

RESEARCH ARTICLE

The effects of asymmetric length trajectories on the initial mechanical efficiency of mouse soleus muscles

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Accepted 19 October 2011

SUMMARY

Asymmetric cycles with more than half of the cycle spent shortening enhance the mechanical power output of muscle during flight and vocalisation. However, strategies that enhance muscle mechanical power output often compromise efficiency. In order to establish whether a trade-off necessarily exists between power and efficiency, we investigated the effects of asymmetric muscle length trajectories on the maximal mechanical cycle-average power output and initial mechanical efficiency (E_i). Work and heat were measured *in vitro* in a mouse soleus muscle undergoing contraction cycles with 25% (Saw25%), 50% (Saw50%) and 75% (Saw75%) of the cycles spent shortening. Cycle-average power output tended to increase with the proportion of the cycle spent shortening at a given frequency. Maximum cycle-average power output was $102.9 \pm 7.6 \text{ W kg}^{-1}$ for Saw75% cycles at 5 Hz. E_i was very similar for Saw50% and Saw75% cycles at all frequencies (approximately 0.27 at 5 Hz). Saw25% cycles had E_i values similar to those of Saw50% and Saw75% cycles at 1 Hz (approximately 0.20), but were much less efficient at 5 Hz (0.08 ± 0.03). The lower initial mechanical efficiency of Saw25% cycles at higher frequencies suggests that initial mechanical efficiency is reduced if the time available for force generation and relaxation during shortening is insufficient. The similar initial mechanical efficiency of Saw50% and Saw75% cycles at all frequencies shows that increasing the proportion of the contraction cycle spent shortening is a strategy that allows an animal to increase muscle mechanical power output without compromising initial mechanical efficiency.

Key words: length trajectory, work loop, skeletal muscle, power output, initial efficiency, activation, heat production, locomotion.

INTRODUCTION

Muscle length trajectory is a major determinant of muscle mechanical power output (Josephson, 1985; Altringham and Johnston, 1990; Askew and Marsh, 1997; Girgenrath and Marsh, 1999). In many activities requiring a high power output, the muscle's length trajectory is asymmetrical, with more than half of the cycle duration spent shortening. For example, length trajectories where the proportion of time spent shortening is 65–75% of the cycle have been observed in the vocalisation muscles of hylid frogs (Girgenrath and Marsh, 1997), the flight muscles of some birds during take-off and slow flight (Askew et al., 2001; Ellerby and Askew, 2007) and the swimming muscles of some species of scallops (Marsh et al., 1992). It has been demonstrated that mechanical power output increases concomitantly with an increase in the proportion of the cycle spent shortening (Askew and Marsh, 1997; Askew and Marsh, 1998). However, in addition to mechanical power output, the rate of metabolic energy expenditure is an important aspect of muscle performance. The energetic cost of an activity dictates an animal's ability to sustain the activity and the amount of energy remaining following the activity for other processes. It would be advantageous for muscle to operate with both a high mechanical power output and a high efficiency. However, it does not appear as though conditions that maximise power correspond to those maximising efficiency (Curtin and Woledge, 1996). Although the energetic consequences of a range of muscle length trajectories (Barclay and Weber, 2004), including those that mimic *in vivo* muscle function (Mellors and Barclay, 2001; Widén

and Barclay, 2006), have been investigated in both skeletal and cardiac muscles, the effects of asymmetric length trajectories optimised for maximal muscle power output on efficiency have not been systematically investigated.

For a given cycle frequency, increasing the proportion of the cycle spent shortening increases the optimal stimulation duty factor (i.e. the stimulation duration relative to the cycle duration), speeds activation and deactivation kinetics, increases strain and reduces the optimal relative shortening velocity (V/V_{\max}) (Askew and Marsh, 1998) under conditions optimised for maximal cycle-average power output. In sinusoidal cycles, increasing stimulation duty factor increases mechanical power output but also reduces mechanical efficiency (Curtin and Woledge, 1996). However, increasing the relative shortening duration in conjunction with an increase in stimulation duty factor may allow power to be increased without the reduction in efficiency previously seen in symmetrical cycles (Curtin and Woledge, 1996).

