The Journal of Experimental Biology 210, 2843-2850
Published by The Company of Biologists 2007
doi:10.1242/jeb.006379

Effects of stretch on work and efficiency of frog (*Rana pipiens*) muscle

Michelle Trinh and Douglas A. Syme*

Department of Biological Sciences, University of Calgary, 2500 University Drive NW, Calgary, Alberta, T2N 1N4, Canada

*Author for correspondence (e-mail: syme@ucalgary.ca)

Accepted 29 May 2007

Summary

Applying a small stretch to active muscle immediately before shortening results in an increase in force and work done during subsequent shortening. The basis of the increase is not fully understood, having important implications for work and efficiency, and how they are influenced through stretch. We used the anterior tibialis muscle of leopard frogs (*Rana pipiens* complex) to measure the oxygen consumed and work done during shortening contractions that were immediately preceded by either a brief stretch (5% muscle length over 25 ms) or an isometric contraction (25 ms duration). Work done by the muscle while shortening following stretch was about 28% greater than work done following an isometric contraction ($P<0.001$). However the net work done during the entire contraction (i.e. accounting for the work required to stretch the muscle) was reduced by 13% if stretch preceded the shortening phase ($P=0.003$). The energy (oxygen) used during a stretch–shorten cycle was the same as for an isometric-shorten contraction ($P=0.34$). Likewise, the efficiency of net work (net work/energy used) was only marginally different between shortening contractions preceded by stretch or an isometric phase ($P=0.07$). Thus, under conditions that were intended to mimic what might occur during animal movement, a stretch that immediately preceded shortening enhanced work during shortening but did not impart a net mechanical or energetic benefit to the contraction. These observations could indicate that stretch simply extends compliant elements that recoil subsequently with some loss of mechanical energy in the process and/or that stretch results in an increase in the number of, and hence work done by, cross bridges during muscle shortening accompanied by a proportionate increase in energy consumed.

Key words: muscle, stretch, shortening, isometric, efficiency, contraction, work, net work, oxygen, energy, leopard frog.

Introduction

The force and work produced by skeletal muscle following stretch are greater than following an isometric contraction (Abbott and Aubert, 1952; Beltman et al., 2004; Bosco et al., 1987; Cavagna et al., 1965; De Haan et al., 1989; Edman et al., 1978; Edman and Tsuchiya, 1996; Ettema et al., 1990; Heglund and Cavagna, 1987; Herzog and Leonard, 2002; Herzog and Leonard, 2005; Lee and Herzog, 2002; Linari et al., 2000; Linari et al., 2003; Rassier et al., 2003). Likewise, work loop studies employing cyclic bouts of stretch and shortening with isolated muscle show that there is an increase in net work output when the phase of muscle activation is such that a brief eccentric period precedes the concentric portion of the contraction (Altringham and Johnston, 1990; Josephson, 1985). Whether the enhanced force and work after stretch result from an elastic mechanism, from increased time for muscle activation, from an effect of stretch resulting in more cross bridges or more force per cross bridge, or from reflex activity in intact preparations, is actively debated (Cavagna et al., 1994; Cavagna et al., 1985; De Haan et al., 1989; Herzog and Leonard, 2002; Herzog and Leonard, 2005; Linari et al., 2004; Linari et al., 2000; Mantovani et al., 1999; Rassier et al., 2003; Syme and Grattan, 2002; Takarada et al., 1997; Tamura et al., 2005; van Ingen Schenau et al., 1997).

An increase in net work with stretch would suggest that the enhanced work is not solely a result of the stretch imparting mechanical energy to elastic elements, as this would not result in a net work gain. The increased work during shortening following stretch may then be due, at least in part, to increased work done by cross bridges, either *via* increased work done by individual cross bridges or more cross bridges (Linari et al., 2004). However, not all contractions preceded by stretch show an increase in net work (De Haan et al., 1989). No change or a reduction in net work with stretch might suggest the effect is entirely due to stretch of the series compliance. As such, the specific protocol employed to measure the effects of stretch on work must be considered carefully.

The effects of stretch on energy used during the contraction are less well studied than the effects on work, but it is important to understand the functional significance of stretch on force and work, and perhaps the underlying mechanism. Several studies have noted no difference between the energy consumed during contractions where shortening was preceded by a stretch *versus* an isometric phase (Bosco et al., 1987; De Haan et al., 1989; Heglund and Cavagna, 1987). In combination with the increased work done after stretch there is an approximately 5–10% absolute increase in the apparent efficiency of work done during shortening. However, these measures have not accounted for the

mechanical energy required to stretch the muscle and are not all what might be considered representative of movements in animals, making interpretation of mechanism and relevance to animal movement difficult.

Net work from cyclic contractions accounts for both the mechanical and metabolic energy used during the entire contraction (extension and shortening). Using this approach it has been demonstrated that the efficiency of net work is increased, sometimes substantially, over contractions where isovelocity shortening is preceded by an isometric phase (Barclay, 1994; Barclay et al., 1993; Curtin and Woledge, 1993c; Woledge and Curtin, 1993). Yet these two types of contractions differ in several important respects, including the relative duration and phases of muscle activation, and the magnitude and trajectory of stretch and shortening, again making direct comparisons difficult.

