle.

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 mean cross-sectional area was calculated as volume divided by length (where volume is given by wet mass divided by density, assuming a density of 1060 kg m^{-3}). Isometric stress could then be calculated as force per unit cross-sectional area.

Experiments were set up and controlled through a PC microcomputer and results analysed on-line using in-house software. Paired *t*-tests were used to compare values measured at L_{max} and L_{opt} from the same muscle, significance being attributed at a probability of $P<0.05$ (Graphpad Instat version 2.02).

For each set of data from each muscle, length–force and length–work curves were fitted using second- or third-order regression lines. From the curves, the values of L_{max} and L_{opt} could then be more precisely determined.

Results

Isometric studies

The mean wet mass of all the muscles used for the work loop experiments was 0.62±0.05 mg (mean ± S.E.M., N=7). The mean cross-sectional area at L_{opt} was 0.17±0.02 mm^2. The average maximum isometric stress was 46.70±4.31 kN m^{-2} at L_{max} and 43.46±3.94 kN m^{-2} at L_{opt}.

Fig. 2A shows the active and passive length–force relationships for the grouped and averaged data from 10 papillary muscles. The data conform to the classic Frank–Starling relationship (e.g. Frank, 1895; Sonnenblick, 1962; ter Keurs *et al.* 1980). Since muscle length at L_{max} varied between different preparations and 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 representation, force recordings for a similar muscle length were averaged. Groupings were of the order of 2 %L_{max} (e.g. $\geqslant$89 % but <91 %L_{max}, mean plotted at 90 %L_{max}). The length–force relationship for a typical muscle is given in Fig. 2B, illustrating that the averaged data give a good representation of the true length–force relationship.

The mean values of isometric force and muscle length at L_{max} were compared with their respective values at L_{opt} using paired *t*-tests (Table 1). Active and passive stresses and muscle length were all significantly lower at L_{opt} than at L_{max} ($P<0.01$). The ratio of passive to active forces at L_{opt} was significantly lower than at L_{max} (non-parametric Wilcoxon signed-rank test, $P<0.01$). Table 1 also includes the mean values of forces and muscle lengths at L_{opt} as a percentage of their values at L_{max}. The mean muscle length for maximum work production (L_{opt}) was approximately 95 % of the length at which maximum force was achieved (L_{max}). Although active force at L_{opt} was still around 93 % of active force at L_{max}, the passive force had fallen to 57 % of its value at L_{max}.

Work loop experiments

Table 1 shows that net work (J kg^{-1}) was significantly greater at L_{opt} than at L_{max}. Fig. 3 illustrates the length–work relationship for the combined data set (data grouped and averaged as for the length–force relationship), showing that