In order to establish whether a trade-off necessarily exists between power and efficiency (Curtin and Woledge, 1996), we investigated the effects of asymmetric muscle length trajectories on the maximal mechanical cycle-average power output and initial mechanical efficiency (E_i) (Barclay and Weber, 2004) of mouse soleus muscles using sawtooth-shaped contraction cycles with 25, 50 and 75% of the cycle spent shortening. We hypothesised that although mechanical power output will increase with increasing relative shortening duration (Askew and Marsh, 1997; Askew and Marsh, 1998), efficiency will remain constant because of the

concurrent changes in relative shortening duration and stimulation duty factor. Mouse soleus muscles were used as both their energetics (Barclay et al., 1993; Barclay, 1994) and mechanical power output during asymmetric contraction cycles (Askew and Marsh, 1997; Askew and Marsh, 1998) have been well characterised. However, it is likely that the findings are generally applicable to all skeletal muscles.

MATERIALS AND METHODS

Female white mice (CD1, Harlan, Bicester, UK) aged 5–9 weeks were killed by cervical dislocation. The skin was stripped from the hind limbs and the legs were removed at the hip. Limbs were placed into Ringer's solution (concentrations in mmol l^{-1} : 118.1 NaCl, 2.7 KCl, 1.5 CaCl_2 , 1.2 KH_2PO_4 , 1.9 MgSO_4 , 25.3 NaHCO_3 and 5.6 glucose, pH 7.4) (Ellerby and Askew, 2007) saturated with 95% O_2 , 5% CO_2 at 3°C. The soleus muscle from each limb was carefully dissected out, leaving both the proximal and distal tendons and a small piece of the tibia attached. One muscle from each mouse was used to determine the physiological properties of the muscles from that animal and an approximation of work-loop parameters required to produce maximal power output for each of the contraction cycles used. The contralateral muscle was initially pinned out at approximately resting length in oxygenated (95% O_2 , 5% CO_2) Ringer's solution and subsequently used to determine the E_i of these cycles under conditions optimised for maximal cycle-average power output.

Determination of physiological properties of the muscle

The first muscle from each animal was attached to a lightweight silver chain (50 mm long, 0.23 g) using silk suture (5-0; SharpPoint, Reading, PA, USA) tied as close to end of the muscle fibres as possible without causing damage. The muscle was suspended vertically in a Perspex flow-through muscle chamber circulated with oxygenated (95% O_2 , 5% CO_2) Ringer's solution at 37°C (Askew and Marsh, 1997). One end of the muscle was secured to the base of the chamber using a stainless steel clip, and the other end was connected to an ergometer (series 305B-LR; Aurora Scientific Inc., Aurora, ON, Canada) via the silver chain. The ergometer was used to control the length trajectory of the muscle and to record muscle force production and length change. Once transferred to the muscle chamber, the muscle was set to approximately resting length and allowed to thermoequilibrate for 30 min.

Stimulation, force production and length change

Muscles were activated using the current generated by a stimulator (S48; Grass, West Warwick, RI, USA), amplified using a stimulus isolation unit (UISO model 236; Hugo Sachs Elektronik, March-Hugstetten, Germany) and delivered via parallel platinum plate electrodes. Supramaximal square-wave stimuli with a pulse width of 0.25 ms duration were used. The resulting muscle force and length change were logged to a PC via a 12 bit A/D converter (DAS-1801AO; Keithley Metrabyte, Cleveland, OH, USA) at 5000 Hz for isometric contractions or $1000f$, where f is cycle frequency, for cyclical contractions.

Isometric properties

A series of isometric twitches, using the stimulation regime detailed above, were elicited at a range of muscle lengths varied in 0.5 mm increments. The muscle length yielding maximum isometric twitch force was determined. Fibre length (L_0) was assumed to be 0.85 times muscle length (Askew and Marsh, 1997). In estimating muscle fibre length from muscle length, no correction was made for

pennation angle as the error is less than 0.1% (Close, 1964). All subsequent contractions were then carried out with resting muscle fibre length set at L_0 unless otherwise specified. Twitches rather than tetani were used to set muscle length in order to preserve muscle performance. In these muscles, tetanic force is only approximately 5% lower at the length giving peak twitch force than at the length giving peak tetanic force (Askew and Marsh, 1998). There is evidence that some muscles operate *in vivo* at lengths below that at which maximum isometric force is generated (Askew and Marsh, 2001). Peak tetanic force was determined from the plateau of a tetanus elicited with a stimulation burst of 450 ms using a stimulation frequency of 150 Hz. Maximal isometric tetanic stress (P_0) was determined from the peak isometric force, L_0 and muscle mass (determined at the end of experiments), assuming a muscle density of 1060 kg m^{-3} (Mendez and Keys, 1960).