The cause and potential magnitude of changes in work and efficiency after stretch remain poorly understood, and it remains unclear if efficiency of contraction *per se* is improved by stretch or if the observations reflect the effects of the timing of activation on work and efficiency (see also Curtin and Woledge, 1996). The objectives of the present study were to determine if a stretch–shorten protocol that broadly mimics what might occur during movement in animals results in an enhancement in work done by the muscles, how the energetic cost of the movement is impacted, and if the results suggest a compliance or cross-bridge-based mechanism. Muscles were activated, either with or without a stretch, and with or without subsequent shortening. The work required to extend the muscle and that done while shortening were measured, along with the oxygen consumed by the muscle. While work done during shortening was increased by stretch, net work and efficiency were not. This is consistent with stretch simply extending series complaint elements, which then recoil during subsequent shortening, but does not exclude an effect of stretch on the number of attached cross bridges as a contributing mechanism.

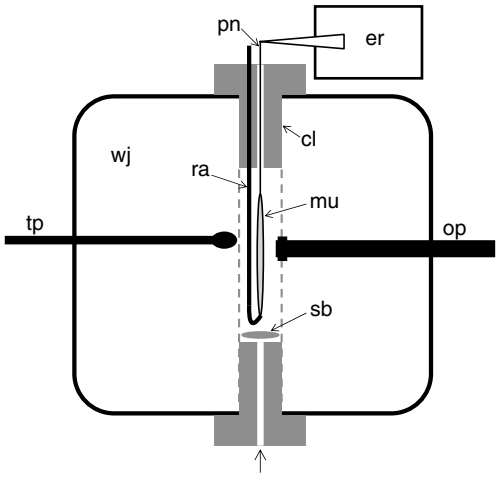

Fig. 1. Experimental apparatus used to measure oxygen consumption and work. Muscle (mu) was mounted in a cylindrical glass chamber (bound by broken lines) surrounded by a temperature controlled water jacket (wj). One end of the muscle was attached to a rigid, stainless-steel arm (ra) secured to the stainless-steel chamber lid (cl). The other end was attached to a stainless-steel pin (pn) that passes through a narrow aperture in the lid and was connected to the arm of an ergometer (er). A glass-encapsulated magnetic stir bar (sb) was used to mix the saline in the chamber. An oxygen probe (op*)* enters the chamber through a sealed side port. A temperature probe (tp) was placed adjacent to the chamber. To flush the chamber, saline entered through a port in the bottom (arrow) and exited through the hole in the chamber lid; diameter of holes in chamber lid and bottom are exaggerated for illustration purposes. Chamber lid and bottom formed a tight seal with the walls of the chamber using rubber O-rings. The rigid arm and pin connected to the ends of the muscle were attached to fine magnet wires outside the chamber (not shown) and used to stimulate the muscle. The rigid arm was insulated along its length with polyethylene tubing to minimize stray current in the chamber.

Materials and methods

Muscle preparation and apparatus

All procedures were approved following animal care guidelines of the Canadian Council on Animal Care and the University of Calgary. Adult leopard frogs (*Rana pipiens* complex) of either sex were killed by decapitation and pithing. A hindleg was removed and placed in a dish containing physiological saline (in mmol l^{-1}: 115 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 6 NaH_2PO_4, 5 glucose, pH 7.5 at 20°C). The anterior tibialis was isolated intact with tendon on either end. A central fascicle (the same fascicle for all experiments) was removed from the muscle, and further dissected to about one third of its original mass. The muscle bundle was then secured in a chamber for measurement of work and oxygen consumption (Fig. 1) similar to previous work (Syme, 1994). The tendon at one end of the bundle was tied using 6-0 silk suture to a stainless steel arm attached rigidly to the chamber lid. The tendon at the other end of the muscle was tied to a stainless steel pin that ran through a small aperture in the chamber lid and was later attached to the arm of an ergometer (model 350, Cambridge Technology, Cambridge, MA, USA). The ties were made within 0.5 mm of the muscle fibres to reduce stray compliance. The muscle/lid was then placed into a glass chamber containing physiological saline such that it formed a tight seal against the walls of the chamber. Air bubbles were removed from the chamber before the lid was inserted. The temperature of the chamber was maintained at 15°C by a water jacket surrounding the chamber and was monitored by a probe that abutted but did not enter the chamber itself. A small, glass-encapsulated, magnetic stir bar in the bottom of the chamber was driven by a magnetic stirrer placed below the chamber.

The height of the ergometer was adjusted to remove visible slack from the muscle, and the horizontal position adjusted so that the pin moved freely through the aperture in the lid. The pin and arm attached to either end of the muscle were connected *via* fine magnet wires to a Grass SD9 stimulator (Grass Inst. Div., Astro-Med, Inc., West Warwick, RI, USA) so that the muscle was stimulated directly end-to-end. The rigid support arm was insulated except at the tip where it contacted the muscle, and the face of the chamber lid was electrically isolated from both pins such that the current path was largely limited to flow through the muscle itself, allowing direct activation *via* the SD9 stimulator. A bipolar stimulus pulse, 0.5 ms duration, was applied to the muscle through the pin/arm. The stimulus voltage was set to 150% of that required to elicit maximal twitch force

(range 20–40 V); direct recording of the stimulus voltage at the chamber and the relatively high area-specific isometric forces produced by the muscle confirmed maximal activation. The muscle was then stimulated tetanically (100 Hz for 100 ms) to remove any potential slack in the ties. Muscle length (*ML*) was then varied systematically until the length giving maximal, isometric twitch force (double pulses) was found, referred to as L_o. L_o was measured using an ocular micrometer on a stereomicroscope.