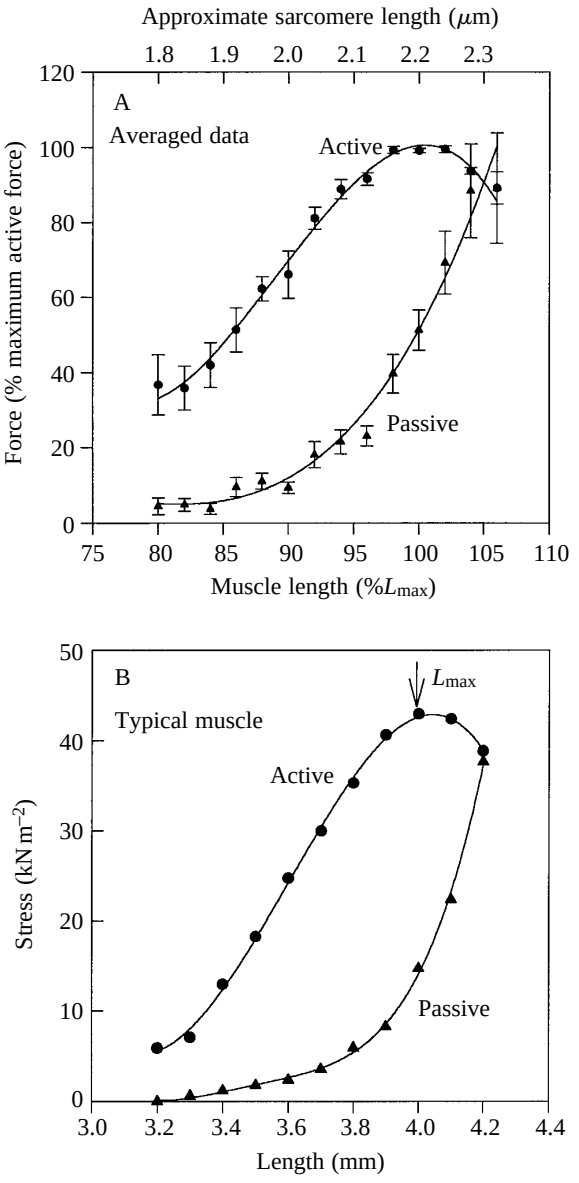

Fig. 2. The length–force relationships (active and passive) for the combined data set (A) and a typical preparation (B). For A, symbols and error bars represent mean ± S.E.M. of active force and passive force. A also illustrates the approximate sarcomere length. Details of how the data were grouped and averaged are given in the results. For each data point, the number of replicates is between three and 10 (from 10 muscles). Curves were fitted using second- or third-order regressions.

L_{opt} was approximately 95 %L_{max}. Representative active and passive work loops, from a typical muscle, are illustrated above the graph.

Fig. 4 shows that SW_a increases with increasing muscle length, but reaches its maximum at L_{max} and then begins to decline. LW_a increases rapidly and non-linearly with increasing muscle length. The decline in W_{net} beyond 95 %L_{max} corresponds to the range over which LW_a increases dramatically with increasing length. Fig. 4 also illustrates the increase in W_{hyst} with increasing muscle length (as derived from the passive loops generated for each length).

Discussion

Isometric experiments

The average isometric stresses at 37 °C, recorded in the present study, are comparable with those reported for cat ventricular trabeculae (Elzinga and Westerhof, 1981) and cat papillary muscles (Yeatman *et al.* 1969) at the same temperature.

The isometric length–force relationship (Fig. 2) can partly be explained by the degree of overlap of the actin and myosin filaments, as described for skeletal muscle (Gordon *et al.* 1966). At L_{max}, there is maximum overlap of the actin and myosin filaments, producing the greatest number of sites for actin–myosin interaction and crossbridge formation. The high passive forces present at L_{max} prevent the stretching of sarcomere lengths beyond the range for maximum force generation (Julian and Sollins, 1975; ter Keurs *et al.* 1980). For our preparations, the ascending limb of the active length–force relationship is probably too steep to be accounted for by myofilament overlap alone. It is therefore likely that length-dependent activation of the contractile elements is also involved (Jewell, 1977).

The length for maximum net work generation

L_{opt} falls close to the middle of the physiological length range over which cardiac muscle operates, i.e. from 12.5 % below L_{max} to L_{max} (Brutsaert and Paulus, 1977). Previous studies in frog (Syme, 1993) and rat (Layland *et al.* 1995) have also found that L_{opt} is shorter than L_{max}, although Syme (1994) could not find a distinct optimum for net work production. Published studies which have applied 'physiological' contractions to isolated cardiac muscle (i.e. using appropriate force and length changes) have also set muscle working length below L_{max} to represent the physiological condition (Elzinga and Westerhof, 1981; Jewell and Hanck, 1987).

Estimation of the sarcomere length range for net work output

Julian and Sollins (1975) reported that the average resting sarcomere length of living rat papillary muscles at L_{max} was 2.23 μm. This measurement was derived from striation patterns observed using microscopy of living muscle and therefore did not involve problems of shrinkage caused by muscle fixation. In the present study, the sarcomere length at L_{max} is therefore assumed to be 2.23 μm. Since sarcomere length is directly related to muscle length within the physiological length range, the sarcomere length for L_{opt} is calculated to be about 2.12 μm (Figs 2, 3). A strain amplitude of ±5 % from this sarcomere length would produce a working range between 2.01 and 2.22 μm. Julian and Sollins (1975) proposed that the high resting force in the region of the length–force relationship approaching L_{max} acts to maintain active sarcomere length within the range 2.0–2.2 μm, in close agreement with the range found to be optimal for net work production in the present study.

