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THE MECHANICAL PROPERTIES OF FAST AND SLOW SKELETAL MUSCLES OF THE MOUSE IN RELATION TO THEIR LOCOMOTORY FUNCTION

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Summary

The mechanical properties of soleus and extensor digitorum longus (EDL) muscles from the mouse were studied using the work loop technique. Under optimum conditions, the EDL produced a maximum mean power output of 107 W kg^{-1} at a cycle frequency of 10 Hz. In comparison, the maximum mean power output of the soleus was 34 W kg^{-1} at 5 Hz cycle frequency. Video analysis of mice determined the stride frequency range to be from 2.87 Hz at a walk to 8.23 Hz at a flat-out gallop, with the trot-to-gallop transition occurring at 5.89 Hz. *In vivo* EDL electromyogram (EMG) activity is recorded primarily during shortening and the muscle operates in a power-generating mode. The soleus is close to isometric when EMG activity is recorded, but mechanical activity persists into the shortening phase. Both muscles are likely to operate over cycle frequency ranges just below, or at, those yielding maximal power. Soleus and EDL produced maximal power output *in vitro* when operating at mean sarcomere lengths of 2.58 μm and 2.71 μm respectively. These lengths are slightly above the plateau of the length–force curve predicted for rat leg muscle (2.3–2.5 μm). The sarcomere length ranges used *in vivo* by the soleus and EDL were determined, by fixing muscles in the extreme active positions predicted from video and cine analysis, to be 2.28–2.57 μm and 2.49–2.88 μm respectively. These ranges are both close to those shown to yield maximum power output *in vitro* and to the plateau of the sarcomere length–force curve.

Key words: locomotion, mammal, muscle mechanics, oscillatory work, sarcomere length, work loops, mouse.

Introduction

Comparative studies of the properties of mammalian muscle *in vitro* are common, but little has been done to relate these findings to *in vivo* function. Extensor digitorum longus (EDL) and soleus muscles have often been used experimentally as representative fast-twitch and slow-twitch muscles respectively. Histochemical fibre-typing, using myosin ATPase staining techniques, has typically shown mouse EDL to consist wholly of type IIA and type IIB fibres in roughly equal proportions (Haida *et al.* 1989; Klueber *et al.* 1989). In contrast, mouse soleus has been shown to consist of only type I and type IIA fibres in varying proportions from 31 to 41 % type I fibres (Dribin and Simpson, 1977; Lewis *et al.* 1982; Carnwath and Shotton, 1987; Haida *et al.* 1989). This variation in percentage of fibre types found in soleus is likely to be caused by the use of different ages, sexes and strains of mice.

Energetic studies have complemented these findings and shown that mouse EDL has far higher basal rates of glycolysis and glyconeogenesis (Bonen *et al.* 1984, 1990), but a lower basal rate of glycogenesis (Bonen *et al.* 1990) than soleus. Measurements of heat production by mouse EDL and soleus during and after short tetani have given further indications of the differences between these muscles (Leijendekker and Elzinga, 1990). The results suggested that, during recovery after a tetanic contraction, soleus tends to use fat as a substrate rather than glycogen, whilst EDL uses oxidative phosphorylation supplemented by a low level of glycogenolysis.

Mechanical studies have also highlighted differences in the properties of EDL and soleus. Mouse EDL has a faster twitch and a lower fatigue resistance than soleus (Brooks and Faulkner, 1991). During isovelocity contractions, shortening by 10 % of L_0 (the length at which the muscle produces maximal force *in vitro*) at a velocity optimal for power generation, EDL produced a higher power output than soleus during the initial shortening phase, 164 W kg^{-1} compared with 62 W kg^{-1}.

Electromyography (EMG) studies on the rat have demonstrated differences between the EDL and soleus in both the total daily time of activation and the activation per stride. EDL motor units are active for 5–22 % of every 24 h period but soleus motor units are active for 22–35 % of the time (Hennig and Lømo, 1985). During locomotion, EMG activity in rat soleus (an ankle extensor) begins just prior to foot contact with the ground and continues until immediately before the

foot lifts off again (Nicolopoulos-Stournaras and Iles, 1984). In contrast, EDL (a toe extensor) has an unusual pattern of EMG activity, unlike the general patterns observed in flexors or other extensors. EDL EMG activity in the rat begins after the foot has left the ground and continues until just after the foot touches down again.

Measurements of glycogen depletion have been used to estimate the proportion of a muscle and the proportion of each fibre population within a muscle that are active at different locomotory speeds. Such studies have indicated that, in superficial extensor muscles of the cat, most of the force produced during walking is due to the activity of slow oxidative (SO) fibres, with a minimal contribution from fast oxidative glycolytic (FOG) fibres (Smith *et al.* 1977). As speed increases to a slow trot, in the cat and the rat, SO and FOG fibres appear to provide the majority of the force produced by the muscle, with a small contribution by fast glycolytic (FG) fibres (Armstrong *et al.* 1977; Sullivan and Armstrong, 1978). This trend continues with further increases in speed throughout the trot, resulting in FG fibres becoming a more important contributor to the force produced by the muscle. At a slow gallop, SO and FOG fibres again become responsible for the majority of force production, with FG fibres becoming increasingly important as speed increases (Armstrong *et al.* 1977). Force production by the gastrocnemius muscle has consequently been found to vary greatly in the cat, by up to threefold, over a large range of running speeds (Smith *et al.* 1977; Walmsley *et al.* 1978). The soleus appears to be activated to a high degree in the cat, for postural maintenance and throughout all locomotory paces, with little variability in force production (Smith *et al.* 1977; Walmsley *et al.* 1978).

In the present study, we compared the isometric properties of mouse EDL and soleus and measured power output using the work loop technique (Josephson, 1985*a*).

High-speed video recordings of running mice were used as the basis of *in vivo* muscle and sarcomere length strain determinations. As EMG data for the mouse were unavailable, rat EMG data (Nicolopoulos-Stournaras and Iles, 1984; Roy *et al.* 1991) were used to determine locomotory muscle activation patterns. The conditions required to produce optimum power output over a range of cycle frequencies *in vitro* were compared with those believed to be operating *in vivo*.

Materials and methods

Muscle mechanics

Female LACA white mice, between 6 and 8 weeks of age, were killed by cervical dislocation. Extensor digitorum longus (EDL) and soleus muscles were rapidly dissected out in frequently changed, oxygenated Ringer's solution (composition in mmol l^{-1}: NaCl, 144; sodium pyruvate, 10; KCl, 6; $MgCl_2$, 1; NaH_2PO_4, 1; $MgSO_4$, 1; Hepes, 10; $CaCl_2$, 2; pH 7.4 at 21 °C) then immersed in fresh oxygenated Ringer's solution. For mechanical experimentation, muscles were placed in a flow-through chamber containing oxygenated Ringer maintained at 35±0.5 °C (pH 7.2). The muscles were attached using small aluminium foil clips to an isometric force transducer (AME 801, SensoNor, Horten, Norway) at one end and to a servomotor at the other end.