A fibre-optic oxygen probe (PSt3 oxygen-sensitive foil) was placed in the chamber through a sealed port (Fig. 1), connected to a Fibox 3 oxygen meter (PreSens Precision Sensing GmbH, Regensburg, Germany) and used to measure the partial pressure of oxygen (P_{O_2}) in the chamber. Preceding each experiment a two-point calibration was performed at 0% and 100% air saturation. A reservoir of saline was gassed with a mixture of 60% nitrogen and 40% oxygen, giving a P_{O_2} of about 200% air saturation. Before measurement began this saline was flushed through the chamber *via* a small, stainless steel port at the base of the chamber and exited through the aperture in the chamber lid (Fig. 1). The inlet tube to the chamber was then closed so that the chamber was functionally sealed. The P_{O_2} of the saline in the chamber declined gradually over the course of the experiment as the muscle consumed oxygen, typically ending near 150% air saturation. The small diameter and long lengths of the inlet/outlet ports limited diffusion of oxygen into and out of the chamber to levels that were undetectable during control experiments. Also, due to the careful insulation of metal parts not in direct contact with the muscle, hydrolysis was not observed during stimulation, and similar stimulation without a muscle in the chamber resulted in no noticeable deflection of the oxygen partial pressure in the chamber.

During experiments the P_{O_2} of the saline was measured every second and logged to computer (Fig. 2). The decline in P_{O_2} of the saline due to resting muscle metabolism was measured for about 20 min to obtain a reliable baseline. The muscle was then activated (see measurement protocols below), causing an increase in the rate of decline of P_{O_2} in the chamber. After stimulation ended the rate of decline of P_{O_2} recovered back to the baseline level over a period of 10–15 min, and the muscle was allowed to recover for 30–60 min to again obtain a reliable baseline. The change in P_{O_2} in the saline as a result of the muscle being activated and doing work was measured by fitting linear regressions to the parallel pre- and post-baseline rates of decline, and calculating the separation between the regressions at a time point approximately mid-way between the beginning and end of each bout of contractions (Fig. 2). Selecting the mid-way point for measurement provided an objective measure of the change in P_{O_2} that would be least biased by any small differences in rates of decline pre- and post-activation.

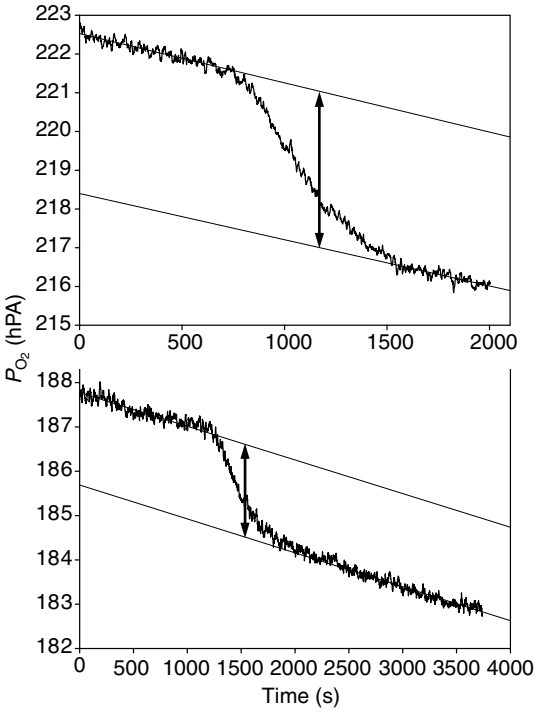

Fig. 2. Records of the partial pressure (P_{O_2}) of oxygen in the chamber during the course of one series of measurements in two different muscles (top and bottom traces). The initial decline in P_{O_2} as a result of resting metabolism in the muscle is followed by a more rapid decline when the muscle is stimulated to do work, which is then followed by a return to the resting rate when the muscle ceases working. The vertical separation between regressions fit to the initial and final resting rates, evaluated about half way between the onset and termination of work (line with arrowheads), is used to measure the change in P_{O_2} that occurred while the muscle was working.

Measurement protocols

Four different protocols were employed, the order of presentation being varied between experiments (Fig. 3). (i) The first protocol was designed to measure the work done and energy consumed when shortening was preceded by an isometric phase of contraction. The muscle was first lengthened to 105% L_o while relaxed. It was then stimulated for 25 ms at a length of 105% L_o and then shortened at a velocity of 1 *ML* s^{-1} for 150 ms with stimulation ending 70 ms after it began. In this way the muscle was activated for 25 ms before shortening commenced, it shortened a total of 15% L_o (from 105–90% L_o) and force declined to resting levels before the end of shortening. (ii) The second protocol was designed to measure the work done and energy consumed when shortening was preceded by a stretch. Stimulation commenced, the muscle was stretched from 100% to 105% L_o over a 25 ms period (i.e. at 2 *ML* s^{-1}) and then shortened at a velocity of 1 *ML* s^{-1} for 150 ms with stimulation ending 70 ms after it began. As in the first protocol, the muscle was activated for 25 ms before shortening commenced except that it was being stretched during this time, the muscle shortened a total of 15% L_o and over the same range of muscle lengths, and force declined to resting levels before the end of shortening. (iii) The third protocol was designed to measure the cost of an isometric contraction. The muscle was first lengthened to 105% L_o while relaxed. The muscle was then held isometric at 105% L_o and stimulated for 100 ms. (iv) The fourth protocol was designed to measure the cost of an isometric contraction preceded by stretch. Stimulation commenced, the muscle was stretched from 100% to 105% L_o over a 25 ms period, and then held isometric with stimulation ending 100 ms after it began.