Force–velocity properties

After-loaded isotonic tetanic contractions were used to determine the force–velocity characteristics of the muscle (Askew and Marsh, 1998). The muscle was stimulated as described for isometric tetanic contractions. Force rose to a predefined level, ranging from approximately 5 to 80% of P_0 , and was then held constant by muscle shortening (controlled by the ergometer). Force (P) and length (L) were recorded as described above. Force was expressed as a proportion of P_0 and muscle length was converted to strain $[(L-L_0)/L_0]$. Strain was differentiated with respect to time in order to determine velocity [V in fibre lengths per second ($L_0 \text{ s}^{-1}$)]. The maximal shortening velocity and the average force produced during this time were recorded. Relative force (P/P_0) was plotted against V and a hyperbolic-linear curve (Marsh and Bennett, 1986) was fitted to the data using the non-linear curve fitting function in IGOR Pro (Version 5.01; Wavemetrics, Lake Oswego, OR, USA). Maximum shortening velocity (V_{max}) was determined by extrapolation of the relationship to zero relative force.

Optimisation of strain and stimulation parameters for sawtooth contraction cycles

Providing the maximal isometric tetanic force of the muscle had not declined below 90% of the initial P_0 , the muscle was further used to determine the strain and stimulation parameters that yielded the maximum mechanical power output for each length trajectory. The work-loop technique (Machin and Pringle, 1960; Josephson, 1985) was used to impose cyclical length changes and a phasic stimulation pattern on the muscle. Force and length change were recorded as above, and cycle-average power output and net work were calculated.

Sawtooth-shaped length trajectories with 25% (Saw25%), 50% (Saw50%) or 75% (Saw75%) of the cycle spent shortening were performed at 1, 5 and 9 Hz (Askew and Marsh, 1998). Strain was set to that which yielded maximal cycle-average mechanical power output. For each muscle length trajectory, the relative shortening velocity (V/V_{max}) was calculated from the V_{max} for each muscle determined from the force–velocity relationship and optimal strain. The optimal relative shortening velocity determined in a series of preliminary experiments was found to be the same as has previously been measured on the same muscles under similar conditions (Askew and Marsh, 1998). In subsequent experiments, we set strain based on the optimal V/V_{max} previously reported (Askew and Marsh, 1998) and V_{max} , determined as described above.

The length trajectory and activation pattern were imposed on the muscle using custom-written software in the program Testpoint (version 5; Capital Equipment Corp., Norton, MA,

USA), which controlled the ergometer (series 305B-LR; Aurora Scientific Inc.) and the stimulator *via* a 12 bit D/A converter (DAS-1801AO; Keithley Metrabyte). Force and length were recorded as described above. Instantaneous power output was determined as the product of force and velocity. Cycle-average mechanical power output was calculated as the average instantaneous power over the course of the cycle. Net work was calculated by multiplying the cycle-average power by cycle duration using Testpoint. Stimulation phase and duration were adjusted to produce maximal cycle-average mechanical power output for each combination of frequency and muscle length trajectory. The optimal stimulation parameters were determined for several muscles and used throughout subsequent experiments. A recovery period of 5 min was allowed between each set of contractions. Control loops, using a sinusoidal length trajectory and constant stimulation parameters that produced approximately maximum power output, were carried out at the beginning of each experiment and then approximately every four to five runs in order to monitor any decline in the muscle's performance. The muscle was no longer used once cycle-average power output had fallen below 80% of the maximal value measured.

Measurement of enthalpy output during sawtooth contraction cycles

The mechanical work output of the muscle is provided by the hydrolysis of ATP. However, only a fraction of the chemical energy released from the hydrolysis of ATP is converted to mechanical work. The remaining energy is liberated as heat (Smith et al., 2005). Therefore, the total enthalpy output of the muscle can be determined by measuring heat and work produced during a contraction. E_i can then be calculated as the ratio between the mechanical work produced and the total enthalpy output (Curtin and Woledge, 1993a; Curtin and Woledge, 1993b; Barclay, 1994). Net work output can be measured in sawtooth contraction cycles as described above. The heat output of the muscle during these contractions can be simultaneously determined from the measurement of temperature change during contraction using an antimony-bismuth thermopile (Mulieri et al., 1977; Barclay et al., 1993).