Muscles were supramaximally stimulated with 1 ms pulses *via* parallel platinum wire electrodes. Muscle length was adjusted to obtain maximal isometric twitch height; this length was defined as L_0 and corresponded to the length yielding maximum tetanic force. Twitch kinetics and the stimulation frequency yielding maximum tetanic force were determined under isometric conditions. The muscle preparation was then subjected to sinusoidal length changes using a constant strain amplitude of ±5 % fibre length, symmetrical about L_0, whilst being phasically stimulated. The stimulation phase shift (phase shift is expressed in degrees, where 0 ° denotes muscle at L_0 and lengthening, and a complete strain cycle is 360 °), and the number of stimuli used was systematically altered at each cycle frequency until the conditions required for maximal power output were established. The muscle was subjected to four oscillatory work cycles per run with a rest of 7 min between runs. A set of control stimulation and oscillation variables, corresponding to those yielding maximal power output from the muscle, were used for every third or fourth run to monitor and allow correction for deterioration in the ability of the muscle to produce power, assuming a linear decline in power output with each run. At low cycle frequencies, where decline in power output was greatest, every other run was used as a control. Changes in force production and displacement of the muscle were controlled, monitored and analysed on-line by a PC microcomputer using in-house software. Work loops were generated to allow determination of muscle power output (Josephson, 1985*a*; Altringham and Johnston, 1990*a*).

Preliminary experiments revealed that, for both muscles, a strain amplitude of ±5.0 %L_0 was nearly optimal for power output over the range of cycle frequencies yielding peak power output. This constant strain amplitude was then used whilst the number of stimuli and their phase shift with respect to the strain cycle were varied to determine the conditions required for maximal power output over a range of cycle frequencies. The effects of optimising strain amplitude at different cycle frequencies and the effects of changing starting length (defined as L_s) were also investigated.

These mechanical experiments were then repeated on muscle without the use of electrical stimulation, to determine the importance of work done against passive elements in the muscle.

Muscle length to fibre length ratios were determined for EDL and soleus after fixation, using 4 % formalin (v/v) in mammalian Ringer, at the length which yielded maximal twitch height.

Kinematics

Video recordings were made of running mice, at 200 frames s^{-1}, to determine the range of stride frequencies and the associated ranges of limb positions used during

locomotion. As a comparison, the limb positions used during locomotion in the rat were determined from high-speed cine film (provided by R. McN. Alexander). As the limb positions used during locomotion by the rat and mouse were essentially the same, and as EMG data for the mouse were unavailable, EMG data from the rat (Nicolopoulos-Stournaras and Iles, 1984) were used to determine when, during the stride, the soleus and EDL are likely to be active. This assumes geometric scaling between mouse and rat, a reasonable assumption (Alexander, 1989).

Sarcomere length at L_0 was determined for soleus and EDL. Both muscles were prepared for isometric study as described earlier. Muscle length was then adjusted to give maximal twitch height and, in some cases, this length was checked by optimisation for maximal tetanus height. Muscle length was measured, and each muscle was removed and fixed at this optimised muscle length, Ringer being replaced with 4 % formalin (v/v) in Ringer. Muscles were fixed overnight, before subsequent dissection of two bundles of fibres from each muscle, which were set in glycerol. Sarcomere length was then determined from the laser diffraction pattern observed using a HeNe laser. Page and Huxley (1963) have shown that sarcomere length does not change in muscles held isometrically during fixation.

$$\text{Sarcomere length} = \lambda/\sin\phi,$$

where ϕ is the angle subtended by the zero- and first-order diffraction lines and λ is wavelength of laser radiation used (0.6328 μm). The ranges of sarcomere lengths used by soleus and EDL when active during locomotion were also determined. Hind limbs of recently killed mice were held in the positions, determined from video analysis, that corresponded to the shortest and longest active muscle lengths during locomotion. When the muscles had gone into rigor, they were fixed and sarcomere length was determined as outlined above.

The muscle length change (strain) waveform used during locomotion was initially estimated by placing limbs into the positions observed during video analysis and measuring passive muscle length. Further analysis showed that the muscle length change waveform obtained showed a high degree of correlation (r^2 values of 0.9 and 0.8 for soleus and EDL respectively) with the change in ankle angle. Ankle angles were determined for at least five pairs of adjacent strides, from two or three different mice, for each gait. Unless stated otherwise, the results are presented as mean ± S.E.M. (number of observations). The ankle angles measured from video analysis were subsequently used to relate sarcomere length to the extreme limb positions used during locomotion, to enable the sarcomere length changes for the limb cycle to be predicted from ankle angle.

Results

Muscle mechanics

The isometric studies demonstrated marked differences in contraction kinetics between soleus and EDL. EDL had much shorter activation and relaxation times than soleus, with both muscles producing a stress of about 230 kN m^{-2} (Table 1). Stimulation frequencies for maximum force were 130 Hz for soleus and 200 Hz for EDL, and all stimuli used in the work loop studies were delivered at these rates. Using the work loop technique, at all but the lowest cycle frequencies, work decreased from the first to the second cycle and then remained constant throughout more than 12 successive cycles for EDL and 20 successive cycles for soleus. The third cycle per run was taken to be representative of the muscle's ability to produce power. Work per cycle produced by soleus at 1 Hz cycle frequency typically decreased by 3.5 % from the first to the second cycle and decreased by 7.0 % from the first to the fourth cycle. At 2 Hz and 4 Hz cycle frequencies, the work per cycle produced by EDL typically decreased by 6.5 % from the first to the second cycle and then remained stable throughout successive cycles. At these low cycle frequencies, the first loop per run was taken as representative of the real capacity of the muscle to produce work prior to the onset of fatigue due to these long tetani.

Muscle preparations were discarded if power output decreased to below 80 % of its starting value. In those experiments where power did decline appreciably, the power output and cycle frequency relationship, loop shape, optimum strain and stimulation variables did not change. The only variables affected were stress and power output. Deterioration of muscle was found to be dependent on the level of work performed in a run, such that runs at low cycle frequency

Table 1. *A comparison of some properties of the extensor digitorum longus (EDL) and soleus muscles*

Property	Soleus	EDL
Mouse body mass (g)	25.79±0.87 (16)	24.76±0.64 (19)
Muscle mass (mg)	6.48±0.47 (13)	7.16±0.34 (18)
Muscle fibre length (mm)	9.13±0.25 (16)	9.05±0.23 (19)
Time to peak twitch force (ms)	22.14±0.53 (16)	9.58±0.28 (18)
Time to 90 % twitch relaxation (ms)	97.65±3.53 (16)	41.03±2.35 (18)
Maximum stress (kN m^{-2})	224.1±10.8 (13)	233.3±7.70 (13)
Maximum power output (W kg^{-1})	34.02±2.11 (13)	107.2±3.00 (12)

Values are means ± S.E.M. (*N*).