The above protocols were designed to approximate the types

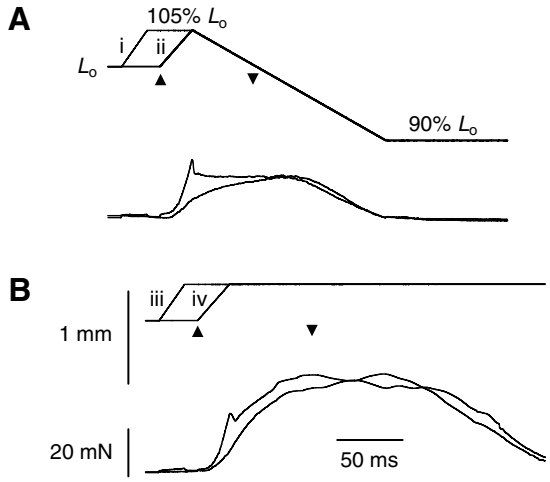

Fig. 3. Examples of the four protocols used to measure work done and cost of the different contractions, as described in Materials and methods. (A) Muscle length (upper traces) and force (lower traces) during shortening contractions preceded by either an isometric phase (protocol i) or stretch (protocol ii). Triangles indicate onset (up) and termination (down) of stimulation. (B) Muscle length (upper) and force (lower) during either a purely isometric contraction (protocol iii) or an isometric contraction preceded by a stretch (protocol iv). L_o is muscle length giving maximal, isometric twitch force. Scale bars indicate muscle length (mm), force (mN) and time (ms).

of eccentric and concentric contractions that might occur during movement in animals, with the following considerations. Effective release of elastic strain energy as external work will have a large impact on efficiency, and is dependent on allowing the muscle to relax fully while still shortening (Lou et al., 1999). Thus, in the protocols described above, the stimulus ended well before shortening ended so that force declined to rest while the muscle was still shortening. The amplitude of muscle stretch (5% of L_o) was chosen to require considerable eccentric work yet not be so great as to exceed concentric shortening work (i.e. net work output was consistently positive). This amplitude may exceed the short-range stiffness of cross bridges (Flitney and Hirst, 1978) and thus could cause forcible detachment of cross bridges; however, it is not unrealistic in the context of animal movement (Biewener et al., 1998), and a considerable portion of the stretch is likely absorbed by compliant elements in the preparation. We did not measure the stiffness of the series compliance and so cannot ascertain the extent of strain on cross bridges imposed by the stretch protocols.

The stimulator and ergometer were controlled by computer using custom software written in LabView 6.1 and a PCI-MIO-16-E4 data acquisition and control card (National Instruments, Austin, TX, USA) with 5 kHz resolution. Muscle force and length and the stimulus were likewise recorded at 5 kHz. The work done or absorbed by the muscle was calculated by integrating force with respect to muscle length over the shortening or stretch portions of the protocol. Net work was the sum of work absorbed during stretch (a negative value) and work done while shortening (a positive value). Net work during the isometric-shorten protocol was equal to the shortening work as no external work was done during the isometric phase.

Stability of the preparations was demonstrated by the lack of change in peak force measured from the first to last trial in each series. At the end of experiments the muscle was removed from the chamber, the tendons were cut from the muscle and surface moisture was removed by blotting with filter paper. The muscle was placed in a small centrifuge tube to prevent desiccation and muscle mass was measured using a Sartorious CP124S analytical balance or Mettler MT5 microbalance. Work and energy consumed (see below) were then standardize to muscle mass (J kg^{-1}).

Energetic cost of contraction

To ensure a large change in P_{O_2} in the chamber that could be resolved reliably yet not promote fatigue or anaerobic metabolism, each contraction protocol was repeated 10 times in sequence with a 30 s rest between each contraction. This, in combination with the extended period over which P_{O_2} was monitored after the bout of contractions had ended, ensured that virtually all energy used by the muscle would be accounted for by the measurement of oxygen consumed. Further, previous studies have demonstrated the adequacy of using oxygen consumption in frog sartorius muscle under stimulus conditions more demanding than the present study (Heglund and Cavagna, 1987), and so it was assumed that contractions were supported exclusively by oxidative metabolism.

The amount of oxygen consumed is directly related to the ATP used and re-synthesized *via* oxidative phosphorylation. This amount was converted to Joule equivalents, as described previously (Syme, 1994). Briefly, oxygen compliance of the saline in the chamber was taken from standard tables (9.50 mg O_2 l^{-1}/101.36 kPa with 21% oxygen at 15°C), multiplied by the chamber volume (2.245 ml) and by the measured change in P_{O_2} (kPa) to obtain mg O_2 used by the muscle during the sequence of contractions. This was converted to moles of O_2 (32 g mol^{-1} O_2) and multiplied by 450 kJ mol^{-1} O_2 (Nelson and Cox, 2005), which is the energy released by oxidation of substrates based on a mixed diet. This yields the metabolic energy released in the muscle during the contractions. Efficiency was calculated by dividing the net work done during the entire contraction (including isometric, stretch and shortening phases) by the total energy released during the contraction.

Statistics

Nine muscles from 9 frogs were used. Values are reported as mean ± s.e.m. Values of mechanical work and metabolic energy consumption displayed in the figures have been normalized to muscle mass and are expressed as J kg^{-1} muscle. However, for statistical tests data were not normalized to muscle mass to eliminate errors associated with measuring mass. Comparisons were made using paired *t*-tests or one-way repeated measures ANOVA where appropriate. $P<0.05$ was considered significant.

Results

Mass of the muscle bundles averaged 5.18±0.63 mg and muscle length 12.8±0.89 mm. Assuming muscle density of 1.05 g cm^{-3}, the average cross sectional area of the preparations was 0.38 mm^2. Isometric tension averaged 379±23.5 kN m^{-2}.