The thermopile was calibrated using the Peltier heating method (Kretzschmar and Wilkie, 1972; Kretzschmar and Wilkie, 1975; Woledge et al., 1985). Each thermocouple was determined to have an output of 79.3 and 78.7 μVK^{-1} , respectively, for the two thermopiles used. The second muscle from each animal was clipped at either end using small aluminium foil clips and positioned so that the centre of the muscle was in contact with the active region of the thermopile (a 4 mm length of thermopile comprising 16 thermocouples). The remainder of the muscle was in contact with the surrounding protecting region (Woledge et al., 1985; Barclay et al., 1993). The thermopile was clamped onto a temperature-controlled brass block that held the internal chamber temperature at 37°C. The muscle was attached at one end to the lever arm of an ergometer (300B-LR Aurora Scientific Inc., Ontario, Canada) and to a moveable stage at the other *via* stainless steel connecting rods (diameter 0.3 mm, mass 63 mg) and the aluminium foil clips. The muscle was irrigated with oxygenated (95% O₂, 5% CO₂) Ringer's solution at 37°C. L_0 was set and control work loops performed as described above. The muscle was stimulated using the apparatus described above with the stimulation pulses delivered *via* fine silver wires soldered onto the stainless steel rods.

In order to make heat measurements, excess Ringer's solution was drained from the chamber and the muscle was blotted. The brass block was sealed and internal temperature was allowed to stabilise. One of

the sawtooth cycles, using the length trajectory and stimulation pattern determined above, was imposed on the muscle. Force and length data were recorded as previously described. The thermopile output was amplified by a factor of 5000, a 100 Hz low-pass filter was applied using a low noise preamplifier (SR560, Stanford Research Systems, Sunnyvale, CA, USA) and data were logged to a PC at 200 Hz *via* a 12 bit A/D converter (KPCI 3104, Keithley Metrabyte). Temperature change was converted to heat output using the heat capacity of the preparation estimated by Peltier heating of the preparation and correcting for heat loss (Woledge et al., 1985). Stimulus heat was measured by simulating a muscle rendered inexcitable and was found to constitute less than 3% of the heat measured in any contraction. Hence, no correction was made, following Barclay (Barclay, 1994). Work and heat per cycle were determined for cycles 2 and 3 and E_i was calculated as the average work produced in these cycles divided by the sum of the average work and heat produced. Work and heat produced solely during the shortening period were determined and the initial mechanical efficiency of shortening ($E_{i,\text{short}}$) was calculated, as for E_i , using these values. One-way ANOVAs and Tukey's honestly significant difference test were carried out in R (R Development Core Team, 2009) to determine whether there was a significant effect of length trajectory on power output and efficiency at each frequency.

Given the radius of the muscles (Table 1), the use of a temperature of 37°C and the duty cycles used (Table 2), it is likely that the centre of the muscles would become anoxic relatively quickly [estimated to be 10–25 s at 35°C (Barclay, 2005)]. The series of contractions used here lasted between 0.6 and 5 s and so the number of contractions that could be performed was severely limited. Muscle performance was monitored by performing control loops after each set of sawtooth contractions, and muscles were no longer used once performance had fallen below 80% of maximal. No decline in muscle mechanical performance was ever noted over the series of five contractions performed. However, a single muscle rarely performed more than two sets of muscle contractions and associated control loops. The control measures used here, although potentially contributing to the decline in muscle performance, ensure that reliable measurements are obtained for the few contraction series performed by each muscle.

RESULTS

All values are reported as means \pm s.e.m. (number of replicates). The mice used in the experiments had a mass of 27.7 \pm 0.8 g ($N=24$). The contractile properties of the muscles used are reported in Table 1.

Rate of energy output during sawtooth length trajectories

Cycle-average power output and rate of heat production are shown in Fig. 1. Cycle-average power output generally increased with the

Table 1. Contractile properties of mouse soleus muscles at 37°C

Property	Mean \pm s.e.m. ($N=24$)
Muscle mass (mg)	5.6 \pm 0.3
Fibre length, L_0 (mm)	11.1 \pm 0.1
Muscle radius (mm)	0.4 \pm 0.008
Maximum isometric force, P_0 (N cm ⁻²)	47.1 \pm 2.2
Maximum shortening velocity, V_{max} (L_0 s ⁻¹)	5.5 \pm 0.2
Net power output (W kg ⁻¹) ^a	44.6 \pm 2.3

N , number of replicates.

^aNet power output was measured using sinusoidal length trajectories at 5 Hz, \pm 0.06 strain; stimulation parameters were optimised to maximise cycle-average power.