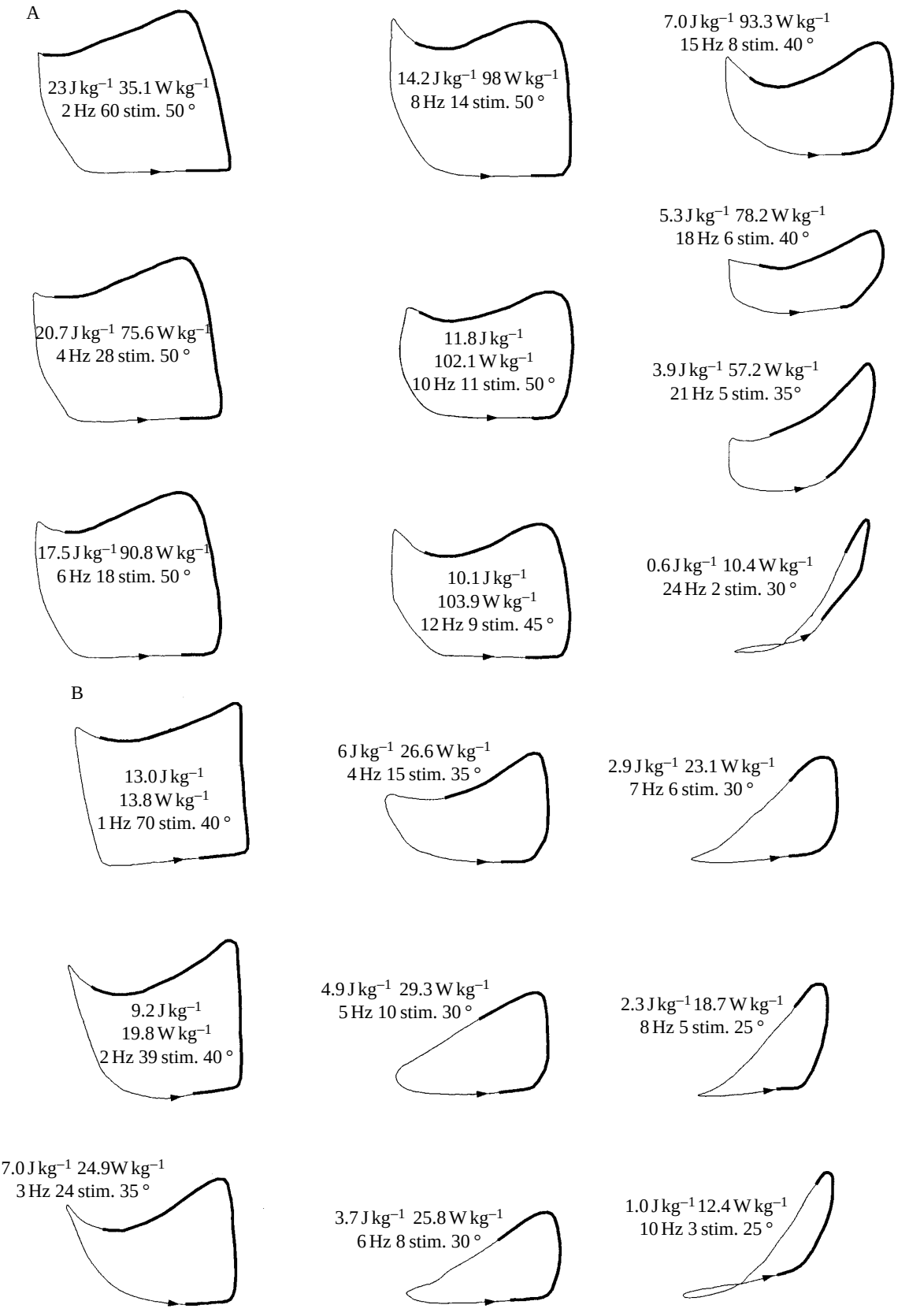

Fig. 1. The effects of cycle frequency on (A) EDL and (B) soleus work loop shape, using constant ±5 %L_0 strain amplitude under conditions for maximal power output. The period of stimulation is shown by the thickened line on each work loop. In each cycle, the work done and the mean power output of all muscles studied are shown. Also given are cycle frequency, number of stimuli and phase shift (in degrees). Arrow shows direction of loop.

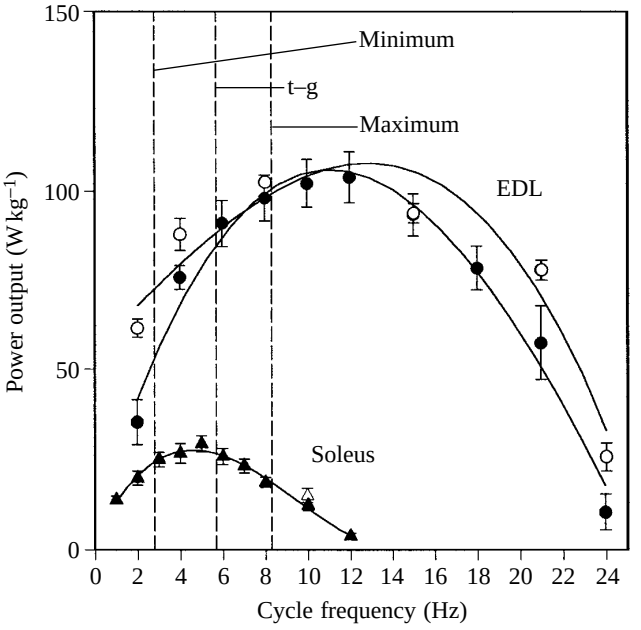

Fig. 2. Effect of cycle frequency on power output. EDL is represented by circles, soleus by triangles. Filled symbols show constant (±5 %) strain, and open symbols show strain optimised at each frequency. The lines are third-order polynomials fitted to the data using a least-squares regression. The vertical lines indicate the limits of the *in vivo* limb cycle frequency range and the trot-to-gallop transition (t–g). Values are means ± S.E.M. Soleus, N>4; EDL, N=4.