The work done while shortening after a stretch was 28%

greater than work done following an isometric contraction (P<0.001) (Fig. 4). However, the work required to stretch the muscle was about one third of the work done during subsequent shortening, so that the net work done during contractions where stretch preceded shortening was only 87% of that done when shortening was preceded by an isometric phase (P=0.003) (Fig. 4).

The metabolic cost of contractions consisting of an isometric phase followed by shortening was not significantly different than the cost of contractions where shortening was preceded by stretch (P=0.34) (Fig. 5). These costs are the sum of that incurred during stretch or isometric contraction and that incurred during shortening. The cost of a 100 ms isometric contraction was 20% greater than the cost of a 25 ms stretch followed by 75 ms isometric contraction (P<0.001) (Fig. 5), such that either the stretch, the ensuing isometric phase, or both, were less costly than a purely isometric contraction.

The apparent efficiency of work done while shortening (work done while shortening divided by the energy consumed during the entire contraction) was about 28% greater if shortening was preceded by stretch *versus* an isometric phase (P=0.002) (Fig. 6). However, this does not account for the work required to stretch the muscle, which would require a measure of the net work output. The efficiency of the net work output (net work done divided by energy consumed during the entire contraction) was only marginally different between contractions preceded by a stretch or isometric phase (P=0.07) (Fig. 6). Thus, any change in net work output as a result of stretch was approximately matched by an equal change in energy used.

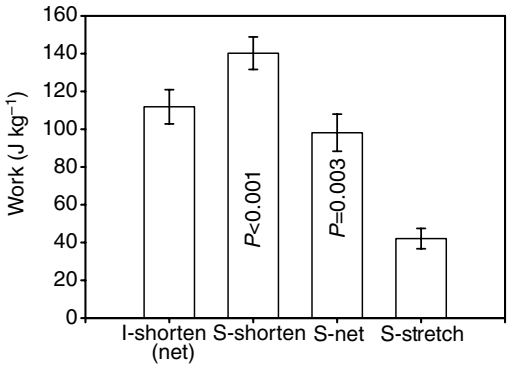

Fig. 4. Cumulative work done during 10 contractions in the protocols described in the Materials and methods and in Fig. 3. 'I', shortening contractions preceded by an isometric phase; 'S', shortening contractions preceded by a stretch; 'shorten', the work done during the shortening portion of the contraction; 'net', the net work done during the entire contraction (note: for contractions preceded by an isometric phase, net work equals shortening work); 'stretch', the work required to stretch the muscle. Work is expressed relative to muscle mass. Values are means ± s.e.m. P values are comparisons with I-shorten using work values uncorrected for muscle mass.

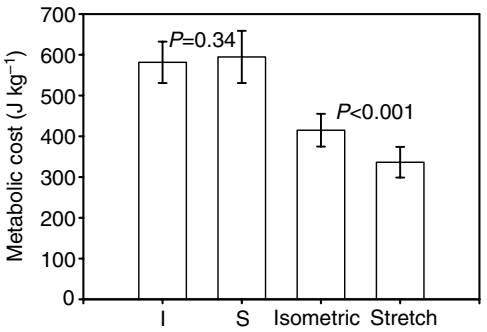

Fig. 5. Cumulative metabolic cost over 10 contractions, derived from oxygen consumption as described in Materials and methods. 'I', shortening contractions preceded by an isometric phase; 'S', shortening contractions preceded by a stretch; 'Isometric', cost of a 100 ms isometric contraction; 'Stretch', cost of a 25 ms stretch followed by 75 ms isometric contraction. Cost is expressed relative to muscle mass. Values are means ± s.e.m. P values compare the measurements that they straddle using values uncorrected for muscle mass.

Discussion

Effect of stretch on work

Stretch before shortening resulted in a significant increase in the work done during shortening (Fig. 4), as has been observed previously (see Introduction). The increase could result from recoil of compliant elements that were subject to strain during the stretch, increased numbers of cross bridges resulting from decreased rates of cross-bridge detachment during stretch, or increased force per cross bridge (e.g. Cavagna et al., 1994; De Haan et al., 1989; Herzog and Leonard, 2002; Linari et al., 2004; Linari et al., 2000). The magnitude of the increase in shortening work after stretch (about 28%, Fig. 4) was similar to that observed in frog sartorius muscle (Heglund and Cavagna, 1987) and rat medial gastrocnemius (De Haan et al., 1989), despite large differences between the studies in the stimulation protocol and the ratio between work required to stretch the muscle and work done during subsequent shortening. It appears that an appropriately small, brief stretch of active muscle may be similarly effective at augmenting work as a large, prolonged stretch.

This apparent limit to the magnitude of increased work elicited as a result of stretch might be expected if the increase

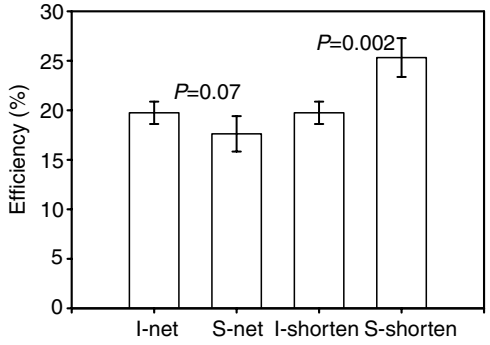

Fig. 6. Efficiency of muscle work measured over 10 contractions, derived from work and oxygen consumption as described in Materials and methods. 'I', shortening contractions preceded by an isometric phase; 'S', shortening contractions preceded by a stretch; 'net', calculations using the net work done during the entire contraction; 'shorten', calculations using the work done during only the shortening portion of the contraction, but still the energy used during the entire contraction. Values are means ± s.e.m. P values compare the two measurements that they straddle.