Table 2. Mean strain amplitude and stimulation parameters producing maximal power output for all length trajectories at all frequencies

Parameter	Frequency								
	1 Hz			5 Hz			9 Hz		
	Saw25%	Saw50%	Saw75%	Saw25%	Saw50%	Saw75%	Saw25%	Saw50%	Saw75%
Strain (\pm)	0.088	0.11	0.17	0.033	0.070	0.091	0.027	0.035	0.053
Phase (ms)	-15	-15	-5	-15	-15	-5	-10	-10	-5
Duration (ms)	230	460	680	40	60	90	20	30	30
D_f	0.23	0.46	0.68	0.20	0.30	0.45	0.18	0.27	0.27
$D_{f,short}$	0.92	0.92	0.91	0.80	0.60	0.60	0.72	0.54	0.36
Delay (ms)	35.0	55.0	75.0	25.0	55.0	65.0	17.8	35.6	58.3

Saw25%, Saw50% and Saw75% are sawtooth-shaped length trajectories with 25, 50 or 75% of the cycle spent shortening, respectively. Phase is the timing of stimulation, expressed relative to peak length; duration is the length of stimulation. D_f and $D_{f,short}$ describe the proportion of the cycle and the proportion of shortening, respectively, spent being stimulated. The delay describes the length of time between the end of stimulation and the beginning of lengthening.

proportion of the cycle spent shortening. Maximum cycle average power output ranged from $13.8 \pm 0.9 \text{ W kg}^{-1}$ ($N=2$) during Saw25% cycles at 9 Hz to $102.9 \pm 7.6 \text{ W kg}^{-1}$ ($N=4$) during Saw75% cycles at 5 Hz. There was a significant effect of length trajectory on cycle-average mechanical power output at 5 ($F=49.8$, d.f.=2, $P<0.001$) and 9 Hz ($F=7.1$, d.f.=2, $P<0.05$), with significant differences between Saw25% and Saw50% cycles ($P>0.01$) and Saw50% and Saw75% cycles ($P>0.001$) at 5 Hz, as well as between Saw25% and Saw75% cycles ($P>0.05$) at 9 Hz (Fig. 1). The values of cycle-average mechanical power output and the relationship between frequency, length trajectory and cycle-average power output are comparable to those reported by Askew and Marsh (Askew and Marsh, 1998).

Initial mechanical efficiency

Initial mechanical efficiency was at a maximum (0.27 ± 0.02 , $N=4$) in Saw50% cycles at 5 Hz. There was no significant effect of length trajectory on efficiency at 1 Hz ($F=0.40$, d.f.=2, $P=0.69$); E_i was approximately 0.2 for all length trajectories. There was a significant effect of length trajectory on E_i at 5 Hz ($F=4.8$, d.f.=2, $P<0.05$) and a tendency towards an effect at 9 Hz ($F=3.4$, d.f.=2, $P=0.10$). E_i appeared to be lower in Saw25% cycles than in Saw50% and Saw75% cycles at 5 and 9 Hz, though the differences were not significant (e.g. 5 Hz, Saw25% vs Saw50% $P=0.059$, Saw25% vs Saw75% $P=0.062$; Fig. 2).

Strain trajectories and stimulation parameters

The strain required to produce the required V/V_{max} (Askew and Marsh, 1998) decreased with increasing frequency and as the

proportion of the cycle spent shortening decreased (Table 2). Strains ranged from a minimum of 0.03 ± 0.002 ($N=2$) for Saw25% cycles at 9 Hz to a maximum of 0.17 ± 0.03 ($N=3$) for Saw75% cycles at 1 Hz.

Stimulus duration producing maximal power increased with increasing proportion of the cycle spent shortening and decreasing frequency, and ranged from 20 ms for Saw25% cycles at 9 Hz to 680 ms for Saw75% cycles at 1 Hz (Table 2). Stimulus phase producing maximal power output was always prior to the beginning of shortening. However, the onset of stimulation was earlier in Saw25% and Saw50% cycles than in Saw75% cycles at all frequencies and in Saw25% and Saw50% cycles at 1 and 5 Hz compared with the same cycles at 9 Hz (Table 2). The earliest stimulation phase was -15 ms, with 0 ms being the start of shortening, for Saw25% cycles at 5 Hz and Saw50% cycles at 1 and 5 Hz. Stimulation duty factor (D_f), the proportion of the cycle spent being stimulated, was maximal for Saw75% cycles at 1 Hz and decreased with decreasing proportion of the cycle spent shortening and increasing frequency. However, given that the proportion of the cycle spent shortening was systematically different between muscle length trajectories, duty factor may not be particularly relevant. It may be more appropriate to consider the stimulation duration as a proportion of shortening, $D_{f,short}$. $D_{f,short}$ was maximal at 1 Hz and decreased with increasing frequency and increasing proportion of the cycle spent shortening (Table 2). The delay between the end of stimulation and the end of shortening decreased with increasing frequency and decreasing proportion of the cycle spent shortening (Table 2).