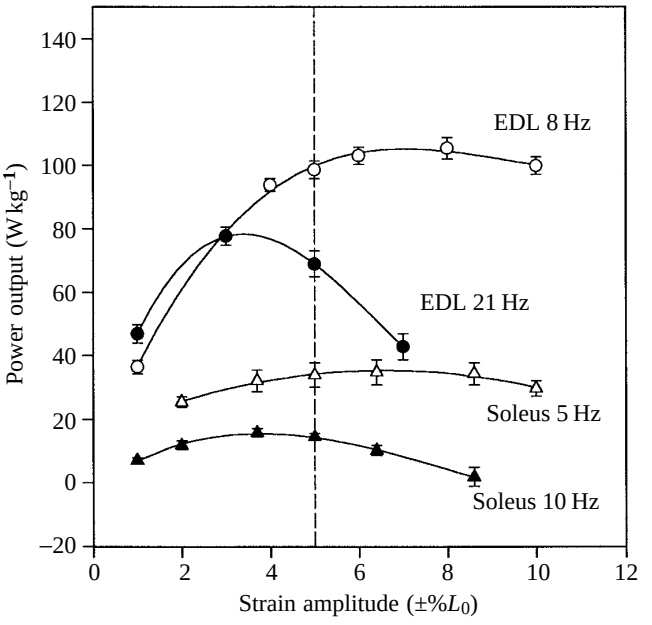

Fig. 3. Effects of strain amplitude on power output of EDL and soleus at different cycle frequencies. The lines are third-order polynomials fitted to the data using a least-squares regression. Values are means ± S.E.M. (N=4).

caused a more rapid decrease in the ability of a muscle to produce power.

The number of stimuli for optimum power output decreased with increasing frequency, from 70 at 1 Hz to 2 at 12 Hz for soleus and from 59 at 2 Hz to 2 at 24 Hz for EDL. Phase also decreased over the same frequency ranges: for soleus, from 40 to 25 °; for EDL, from 50 to 30 °. These results are summarised in Fig. 1.

Over a broad range of cycle frequencies using constant strain amplitude (±5 %L_0), EDL consistently produced a higher maximum power than soleus (Fig. 2). Power output was found to be strongly dependent on cycle frequency: with increasing cycle frequency, power ouput rose to a broad peak before it decreased again. Peak power output for EDL was at cycle frequencies of 10–12 Hz, whereas peak power output for soleus was at 4–6 Hz.

Optimal strain amplitude decreased with increasing cycle frequency. At cycle frequencies corresponding to maximal power output, a broad range of strain amplitudes yielded near maximal power output (Fig. 3). At higher cycle frequencies, variation in strain amplitude had a more marked effect on muscle power output, with optimum strain amplitude decreasing to approximately ±4 %L_0 at cycle frequencies of 15 Hz and 10 Hz for EDL and soleus respectively. Strain optimisation resulted in a slight flattening of the power output against cycle frequency curve, relative to that produced using constant strain, but peak power output was produced at the same cycle frequency (Fig. 2).

At low cycle frequencies, power output increased with increasing strain amplitude; however, a point was reached where the strain was so great that power output could not be sustained, owing to muscle damage. This typically occurred at strains above ±10 %. Power output of EDL at 2 Hz cycle frequency was highest at a strain amplitude of ±12 %L_0 (10 % greater than at ±9 %L_0), but this resulted in damage to the muscle and a subsequent drop in power output.

Starting lengths (L_s) greater or less than L_0 resulted in a marked reduction in power output in both EDL and soleus (Fig. 4).

The work done on passive muscle under imposed cyclical length changes was only 1–2.5 % in EDL and less than 4 % in soleus of the work done by active muscle. Passive work done on EDL and soleus only became substantially higher when L_s was increased, the passive work then becoming about 20 % of the work done by active muscle at an L_s 20 % above L_0, or when the strain amplitude was far higher than that producing maximum power output (Fig. 5). The size of the passive loops increased with length change velocity, i.e. with increases in both cycle frequency and strain amplitude.

Kinematics

A stride frequency range of 2.87±0.09 Hz (N=5) for a slow walk to 8.23±0.07 Hz (N=3) for a flat-out gallop was determined for running mice of 23.7±0.76 g body mass (N=9). The trot–gallop transition occurred at a stride frequency of 5.89±0.10 Hz (N=7).

Soleus and EDL were found to have slightly different

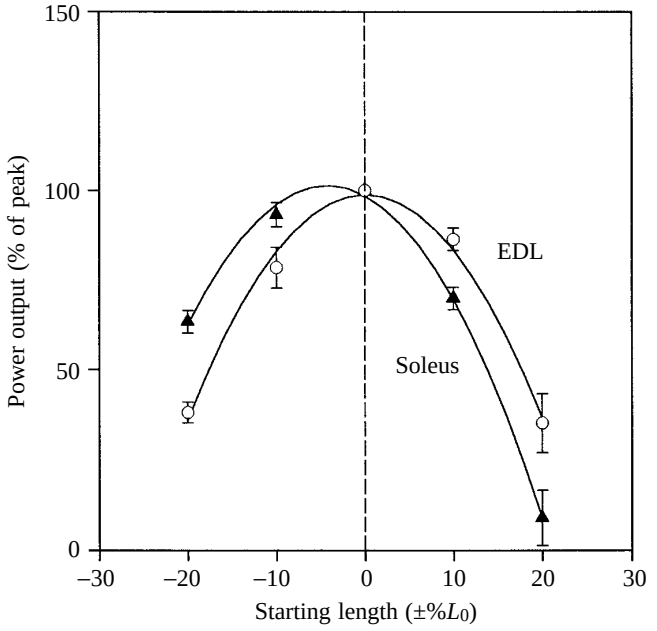

Fig. 4. Effects of starting length variation on power output for soleus (triangles, N=4) and EDL (circles, N=3), with all other conditions optimised for peak power output. The lines represent second-order polynomials fitted to the data by a least-squares regression. Values are means ± S.E.M. All data are normalised to a peak power output of 100 %.

sarcomere lengths at L_0 of 2.58±0.07 and 2.71±0.04 μm respectively (Fig. 6). The sarcomere length ranges determined for soleus and EDL when active during locomotion were found to be close to the values determined for L_0, 2.28±0.04 to 2.57±0.03 μm and 2.49±0.07 to 2.88±0.09 μm respectively (Fig. 6). The strain waveforms varied slightly with changes in gait, but were essentially the same as those shown for the trot (Fig. 7).

EDL and soleus fibre lengths were determined to be 75 % and 85 %, respectively, of their muscle length (tendon to tendon) measurement, values similar to those reported by Crow and Kushmerick (1982) and Leijendekker and Elzinga (1990).

Discussion

Basic in vitro *mechanical properties*

Soleus and EDL both produced similar maximal isometric stress values of 224 and 233 kN m^{-2} respectively; these values are in close agreement with the values of 215 and 219 kN m^{-2} found for mice by Leijendekker and Elzinga (1990) and 245 and 237 kN m^{-2} determined by Brooks and Faulkner (1991). The twitch kinetics determined for EDL and soleus are also similar to kinematics quoted in the literature. The maximal power outputs determined using the work loop technique (107 and 34 W kg^{-1} for EDL and soleus respectively) are lower than those determined from isovelocity releases by Brooks and Faulkner, 1991; 164 and 67 W kg^{-1} for EDL and soleus respectively). However, *in vivo* power output is likely to be overestimated by isovelocity contractions of maximally activated muscle. *In vivo,* muscles are only active for part of each locomotory cycle and spend about half of the cycle being stretched during the periods of inactivity between the power-generating shortening phases. Muscle power output is determined by the level of work per cycle that a muscle can produce and is dependent on cycle frequency, maximal stress and the force–velocity relationship. EDL would therefore be expected to produce a higher power output than soleus as its faster activation and relaxation times enable it to produce similar levels of work to soleus but at higher cycle frequencies.