is a result of extension of the series compliance. Studies on mouse muscle subject to stretch and then rapid shortening suggest that recoil of an un- or lightly damped compliance in the muscle accounts for the vast majority of the increase in work during shortening after stretch, and upwards of 60% of the total work done (Syme and Grattan, 2002). The enhancement of work caused by stretch would be limited by the short-range stiffness and life of an attached cross bridge (see Syme and Grattan, 2002), such that for stretches larger than a certain size the strain energy imparted during stretch is lost internally if cross bridges detach before the compliance recoils against the external load (see also Lou et al., 1999). With stretches larger than this limit more work would be required to extend the muscle, but they would not result in more work done during subsequent shortening.

If stretch simply extends the series compliance, then despite an increase in shortening work after stretch the net external work done during stretch–shorten contractions would at best be equal to that following an isometric contraction, and likely less. In agreement, under the conditions of this study the work required to stretch the muscle was slightly greater than the increase in shortening work elicited by the stretch, such that the net work done during the complete stretch–shorten cycle was reduced (Fig. 4). This is similar to results of De Haan et al. (De Haan et al., 1989) who, as in the present study, chose a stretch–shorten and stimulation protocol intended to mimic what might occur during movement. These observations suggest that the upper boundary of energy that can be stored in the series compliance is reached or closely approached during such movements, such that a stretch–shorten cycle will increase work during shortening but will likely decrease the net mechanical work produced by the muscle to a small extent.

Alternatively, a number of observations suggest that stretch results in an increased number of attached cross bridges, inferred from increased muscle stiffness (Cavagna et al., 1985; Herzog and Leonard, 2000; Linari et al., 2000; Mantovani et al., 1999), direct inference of more attached heads despite unaltered strain of the heads (Linari et al., 2004), and transient shortening against maximal isometric force after stretch (Cavagna et al., 1994). This would argue against strain of compliant elements as the sole consequence of stretch. If the enhanced shortening work were due to additional cross bridges as a result of stretch, or more force per cross bridge, then an increase in net work might be expected, which was not observed (Fig. 4). However, a number of factors could lead to a reduction in net work with stretch even with additional cross bridges, including the unknown relationship between the work required to extend muscle and that potentially released by additional cross bridges, and detachment of cross bridges during stretch with the accompanying loss of their ability to contribute to work during subsequent muscle shortening. Further, even if much of the increased work after stretch may be attributed to recoil of strained compliances, this does not preclude recruitment of additional cross bridges during stretch and subsequent strain of compliances that they support as an important mechanism in enhancing work after stretch. Thus, it is not possible to conclude with certainty which mechanism is responsible for the increased work after stretch by analyzing these aspects of mechanical work alone.

Effect of stretch on energy used

The reduced oxygen consumption with stretch compared with that during isometric contractions (Fig. 5) is consistent with previous observations (Beltman et al., 2004; Curtin and Davies, 1975; Stainsby, 1976). The reduced cost may be attributed to forcible detachment of cross bridges (Flitney and Hirst, 1978) perhaps with subsequent rapid reattachment (Linari et al., 2004), or reduced rates of cross-bridge detachment during stretch (Huxley, 1957); for further discussion see Woledge et al. (Woledge et al., 1985). Reduced detachment during stretch would result in reduced energy consumption, increased numbers of attached cross bridges at the onset of shortening, and then perhaps more work done and energy consumed during shortening. This is also consistent with the observations that stretch resulted in reduced oxygen consumption (when preceding an isometric contraction and so presumably also when preceding shortening) and more work during shortening (Figs 4 and 5).

However, despite a predicted decrease in energy use during stretch, there was no difference in the total cost of contraction whether preceded by stretch or an isometric phase (Fig. 5), as has been observed by others (Beltman et al., 2004; De Haan et al., 1989; Heglund and Cavagna, 1987). The lack of reduction in energy consumed during shortening contractions preceded by stretch might lead to the assumption that the reduced rate of energy use during stretch was closely offset by an increased rate during shortening; while seeming unlikely, this may have occurred within the range of measurement error. Regardless, a small or insignificant effect of a brief stretch preceding shortening on total energy use is a consistent observation, having consequences for the use of stretch–shortening types of contractions during movement, discussed below.

Effect of stretch on efficiency

Efficiency of net work with and without stretch averaged about 18% and 20%, respectively (Fig. 6). This tends to be less than that observed during cyclic contractions (i.e. preceded by stretch) in mouse fast/slow muscle [52% in soleus and 34% in EDL (Barclay, 1994)], dogfish white muscle [41% (Curtin and Woledge, 1993a)] and dogfish red muscle [51% (Curtin and Woledge, 1993b)]. The differences in efficiency will reflect procedural differences between the studies (type of preparation, rates and magnitudes of stretch, stimulation protocol) and the measure of energy use that is employed in calculating efficiency. The cited studies utilized initial heat production, which yields the efficiency of the muscle at converting energy released by ATP hydrolysis into external mechanical work. The present study utilized the total energy available from substrates *via* oxidation, which incorporates the efficiency of associated substrate catabolism and ATP synthesis and presumes perhaps 40–50% more energy available to the muscle. This will reduce the calculated efficiency of contraction by about half, making our measures comparable to those using initial heat. Measures of efficiency from locust flight muscle undergoing sinusoidal strain and using the caloric equivalent of energy released from oxidation of a mixed diet (20.1 kJ l^{-1}oxygen, similar to the present study) gave efficiencies of 4–10% (Josephson and Stevenson, 1991); this is lower than our measure but not

unexpected, given the very high operating frequency of the locust muscle.