Table 1. *Mean values for papillary muscle lengths, active and passive isometric stresses, passive-to-active force ratio and net work measured at* L_{max} *and* L_{opt}

	Mean value at L_{max}	Mean value at L_{opt}	Value at L_{opt} as % of value at L_{max}	Significance
Muscle length (mm)	3.65±0.24 (10)	3.48±0.23 (10)	95.3	P<0.0001
Active stress (kN m^{-2})	46.70±4.31 (7)	43.46±3.94 (7)	93.1	P=0.0023
Passive stress (kN m^{-2})	25.40±3.13 (7)	14.49±1.96 (7)	57.0	P=0.0004
Passive:active force ratio	0.49±0.06 (10)	0.30±0.04 (10)	61.2	P=0.002*
Net work (J kg^{-1})	1.26±0.15 (7)	1.48±0.15 (7)	117.5	P=0.010

For comparison, length, force and work measured at L_{opt} are expressed as a percentage of their values at L_{max}.
Differences in variables measured at L_{opt} and L_{max} were assessed statistically using paired Student's t-tests.
*Passive-to-active force ratio at L_{opt} and L_{max} are compared using a paired non-parametric Wilcoxon signed-rank test.
Statistical significance is attributed when P<0.05.
Values are means ± S.E.M. (N).

Static passive properties

It is widely known that cardiac muscle exhibits a significant resting force, i.e. stiffness increases when muscle is stretched beyond 95 %L_{max} (Brutsaert and Paulus, 1977). In cardiac papillary muscles and trabeculae, the large parallel elastic component has mainly been attributed to extracellular connective tissue, composed principally of collagen (Allen and Kentish, 1985; Kentish *et al.* 1986; Brady, 1991). However, parallel elasticity is also observed in skinned cells (Kentish *et al.* 1986) and is often associated with titin (connectin) filaments, connecting the thick filaments to the Z line (Allen and Kentish, 1985; Horowits *et al.* 1987; Brady, 1991).

Active and passive forces in the determination of work output

Active force at L_{opt} is 93 % of active force at L_{max}, whereas passive force at L_{opt} is only 57 % of its value at L_{max}. The amount of work required to lengthen the muscle (LW_a or LW_p, see Fig. 1A,B) increases with increasing muscle length (Fig. 4) because of the increased muscle stiffness beyond 95 %L_{max} (Kentish *et al.* 1986). Since W_{net} is the difference between SW_a and LW_a, the decline in W_{net} beyond 95 %L_{max} can largely be explained by the dramatic increase in LW_a over this length range. It would therefore appear that the interaction between dynamic active and passive forces during a sinusoidal strain cycle is of great importance in determining the net work that can be done during that cycle.

Passive work loops and net energy loss

It is apparent that muscle resting length has a great effect on passive work as illustrated by a steeper lengthening limb in the passive loop (Fig. 3). A similar effect has been noted for rat diaphragm (Syme, 1990) and gastrocnemius medialis muscles (Heerkens *et al.* 1987).

Muscles demonstrate both elastic and viscous properties (Syme, 1990). During lengthening, the elastic and viscous components both resist the stretch, but on shortening, elastic components recoil whereas viscous elements resist the length change. Consequently, more energy is expended in stretching the muscle than is released during shortening, resulting in a net energy loss (W_{hyst}). In general, energy storage and release are attributed to the elastic properties whereas energy loss is due to the viscous properties.

For rat papillary muscles, it was demonstrated that net energy loss (W_{hyst}) increased with increasing muscle length, as