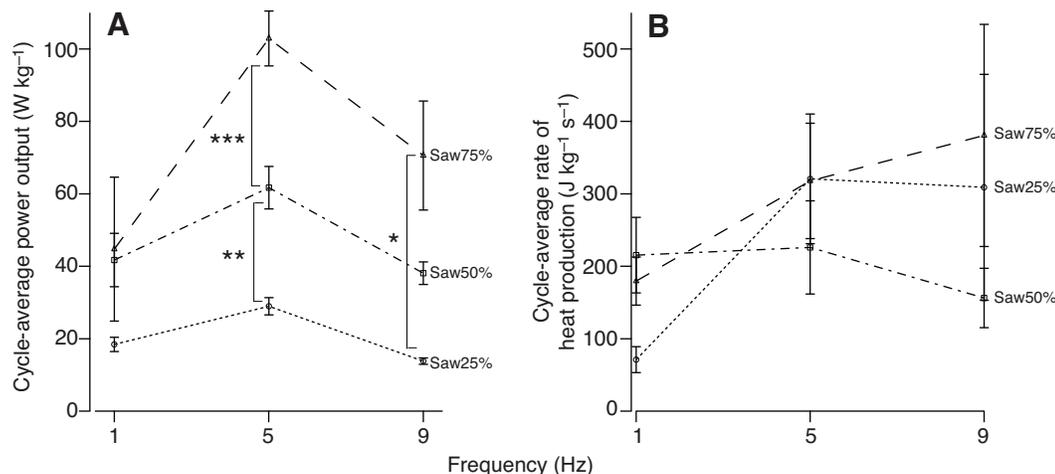


Fig. 1. The effects of frequency and length trajectory on cycle-average mechanical power output (A) and cycle-average rate of heat production (B) in mouse soleus muscles. Data are means \pm s.e.m. ($N=4$, 3 and 3 at 1 Hz; 4, 4 and 4 at 5 Hz and 2, 3 and 3 at 9 Hz, for Saw25%, Saw50% and Saw75% length trajectories, respectively). Saw25%, Saw50% and Saw75% are sawtooth-shaped length trajectories with 25, 50 or 75% of the cycle spent shortening, respectively. Asterisks denote significant differences between the respective length trajectories (* $P<0.05$; ** $P<0.01$; *** $P<0.001$).

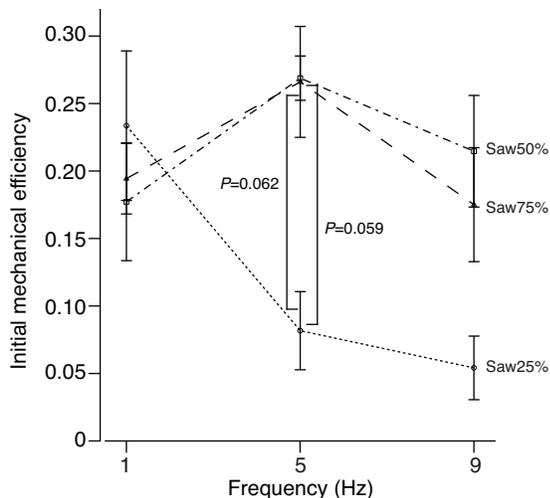


Fig. 2. The effects of length trajectory on initial mechanical efficiency in mouse soleus muscles. Data are means \pm s.e.m. for two to four muscles (see Fig. 1 legend). Trends in differences between the sawtooth contraction cycles are highlighted and *P*-values are presented.

Shortening efficiency

In order to gain insight into the differences in efficiency between different length trajectories at 5 Hz, mechanical work output and enthalpy change were measured solely during the shortening portion of the cycle (Curtin and Woledge, 1996) and the initial mechanical efficiency of shortening ($E_{i,short}$) was calculated. There was no significant difference in $E_{i,short}$ with length trajectory at 5 Hz ($F=0.50$, d.f.=2, $P=0.62$; Fig. 3), suggesting that the lower efficiency seen in Saw25% cycles at 5 Hz may be due to increased energy turnover during lengthening. Force and heat production was commonly seen to continue into lengthening in Saw25% cycles at 5 Hz whereas force had declined closer towards resting level and heat production had ceased at the end of shortening in cycles with longer shortening durations (Fig. 4).