Fig. 8 shows the maximum power output for EDL and soleus in relation to reported values of maximum power output derived using the work loop technique. The power output values were determined at the normal working temperature of the muscle and at the cycle frequency for which power output was maximal. Three-dimensional plots have been used to illustrate the marked interdependence between temperature and cycle frequency, with a trend towards increased power output with increased temperature and cycle frequency.

The power output of both EDL and soleus was influenced by a number of factors. The number of stimuli for maximum power output decreased with increasing cycle frequency, and the phase shift between length change and onset of stimulation also decreased. Strain amplitude decreased with increasing cycle frequency, but the effects were small at all but the highest and lowest frequencies used: a near optimum power output against cycle frequency curve was obtained at a constant ±5 % strain. The effects of these variables on power output were similar to those observed and discussed in previous studies (e.g. Josephson, 1985*a*; Altringham and Johnston, 1990*a*,*b*).

At high cycle frequencies, the ability of EDL and soleus to maintain force during shortening was reduced as a result of constraints imposed by the force–velocity relationship, resulting in a decrease in the work done per cycle. Shortening deactivation, which is thought to result from a transient decrease in the affinity of troponin C for calcium (Ekelund and Edman, 1982), may also be a contributing factor. Increased cycle frequency therefore results in increased power output until an optimum is reached; thereafter, the decrease in the work done per cycle counteracts the gain made by the increased frequency.

Optimum strain amplitude for both muscles was found to decrease as cycle frequency increased. For a given cycle frequency, decreasing the strain amplitude results in a corresponding reduction in shortening velocity. The deleterious effects of increasing velocity, with increasing cycle frequency, can therefore be counteracted by reducing strain amplitude. However, reducing strain amplitude also reduces work, so there will be an optimum strain at each cycle frequency. These effects of strain are similar to those reported for crab scaphoganite levator muscle (Josephson and Stokes, 1989).

Optimisation of strain amplitude had little effect on the overall shape of the power output against cycle frequency curves, relative to using a constant ±5 %L_0, with the frequency

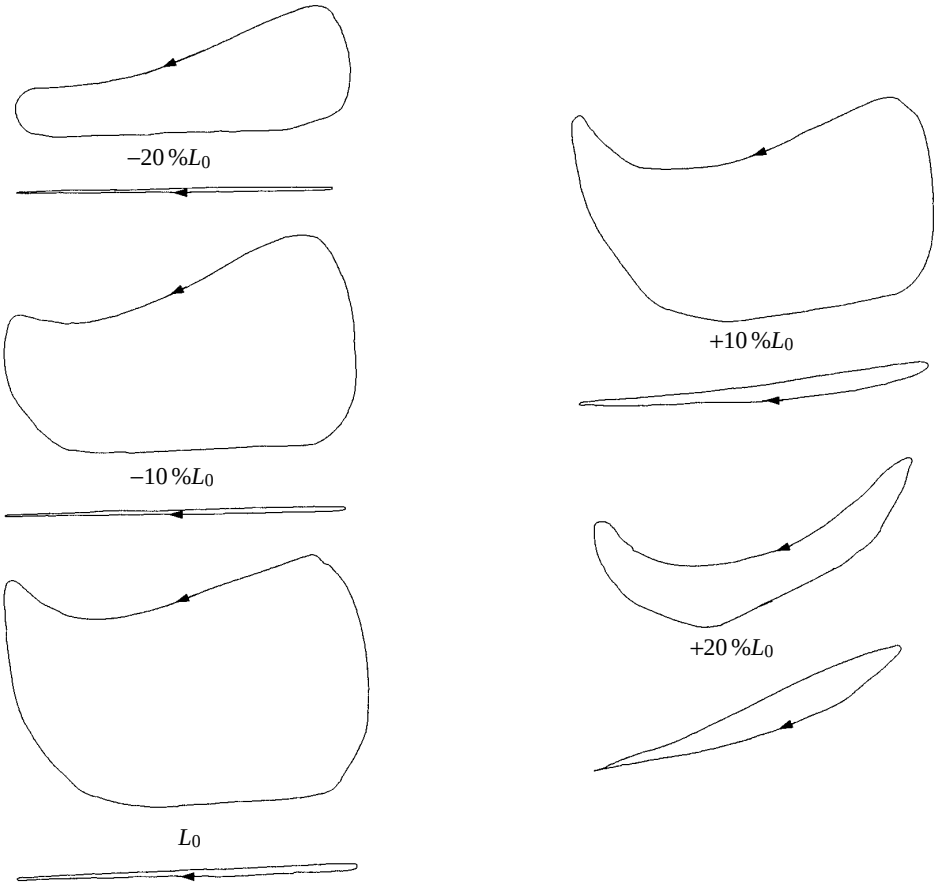

Fig. 5. The effect of starting length on work loop shape, with counterclockwise active work loops and clockwise passive (unstimulated) work loops, of EDL at 8 Hz cycle frequency with 14 stimuli and a phase shift of 50 °. Lengths are shown as %L_0.

for peak power remaining unchanged. Strain optimisation did, however, increase power output at cycle frequencies above and below those that produced peak power output, causing the curve to become slightly more flattened.

The low level of work done against passive elements was of a similar magnitude to that found in rat gastrocnemius and diaphragm muscles (Heerkens *et al.* 1987; Syme, 1990). Passive viscoelastic work only became substantial when the starting length of the muscle was increased, work absorbed becoming about 20 % of the work done at an L_s 20 % above L_0, or when the strain amplitude was far higher than that optimal for power output. Work done against passive viscoelastic elements was consequently ignored in any determinations of muscle power output at L_0.

Variation in the starting length (L_s) of soleus and EDL caused a marked reduction in power output (Fig. 4). As L_s was increased above L_0, the passive forces in the muscle increased, as would be predicted from previous reports (Wells, 1965; Altringham and Bottinelli, 1985). With increased passive forces in muscle there is a resultant decrease in power output due to the greater resistance to muscle lengthening (Heerkens *et al.* 1987). The increases in passive forces were also accompanied by an increase in the contraction time of the muscle, a phenomenon previously observed in crab muscle (Josephson and Stokes, 1989), and a consequent reduction in the work done per cycle.

How much work can a muscle do?