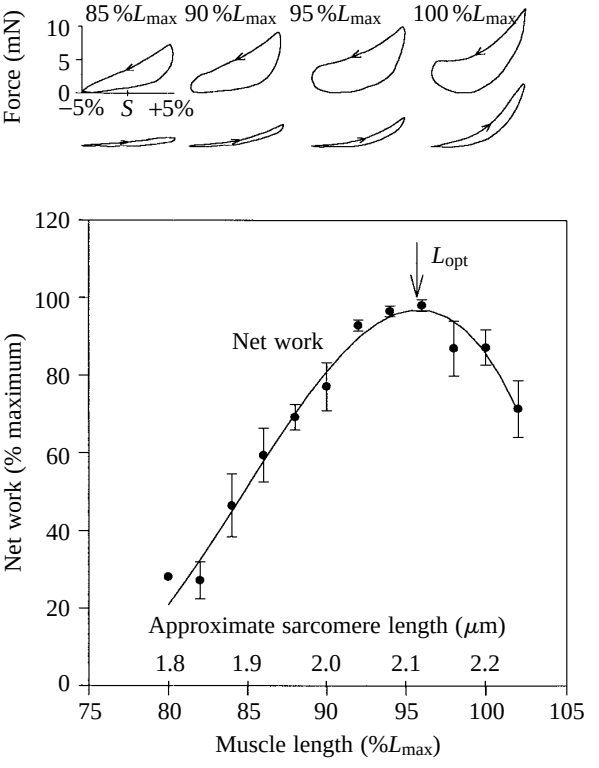

Fig. 3. The length–net work relationship for the combined data set (data grouped and averaged as explained in Fig. 2) together with the corresponding sarcomere length. L_{opt}, the length for maximum net work production, is indicated. Symbols and error bars represent mean ± S.E.M. The number of replicates is between three and nine (from 10 muscles). The curve was fitted using a third-order regression. Examples of loop shapes, active and passive, are illustrated for a typical muscle preparation. The loops are labelled according to their starting length (S) around which cyclical contraction was imposed (i.e. 85 %L_{max}, etc.).

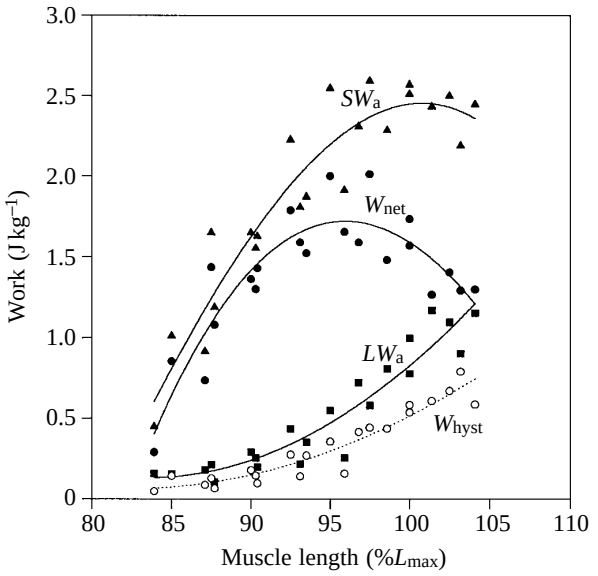

Fig. 4. The relationship between muscle length and SW_a, W_{net} and LW_a (derived from the active loops of three muscles) and W_{hyst} (derived from the passive loops of the same muscles). Curves are fitted using third-order regressions.

previously reported for rat diaphragm (Syme, 1990) and gastrocnemius medialis (Heerkens *et al.* 1987). The increase in W_{hyst} with increasing muscle length relates to a change in the viscous properties of the muscle since the viscous characteristic of cardiac muscle is known to be alinear and increases with increasing muscle length (Loeffler and Sagawa, 1975; Noble, 1977*a*).

The magnitude of the energy loss measured for rat papillary muscles is greater than has been reported for other types of striated muscle (e.g. Josephson, 1985; Heerkens *et al.* 1987; Syme, 1990). At lengths approaching L_{max}, W_{hyst} is between 20 and 30 % of SW_a, compared with 10–15 % at L_{opt}. In insect flight muscle (which also has a relatively high stiffness), it was found that the work absorbed per cycle (hysteresis) was about 10 % of the work (SW_a) produced by the muscle under optimum conditions of length oscillation and phase (Josephson, 1985). Josephson (1985) proposed that work-absorbing components were probably present in active as well as passive muscle. Therefore, in the present study, it was assumed that the dynamic viscous properties (and therefore energy losses) behaved similarly during active loops and during passive loops. It is possible, therefore, that the increase in W_{hyst} with increasing muscle length may also contribute to the decline in W_{net} observed in rat papillary muscle at lengths exceeding 95 %L_{max}, although the dramatic increase in LW_a is the most important factor (as described above).