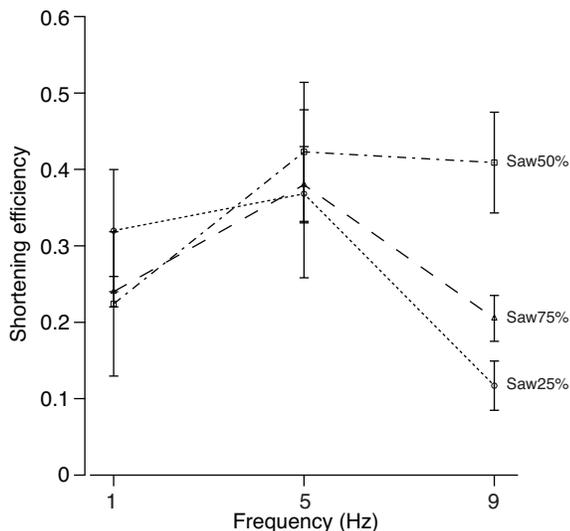


Fig. 3. The effects of length trajectory on shortening efficiency in mouse soleus muscles. Data are means \pm s.e.m. for two to four muscles (see Fig. 1 legend).

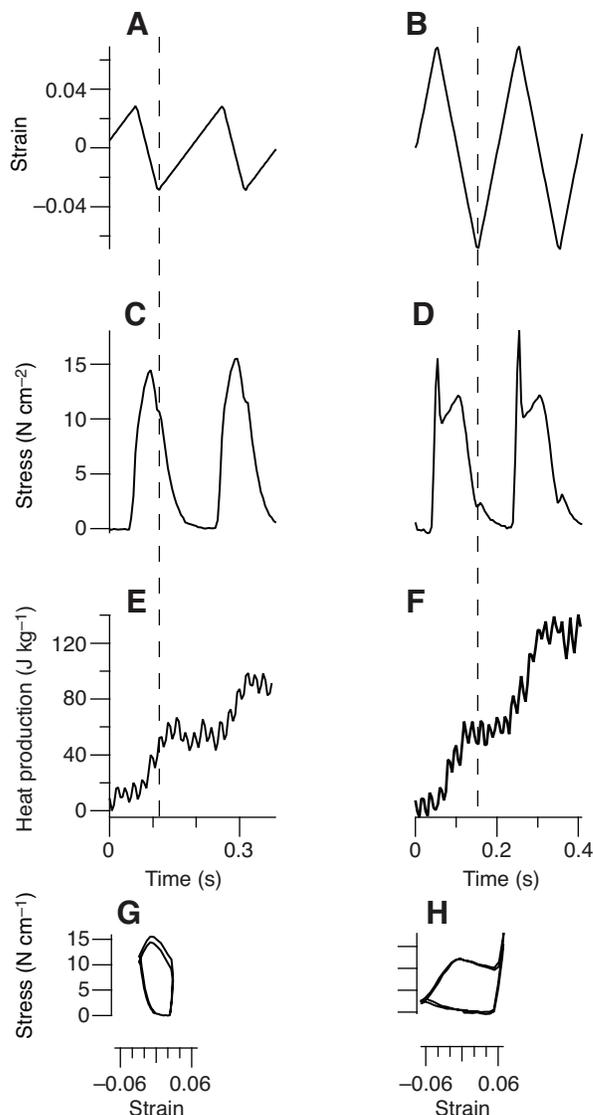


Fig. 4. Example strain (A,B), stress (C,D) and heat (E,F) traces and work loops (G,H) for mouse soleus muscles subjected to Saw25% (A,C,E,G) and Saw50% (B,D,F,H) cycles at 5 Hz. The dashed lines highlight the end of shortening.

DISCUSSION

Power output and length trajectory

The finding that increasing the proportion of the cycle spent shortening increases the cycle-average mechanical power output of a muscle (Askew and Marsh, 1997; Askew and Marsh, 1998) has been confirmed here (Fig. 1). The magnitude of power output measured in these experiments is comparable with that previously reported for mouse soleus muscle performing the same length trajectories (Askew and Marsh, 1998).

Initial mechanical efficiency and length trajectory

In line with our hypothesis, there was no difference in initial mechanical efficiency with length trajectory at 1 Hz or between Saw50% and Saw75% cycles at 5 and 9 Hz. However, in contrast to this hypothesis, initial mechanical efficiency was lower in Saw25% cycles than in Saw50% and Saw75% cycles at 5 Hz and showed a tendency to be lower at 9 Hz (Fig. 2). The values of E_i

measured in symmetrical cycles (Saw50%) are comparable with those previously measured in mouse soleus muscle (Barclay and Webber, 2004).