The maximum work that should be produced by a muscle in a single cycle has been calculated by Alexander (1992) to be 70 J kg^{-1}, using the following equation:

$$\text{Maximum work (per unit mass)} = \frac{\text{maximum stress} \times \text{maximum normal } \textit{in vivo} \text{ strain}}{\text{muscle density}} .$$

A number of assumptions were made: maximum normal *in vivo* strain amplitude is ±12.5 %, muscle activation is instantaneous and maximal, muscle relaxation is instantaneous, there is no decline in force during muscle shortening and the muscle exerts a maximum stress of 300 kN m^{-2}.

The maximum work produced by mouse EDL in a single cycle was 32 J kg^{-1} using an optimised strain amplitude of ±12 %. Why is there a discrepancy between the observed and the predicted work done per cycle? First, the muscle does not activate and relax instantaneously, so that for part of the work-producing phase of the cycle it is only partially active. Second, at a cycle frequency of 2 Hz and a strain amplitude of ±12 % (the measured optimum strain in our experiments, and the maximum strain Alexander predicts to be used in normal movement), force declines during shortening as a result of the effects of the force–velocity relationship and shortening deactivation. At cycle frequencies lower than 2 Hz, EDL

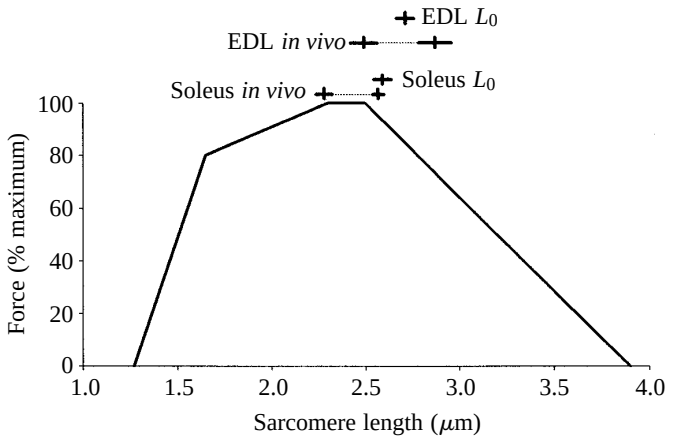

Fig. 6. The sarcomere length–force curve for rat limb muscle (solid line, Page and Huxley, 1963). Also given are sarcomere lengths (mean ± S.E.M.) of L_0 values determined in this study from isometric twitches and tetani for EDL and soleus (these also correspond to the lengths for maximum power output), and the maximum and minimum lengths determined during locomotion for EDL and soleus.

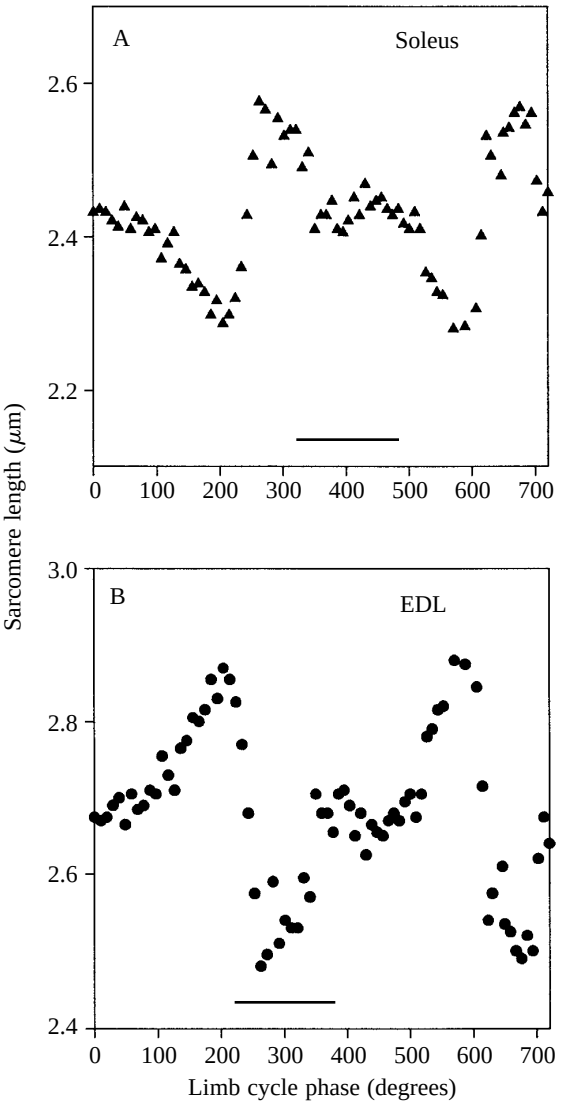

Fig. 7. (A) Sarcomere length changes determined from the ankle angle at a trot over two strides for soleus. Limb cycle phases of 0 and 360° correspond to limb touchdown. (B) Sarcomere length changes determined from the ankle angle at the trot for EDL (two subsequent strides). Limb cycle phases of 0 and 360° correspond to limb touchdown. The bars show EMG timing from Nicolopoulos-Stournaras and Iles (1984).

rapidly fatigues, and at lower strain amplitudes work output drops. Finally, maximum stress is only achieved under isometric conditions. Stress decreases with increasing cycle frequency from 87% of the maximal experimental isometric stress at 2 Hz to 52% at 24 Hz. The measured highest work value of 32 J kg^{-1} can be increased to 42 J kg^{-1}, by assuming that activation is instantaneous and that force does not decrease during shortening, and to 48 J kg^{-1} if isometric stresses are used. If Alexander's maximal stress value of 300 kN m^{-2} is also included, rather than the measured 233 kN m^{-2}, the theoretical maximal work value of 70 J kg^{-1} is reached.

Effects of fibre type heterogeneity

These experiments were carried out on whole soleus and EDL muscles. Whilst these can be described as slow and fast twitch muscles, respectively, neither is homogeneous with respect to fibre type (EDL consisting of type IIA and IIB fibres in roughly equal proportions; soleus consisting of 31–41% type I fibres, the remainder being type IIA). Preparations of a single fibre type have yet to be isolated from these muscles, and practical considerations make the whole muscle a more appropriate preparation.

Martin *et al.* (1988) studied the variation in activity of marker enzymes for oxidative (succinate dehydrogenase, SDH) and glycolytic (α-glycerophosphate dehydrogenase, α-GDP) metabolism in the cat tibialis anterior muscle. A comparison was made between whole muscle and single motor unit variation. Interfibre coefficients of variation were calculated, based on the mean and standard deviation, for single motor units; these were 55% and 81% (SDH and α-GDP respectively) of those for whole muscle, indicating substantial fibre type heterogeneity within motor units (but see Nemeth *et al.* 1986, using different enzymes). This observation implies that caution should be exercised in the interpretation of histochemical and biochemical results to determine the functional properties of a muscle. These findings certainly question the need for preparations of a single fibre type in experiments aimed at studying *in vivo* function.