Despite the constancy of metabolic energy consumed (Fig. 5) and the decrease in net work done (Fig. 4) in contractions preceded by a stretch *versus* an isometric phase, there was not an associated decrease in the efficiency of net work done in contractions preceded by stretch (Fig. 6). By contrast, De Haan et al. (De Haan et al., 1989) observed a 48% reduction in efficiency of contractions in rat medial gastrocnemius when preceded by stretch, the result of a near 50% drop in net work done with no change in energy consumed. The much smaller reduction in net work after stretch in the present study (only 13%) may account for the lack of a statistically significant change in efficiency. Contractions preceded by stretch were less efficient on average than those preceded by an isometric phase, with the difference approaching significance (P=0.07), and the power of the test was relatively low (0.35), making it plausible that efficiency is decreased with stretch but simply out of the statistical resolution of this study.

Of interest, De Haan et al. estimated that under the conditions of their study elastic strain energy imparted during stretch would contribute about 20% to work done during shortening (De Haan et al., 1989). Assuming recoil of elastic elements would not elevate metabolic rate during shortening (e.g. Beltman et al., 2004), there might be expected an approx. 20% relative increase in the apparent efficiency of shortening work (apparent in that the work required to extend the muscle was not included in the calculation). De Haan et al. report a 40% increase in apparent efficiency of shortening work after stretch (De Haan et al., 1989), which suggests that recoil of strained compliance may account for a larger fraction of the enhanced work than estimated. We noted a 28% increase in apparent efficiency of shortening work after stretch (Fig. 6) in the face of a stretch that imparted enough energy to account for an approx. 30% increase in shortening work if all recovered. The close match of these values might suggest that almost all of the extra work done after stretch was from recoil of the strained series compliance.

In summary, under conditions that were selected to be reasonable estimates of what might occur during counter-movements in animals, (i) the work required to stretch the muscle was about one third of the work done during subsequent shortening, (ii) stretch resulted in about one third more work being done during subsequent shortening, but (iii) slightly less net work was done over the entire contraction. Based on stretching and isometric contractions it is expected that the stretch should reduce energy use, but this seems to have been offset by increased energy use during shortening such that stretch did not affect the total energy used during the contraction. The efficiency of work was not affected by stretch. We conclude that stretch of active muscle may increase the work available to power the concentric phase of movement but it does not increase the overall net mechanical work done. Efficiency of work does not appear to be markedly altered by stretch, being either unchanged or slightly reduced, making counter-movements effective at improving concentric work without a substantial metabolic cost. The present results do not distinguish quantitatively between recruitment of additional cross bridges *versus* increasing strain of the series compliance as mechanisms whereby stretch affects work and efficiency during stretch–shorten contractions, but do suggest that both contribute, with recoil of strained compliances perhaps being predominant.

This work was supported by an NSERC Discovery grant to D.A.S.

References

Abbott, B. C. and Aubert, X. M. (1952). The force exerted by active striated muscle during and after change of length. *J. Physiol.* **117**, 77-86.

Altringham, J. D. and Johnston, I. A. (1990). Modelling muscle power output in a swimming fish. *J. Exp. Biol.* **148**, 395-402.

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

Barclay, C. J., Constable, J. K. and Gibbs, C. L. (1993). Energetics of fast- and slow-twitch muscles of the mouse. *J. Physiol.* **472**, 61-80.

Beltman, J. G., van der Vliet, M. R., Sargeant, A. J. and de Haan, A. (2004). Metabolic cost of lengthening, isometric and shortening contractions in maximally stimulated rat skeletal muscle. *Acta Physiol. Scand.* **182**, 179-187.

Biewener, A. A., Konieczynski, D. D. and Baudinette, R. V. (1998). *In vivo* muscle force–length behavior during steady-speed hopping in tammar wallabies. *J. Exp. Biol.* **201**, 1681-1694.

Bosco, C., Montanari, G., Tarkka, I., Latteri, F., Cozzi, M., Iachelli, G., Faina, M., Colli, R., Dal Monte, A., La Rosa, M. et al. (1987). The effect of pre-stretch on mechanical efficiency of human skeletal muscle. *Acta Physiol. Scand.* **131**, 323-329.

Cavagna, G. A., Saibene, F. P. and Margaria, R. (1965). Effect of negative work on the amount of positive work performed by an isolated muscle. *J. Appl. Physiol.* **20**, 157-158.

Cavagna, G. A., Mazzanti, M., Heglund, N. C. and Citterio, G. (1985). Storage and release of mechanical energy by active muscle: a non-elastic mechanism? *J. Exp. Biol.* **115**, 79-87.

Cavagna, G. A., Heglund, N. C., Harry, J. D. and Mantovani, M. (1994). Storage and release of mechanical energy by contracting frog muscle fibres. *J. Physiol.* **481**, 689-708.

Curtin, N. A. and Davies, R. E. (1975). Very high tension with very little ATP breakdown by active skeletal muscle. *J. Mechanochem. Cell Motil.* **3**, 147-154.

Curtin, N. A. and Woledge, R. (1993a). Efficiency of energy conversion during sinusoidal movement of white muscle fibres from the dogfish *Schyliorhinus canalicula*. *J. Exp. Biol.* **183**, 137-147.