Asymmetric length trajectories with more than 50% of the cycle duration spent shortening

In line with our hypothesis, increasing relative shortening duration from 50 to 75% did not have any effect on E_i at any frequency (Fig. 2). It appears that increasing relative shortening duration above 50% offsets the potentially efficiency-reducing effect of increasing stimulation duty factor (Table 2) (Curtin and Woledge, 1996; Lichtwark and Wilson, 2005). This is not surprising as the detrimental effect of increasing stimulus duty factor is thought to be due to the continuation of energy turnover into lengthening (Curtin and Woledge, 1996). Therefore, for the purposes of asymmetric cycles in particular, it may be better to think of stimulus duration in terms of the proportion of shortening ($D_{f,short}$), rather than the proportion of the cycle spent being stimulated, when predicting the effect that it will have on efficiency. In this case, $D_{f,short}$ is very similar between Saw50% and Saw75% cycles at all frequencies (Table 2). The finding that efficiency is not affected by other factors such as the velocity and magnitude of stretch supports previous work demonstrating that although active stretch enhances force production (Fenn, 1924; Altringham and Johnston, 1990; Herzog and Leonard, 2000; Herzog and Leonard, 2002; Linari et al., 2003), it does not alter efficiency (De Haan et al., 1989; Trinh and Syme, 2007).

The finding that increasing relative shortening duration above 50% increases mechanical power output without compromising efficiency suggests that there does not necessarily have to be a trade-off between muscle power output and efficiency. It appears that the use of asymmetric contraction cycles to enhance muscle power output is preferable to other strategies (e.g. increasing operating frequency or activation duty factor) as efficiency is not detrimentally affected. This may be an additional explanation for the observation that asymmetric length trajectories with prolonged shortening durations are utilised in many high power output systems (Marsh et al., 1992; Girgenrath and Marsh, 1997; Askew et al., 2001; Ellerby and Askew, 2007). However, it should be noted that although initial mechanical efficiency is not reduced by prolonging relative shortening duration, increasing mechanical power output will still increase the rate at which metabolic substrates are used and so may limit the endurance (Askew and Marsh, 2002).

Asymmetric length trajectories with less than 50% of the cycle duration spent shortening

In contrast to our hypothesis, at 5 Hz and, to a lesser extent, 9 Hz, E_i was lower in Saw25% cycles than in Saw50% and Saw75% cycles. Given the lack of a significant difference in E_i between Saw50% and Saw75% cycles, the reasons for this reduced efficiency in Saw25% cycles are not immediately clear. However, the relatively high E_i maintained at 1 Hz indicates that the low efficiencies in Saw25% cycles at higher frequencies may be due to the reduced time available for shortening. Closer examination reveals that the low relative shortening duration means that $D_{f,short}$ is higher in Saw25% cycles than in Saw50% or Saw75% cycles at higher frequencies (Table 2). Examination of energy turnover in the shortening phases of the cycle confirms previous findings (Curtin and Woledge, 1996) that stimulation continuing further into shortening increases energy turnover during lengthening (Fig. 4). The lack of a significant effect of length trajectory on $E_{i,short}$ (Fig. 3) illustrates that the lower efficiency in Saw25% cycles at higher

frequencies is likely to be due largely to changes in energy turnover during lengthening rather than from a change in the initial mechanical efficiency during shortening.

Conclusions

Increasing the relative shortening duration of a muscle beyond half of the contraction cycle duration increases its cycle-average mechanical power output with no change in initial mechanical efficiency. Hence, such asymmetrical cycles are well suited to behaviours that demand a high power output as they provide a means by which to enhance mechanical power output without compromising efficiency. Decreasing relative shortening duration below half of the contraction cycle not only decreased mechanical power output of the muscle but, at higher frequencies, also reduced initial mechanical efficiency compared with symmetrical cycles. This finding highlights the importance of muscles having sufficient time to relax before lengthening in order to maximise contractile efficiency. Hence, asymmetric contraction cycles with increased relative shortening duration may not only increase mechanical power output but also, at high contraction frequencies, contribute to keeping energy costs low.

ACKNOWLEDGEMENTS

We are most grateful to Prof. Chris Barclay for his supply of thermopiles, Prof. Nancy Curtin for her assistance with thermopile setup and advice and two anonymous referees for their comments. G.N.A. thanks Prof. Rich Marsh for stimulating his initial interest in asymmetrical muscle length trajectories and their implications for muscle energetics.

FUNDING

This research was supported by a Biotechnology and Biological Sciences Research Council Doctoral Training Grant Studentship [BB/D526488/1 to N.C.H.].

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