Sarcomere length in vivo

Variation in starting length, L_s, will change the degree of overlap between thick and thin filaments, altering the range of sarcomere lengths over which the muscle is operating and affecting the ability of the muscle to produce force (Gordon *et al.* 1966; Altringham and Bottinelli, 1985). The thick filament length of 1.6 μm taken from rat muscle (Page and

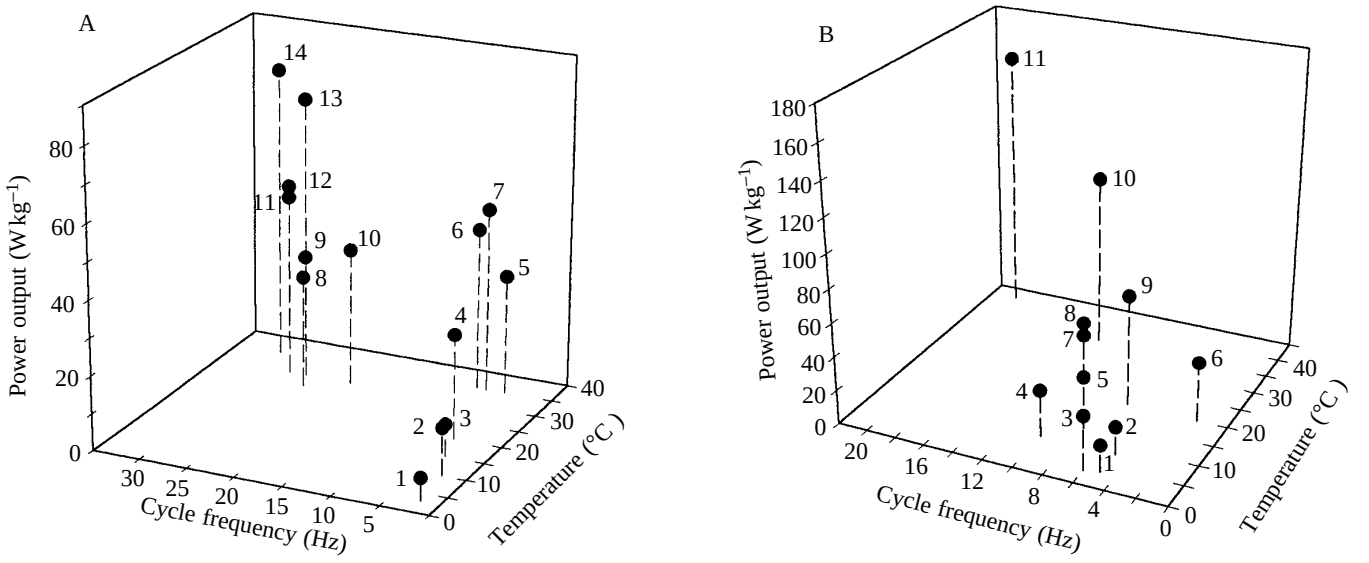

Fig. 8. (A) Relationships between *in vitro* maximal power output, *in vitro* cycle frequency for maximal power output (unless otherwise stated) and temperature of aerobic muscle. (1) Sculpin (*Myoxocephalus scorpius*) slow myotomal muscle (Altringham and Johnston, 1990*a*). (2) Scup (*Stenotomus chrysops*) red muscle under *in vivo* operating conditions (Rome and Swank, 1992). (3) Crab (*Carcinus maenas*) respiratory muscle (Stokes and Josephson, 1988). (4) Scup (*Stenotomus chrysops*) red muscle (Rome and Swank, 1992). (5) Mouse (*Mus musculus*) soleus (this study). (6) Rat (*Rattus norvegicus*) diaphragm (Altringham and Young, 1991). (7) Mouse (*Mus musculus*) diaphragm (Altringham and Young, 1991). (8) Rhinoceros beetle (*Orycetes rhinoceros*) (Machin and Pringle, 1959). (9) Tettigoniid (*Neoconocephalus triops*) flight and singing muscle (Josephson, 1985*b*). (10) Locust (*Schistocerca americana gregaria*) flight muscle under *in vivo* operating conditions (Mizisin and Josephon, 1987). (11) Locust (*Schistocerca americana gregaria*) flight muscle (Mizisin and Josephson, 1987). (12) Locust (*Schistocerca vitens*) flight muscle (Mizisin and Josephson, 1987). (13) Tettigoniid (*N. triops*) flight muscle (Josephson, 1985*a*). (14) Tobacco hawkmoth (*Manduca sexta*) flight muscle (Stevenson and Josephson, 1990). (B) Relationships between *in vitro* maximal power output, *in vitro* optimal cycle frequency and temperature of anaerobic muscle. (1) Cod (*Gadus morhua*) fast myotomal muscle (Moon *et al.* 1991). (2) Cod, 60 cm body length (Anderson and Johnston, 1992). (3) Sculpin fast myotomal muscle (Altringham and Johnston, 1990*a*). (4) Cod, 10 cm body length (Anderson and Johnston, 1992). (5) Saithe fast myotomal muscle from 0.65 body lengths (Altringham *et al.* 1993). (6) Frog (*Rana pipiens*) sartorius (Stevens, 1988). (7) Saithe (*Pollachius virens*) fast myotomal muscle from 0.5 body lengths (Altringham *et al.* 1993). (8) Saithe fast myotomal muscle from 0.35 body lengths (Altringham *et al.* 1993). (9) African clawed toad (*Xenopus laevus*) sartorius (T. Morris, R. S. James and J. D. Altringham, in preparation). (10) Mouse EDL. (11) Desert iguana (*Dipsosaurus dorsalis*) (Swoap *et al.* 1993).

Huxley, 1963) is likely to be correct because lengths have been found to vary little between frog (Page and Huxley, 1963), tortoise (Page, 1968), rabbit psoas (Page and Huxley, 1963), rhesus monkey and human leg muscles (Walker and Schrodt, 1973). Thin filament lengths are more variable, ranging from 2.64 μm in human EDL, sartorius and gastrocnemius to 2.41 μm in rhesus monkey EDL, sartorius and gastrocnemius (Walker and Schrodt, 1973) and 2.24 μm in rabbit psoas (Page and Huxley, 1963). Page and Huxley (1963) obtained a value of 2.30 μm for rat leg muscle, assuming that actin periodicity is the same in all animals. For the purpose of this study, this value from rat leg muscle was used as it was thought to be the best estimate of thin filament length in mouse EDL and soleus.