Curtin, N. A. and Woledge, R. C. (1993b). Efficiency of energy conversion during sinusoidal movement of red muscle fibres from the dogfish *Scyliorhinus canicula*. *J. Exp. Biol.* **185**, 195-206.

Curtin, N. A. and Woledge, R. C. (1993c). Power and efficiency: how to get the most out of striated muscle. *Adv. Exp. Med. Biol.* **332**, 729-733; discussion 733-734.

Curtin, N. A. and Woledge, R. (1996). Power at the expense of efficiency in contraction of white muscle fibres from dogfish *Scyliorhinus canicula*. *J. Exp. Biol.* **199**, 593-601.

De Haan, A., Van Ingen Schenau, G. J., Ettema, G. J., Huijing, P. A. and Lodder, M. A. (1989). Efficiency of rat medial gastrocnemius muscle in contractions with and without an active prestretch. *J. Exp. Biol.* **141**, 327-341.

Edman, K. A. and Tsuchiya, T. (1996). Strain of passive elements during force enhancement by stretch in frog muscle fibres. *J. Physiol.* **490**, 191-205.

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

Ettema, G. J., Huijing, P. A., van Ingen Schenau, G. J. and de Haan, A. (1990). Effects of prestretch at the onset of stimulation on mechanical work output of rat medial gastrocnemius muscle–tendon complex. *J. Exp. Biol.* **152**, 333-351.

Flitney, F. W. and Hirst, D. G. (1978). Cross-bridge detachment and sarcomere 'give' during stretch of active frog's muscle. *J. Physiol.* **276**, 449-465.

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

Herzog, W. and Leonard, T. R. (2000). The history dependence of force production in mammalian skeletal muscle following stretch-shortening and shortening-stretch cycles. *J. Biomech.* **33**, 531-542.

Herzog, W. and Leonard, T. R. (2002). Force enhancement following stretching of skeletal muscle: a new mechanism. *J. Exp. Biol.* **205**, 1275-1283.

Herzog, W. and Leonard, T. R. (2005). The role of passive structures in force

enhancement of skeletal muscles following active stretch. *J. Biomech.* **38**, 409-415.

Huxley, A. F. (1957). Muscle structure and theories of contraction. *Prog. Biophys. Biophys. Chem.* **7**, 255-318.

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

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

Lee, H. D. and Herzog, W. (2002). Force enhancement following muscle stretch of electrically stimulated and voluntarily activated human adductor pollicis. *J. Physiol.* **545**, 321-330.

Linari, M., Lucii, L., Reconditi, M., Casoni, M. E., Amenitsch, H., Bernstorff, S., Piazzesi, G. and Lombardi, V. (2000). A combined mechanical and X-ray diffraction study of stretch potentiation in single frog muscle fibres. *J. Physiol.* **526**, 589-596.

Linari, M., Woledge, R. C. and Curtin, N. A. (2003). Energy storage during stretch of active single fibres from frog skeletal muscle. *J. Physiol.* **548**, 461-474.

Linari, M., Bottinelli, R., Pellegrino, M. A., Reconditi, M., Reggiani, C. and Lombardi, V. (2004). The mechanism of the force response to stretch in human skinned muscle fibres with different myosin isoforms. *J. Physiol.* **554**, 335-352.

Lou, F., Curtin, N. A. and Woledge, R. C. (1999). Elastic energy storage and release in white muscle from dogfish *Scyliorhinus canicula*. *J. Exp. Biol.* **202**, 135-142.

Mantovani, M., Cavagna, G. A. and Heglund, N. C. (1999). Effect of stretching on undamped elasticity in muscle fibres from *Rana temporaria*. *J. Muscle Res. Cell Motil.* **20**, 33-43.

Nelson, D. L. and Cox, M. M. (2005). Oxidative phosphorylation and photophosphorylation. In *Lehninger Principles of Biochemistry*, 4th edn (ed. J. O'Neill), pp. 690-750. New York: W. H. Freeman.

Rassier, D. E., Herzog, W., Wakeling, J. and Syme, D. A. (2003). Stretch-induced, steady-state force enhancement in single skeletal muscle fibers exceeds the isometric force at optimum fiber length. *J. Biomech.* **36**, 1309-1316.

Stainsby, W. N. (1976). Oxygen uptake for negative work, stretching contractions by *in situ* dog skeletal muscle. *Am. J. Physiol.* **230**, 1013-1017.

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

Syme, D. A. and Grattan, M. J. (2002). Effects of stretch on work from fast and slow muscles of mice: damped and undamped energy release. *Can. J. Physiol. Pharmacol.* **80**, 887-900.

Takarada, Y., Iwamoto, H., Sugi, H., Hirano, Y. and Ishii, N. (1997). Stretch-induced enhancement of mechanical work production in frog single fibers and human muscle. *J. Appl. Physiol.* **83**, 1741-1748.

Tamura, Y., Saito, M. and Nagato, R. (2005). A new motor model representing the stretch-induced force enhancement and shortening-induced force depression in skeletal muscle. *J. Biomech.* **38**, 877-884.

van Ingen Schenau, G. J., Bobbert, M. F. and De Haan, A. (1997). Does elastic energy enhance work and efficiency in the stretch-shorten cycle? *J. Appl. Biomech.* **13**, 389-415.

Woledge, R. C. and Curtin, N. A. (1993). The efficiency of energy conversion by swimming muscles of fish. *Adv. Exp. Med. Biol.* **332**, 735-743; discussion 744-747.

Woledge, R., Curtin, N. A. and Homsher, E. (1985). *Energetic Aspects of Muscle Contraction*. Toronto: Academic Press.