It has been suggested that the increase in hysteresis with increasing length may be due to an alteration in fibre dimensions, which alters the viscous properties of the muscle (Heerkens *et al.* 1987; Syme, 1990). It has also been proposed that the cytoskeletal protein titin (connectin) may be responsible for the viscous load during shortening (ter Keurs and de Tombe, 1993). Titin is a huge, striated muscle protein that binds to the thick filaments, linking them to the Z disc, and is important in positioning the thick filaments within the sarcomere (Horowits *et al.* 1989). The titin molecule consists of a series of large macromolecular domains coupled to each other by shorter protein domains. The large macromolecular domains can be enormously extended by unfolding and it is this property which led to the suggestion that titin may account for the viscous load of shortening cardiac muscle (ter Keurs and de Tombe, 1993). The authors emphasized that further investigation was required to support this proposal. However, for the purposes of the present study, one could speculate that increasing muscle length may result in the deformation and unfolding of the titin macromolecular domains, which may explain the observed increase in energy loss (hysteresis) with increasing muscle length. The greater energy losses for rat papillary muscles compared with skeletal muscles (Heerkens *et al.* 1987; Syme, 1990) could be related to the greater titin content in cardiac muscle compared with skeletal muscle (Brady, 1991).

Conclusions and physiological significance

This study demonstrates that cardiac muscle produces maximum net work at approximately 95 %L_{max} (corresponding to a sarcomere length of 2.1 μm) when performing work loops with a strain amplitude, cycle frequency and stimulation phase shift that approximately simulate the *in vivo* contraction. The resulting length–work relationship showed that the range of muscle lengths (sarcomere lengths) over which maximum work was produced correlated well with the *in vivo* operating length range for cardiac muscle.

The interaction between the active and passive properties has been found dramatically to influence work output of cardiac muscle. The steep increase in stiffness observed in cardiac muscle at lengths exceeding 95 %L_{max} appears to be largely responsible for the decline in net work production. At lengths giving maximal (or near maximal) force production, stiffness is lower in skeletal muscle than in cardiac muscle. This difference can be attributed to the ultrastructure of the muscle cell since cardiac muscle contains more connective tissue than skeletal muscle (Spiro and Sonnenblick, 1964). Skinned cardiac cells also exhibit greater passive forces than corresponding skeletal muscle cells and this has been associated with the larger proportion of cytoskeletal proteins (especially titin) in cardiac muscle compared with skeletal muscle (Brady, 1991).

The influence of muscle length on work and power production has been studied in other striated muscles (e.g. Josephson and Stokes, 1989; Syme, 1994; James *et al.* 1995). A similar dependence of work output on average muscle length was reported for crab scaphognathite muscle (Josephson and Stokes, 1989), in which the optimum length for work output fell on the ascending limb of the isometric length–force curve, not far from the maximum. In addition, the length–force curve of the crab scaphognathite muscle was observed to be quite narrow, with an especially steep ascending limb (Stokes and

Josephson, 1988), which is comparable to the steep ascending limb of mammalian cardiac muscle length–force relationships (Allen and Kentish, 1985). In contrast, frog ventricular muscle showed no distinct optimum length for work output (Syme, 1994). Instead, power output increased with increasing muscle length and appeared to reach a plateau. Furthermore, in many work loop studies, skeletal muscles tend to yield a maximum value of work/power around the length for maximum force production (generally called L_0), where passive force remains relatively low (e.g. Syme and Stevens, 1989; Barclay, 1994; James *et al.* 1995). It therefore seems that the degree to which net work production is influenced by muscle length differs from muscle to muscle and the relative influence of passive mechanical properties will probably vary depending on the muscle in question.

The normal ventricle appears to operate at an end-diastolic sarcomere length of 2.0–2.1 μm, where passive force is low. However, acute changes in the dimensions of the heart that occur during dilatation (Winegrad, 1980) may force the muscle to operate in a less favourable range of the passive length–force relationship (i.e. beyond 95 %L_{max}), with a corresponding decline in work production and power output. Passive myocardial properties themselves may be altered, for example as a result of collagen accumulation resulting from muscle damage or a pathological condition (e.g Bartošová *et al.* 1969; Buccino *et al.* 1969). The ventricular wall may become stiffer as a result of thickening or material changes. Thickening of the ventricle is observed in many conditions of cardiac hypertrophy, and an increase in material stiffness may be observed in, for example, clinical ischaemia or constrictive cardiomyopathy (Noble, 1977*b*). A stiffer ventricle is then more resistant to filling, requiring an increased atrial pressure (Noble, 1977*b*). On the basis of the results of the present study, one could speculated that stiffening of the ventricle may impair net work production.

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