The sarcomere lengths measured in this experiment for soleus and EDL at L_0 (Fig. 6) indicate that, during these *in vitro* studies, EDL is operating at lengths slightly above the plateau of the rat muscle sarcomere length–force curve, but soleus is operating at lengths corresponding to the plateau of the rat muscle sarcomere length–force curve. The length at which EDL produces maximal force corresponds to the length for maximal power output (Fig. 4) and the length at the middle of the *in vivo* range (Fig. 6). However, although the length at the middle of the *in vivo* range in soleus does correspond to the

Table 2. *Strain amplitudes and* V/V_{max} *ranges for soleus and extensor digitorum longus (EDL) at different gaits used by the mouse*

	Strain amplitude (% mean length)	V/V_{max} range
Soleus		
Walk	±4.23 (±0.24, *N*=2)	0.10–0.13
Trot	±5.40 (±0.41, *N*=4)	0.20–0.31
Gallop	±6.05 (±0.75, *N*=3)	0.34–0.48
EDL		
Walk	±5.46 (±0.44, *N*=2)	0.12–0.15
Trot	±6.53 (±0.41, *N*=4)	0.24–0.39
Gallop	±7.26 (±0.76, *N*=3)	0.37–0.52

Values are means (±S.E.M., *N*).
See text for further details.

length found *in vitro* for maximal power production, it is 95 % of the *in vitro* length for maximal force production.

Other studies have also shown that the ranges of sarcomere lengths used during locomotion correspond to the plateau region of the relevant length–force curves; for example, carp muscle during swimming (Rome and Sosnicki, 1991), rabbit leg muscles during the gallop (Dimery, 1985), human thigh muscles during the walk (Cutts, 1989) and bird wing muscles during flight (Cutts, 1986).

It seems highly unlikely that the sarcomere lengths for EDL, when optimised for maximal isometric force production, would correspond to anything other than points on the plateau of the sarcomere length–force curve. It is therefore possible that the actin filaments in the mouse EDL are longer than in rat leg muscle.

Effects of tendon elasticity

In determining *in vivo* sarcomere length changes, we had to consider the effects of tendon elasticity. A comparison of the dimensions of kangaroo rat tendons with those of kangaroos and wallabies (Biewener *et al.* 1981) suggests that leg tendon elasticity in small mammals due to the stresses involved in locomotion is low, resulting in tendon length changes of less than 5 %. However, in the present study, precautions were still taken to minimise errors due to tendon extension. For work loop experiments, the aluminium clips were attached as near to the muscle as possible; therefore, any measurements of strain amplitude would involve negligible tendon elasticity error.

James *et al.* (1995) demonstrate that, when tendon compliance is accounted for in *in vitro* measurements of sarcomere length, shorter sarcomere lengths are predicted when the tendon is under stress. However, the muscle length change pattern remains largely unaltered and the greatest error in sarcomere length of 0.01 μm (less than 0.13 % of mean sarcomere length) occurs briefly at high stress, late in the muscle shortening phase. Tendon compliance would therefore have no significant effect on the results or interpretation.

Relating in vitro *properties to* in vivo *muscle function*

The 2.87–8.23 Hz stride frequency range observed in the mouse is comparable to the range of 3.0–8.1 Hz previously reported by Heglund *et al.* (1974) for a 30 g mouse. Their value of 5.8 Hz for the trot–gallop transition is similar to our observed value of 5.89 Hz.

When *in vitro* power output is related to *in vivo* function, a number of important relationships emerge. Stride frequencies below the trot–gallop transition (2.87–5.89 Hz) correspond to the *in vitro* cycle frequencies over which soleus produces 85–100 % of maximal power output. Over the entire range of stride frequencies observed, 2.87–8.23 Hz, soleus produces between 60 and 100 % of its maximal *in vitro* power output. Peak muscle efficiency was found to occur in frog sartorius muscle over a range of V/V_{max} values of 0.23–0.35 (Kushmerick and Davies, 1969; Woledge *et al.* 1985). The maximum shortening velocities have been determined using after-loaded isotonic contractions to be 4.8 and 10.4 fibre lengths s^{-1} for mouse soleus and EDL respectively (Brooks and Faulkner, 1988). Therefore, *in vivo* soleus operates within the above V/V_{max} range cited above (V determined as average velocity during shortening) at the trot (Table 2). Brooks and Faulkner (1988) determined that soleus produces maximal power output between the V/V_{max} values of 0.25 and 0.29, which are also within the range determined for the mouse at the trot.

As running speed increases, faster motor units are gradually recruited, and with increasing speed the pattern of muscle recruitment spreads through the muscle group from the deep soleus outwards, through the plantaris, to the superficial gastrocnemius (Armstrong, 1981). This pattern of activity is reflected by the fibre types of these three muscles in the rat, with a predominance of first fast oxidative glycolytic (FOG) then fast glycolytic (FG) fibres from soleus to gastrocnemius (Armstrong and Phelps, 1984). With increased running speed, the fraction of the stride for which the foot is on the ground decreases so that the feet must exert larger forces on the ground to maintain the average level of force needed to match the body weight (Alexander, 1985). Therefore, with increasing speed, a greater muscle mass would need to be active to produce the necessary force.

The integrated EMG (IEMG) activity of soleus, corresponding to the level of muscle activity, has been shown to decrease with increasing running speed in the rat, whereas the IEMG for the medial gastrocnemius remains relatively constant (Roy *et al.* 1991). However, when the severity of exercise is increased, by inclining the treadmill, the IEMG of gastrocnemius increases with running speed. It is therefore probable that, as running speed increases, the actions of the plantaris and gastrocnemius become more important because they are likely to produce maximal power at higher stride frequencies than the soleus.

Under conditions corresponding to the upper stride frequency range, EDL works at frequencies approaching those yielding maximal power output. However, the strain waveform determined for EDL *in vivo* is such that EDL effectively shortens at approximately twice the velocity experienced during a sinusoidal waveform (Fig. 7B). This rapid shortening enables EDL to shorten with a V/V_{max} range during the trot (0.24–0.39) that would correspond both to the range predicted for maximal efficiency 0.23–0.35 (Kushmerick and Davies, 1969; Woledge *et al.* 1985), and to the range of 0.24–0.32 determined for maximal *in vitro* power output for EDL (Brooks and Faulkner, 1988).

Limb positions measured in the mouse during locomotion, from analysis of video recordings, were found to be very similar to those determined for the rat from analysis of high-speed film. It was therefore assumed that rat EMG data would give a reasonable indication of the EMG activity in the mouse during the same gaits.

When the muscle strain waveforms for EDL and soleus during locomotion are related to EMG data (Nicolopoulos-Stournaras and Iles, 1984), both EDL and soleus are found to

be active primarily during shortening (Fig. 7). It should be remembered that mechanical activity will be delayed slightly relative to EMG activity. In larger mammals, such as the dog, extensors have been shown typically to undergo active stretch prior to shortening (Tokuriki, 1974; Goslow *et al.* 1981). This active stretch is thought to be important in the role of elastic storage of energy. However, in small mammals, tendon elasticity has been shown to be low (Biewener *et al.* 1981), suggesting that elastic storage of energy would be minimal. The soleus, when active, initially shortens, then undergoes a nearly isometric phase before further shortening. This nearly isometric phase corresponds to the period just after touchdown of the limb, when the soleus is likely to be involved in limb stabilisation.

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