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EFFICIENCY OF FAST- AND SLOW-TWITCH MUSCLES OF THE MOUSE PERFORMING CYCLIC CONTRACTIONS

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Summary

The mechanical efficiency of mouse fast- and slow-twitch muscle was determined during contractions involving sinusoidal length changes. Measurements were made of muscle length, force production and initial heat output from bundles of muscle fibres *in vitro* at 31 °C. Power output was calculated as the product of the net work output per sinusoidal length cycle and the cycle frequency. The initial mechanical efficiency was defined as power output/(rate of initial heat production + power output). Both power output and rate of initial heat production were averaged over a full cycle of length change. The amplitude of length changes was ±5 % of muscle length. Stimulus phase and duration were adjusted to maximise net work output at each cycle frequency used. The maximum initial mechanical efficiency of slow-twitch soleus muscle was 0.52±0.01 (mean ±1 S.E.M. N=4) and occurred at a cycle frequency of 3 Hz. Efficiency was not significantly different from this at cycle frequencies of 1.5–4 Hz, but was significantly lower at cycle frequencies of 0.5 and 1 Hz. The maximum efficiency of fast-twitch extensor digitorum longus muscle was 0.34±0.03 (N=4) and was relatively constant (0.32–0.34) over a broad range of frequencies (4–12 Hz). A comparison of these results with those from previous studies of the mechanical efficiency of mammalian muscles indicates that efficiency depends markedly on contraction protocol.

Introduction

The ability to maintain a given level of muscular activity depends on the balance between the energy demands of the activity and the metabolic capacity of the exercising muscle to supply energy (for reviews, see Holloszy and Coyle, 1984; Westerblad *et al.* 1991; Barclay, 1993). If the energetic demands exceed the capacity to supply energy, the level of activity cannot be sustained and the exercising muscles fatigue. An important factor in the balance between the rate of energy use and the rate at which energy can be supplied is the efficiency with which muscles can convert chemical energy into mechanical energy. The efficiency of isolated muscle contraction has been measured in muscles from many species (for a brief review, see Curtin and Woledge, 1991). Typically, efficiency has been measured with muscles fully activated (i.e. during a tetanus) and shortening either at a constant velocity or against a constant

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load (e.g. Barclay *et al.* 1993). Such protocols bear little relationship to the type of contractions that occur during locomotion (for a review, see Johnston, 1991). A more realistic protocol, in which muscles were both stretched and shortened, has been used in several investigations of the efficiency of shortening of isolated mammalian muscle. Stretch of a contracting muscle, as often occurs during locomotion, increases the efficiency of subsequent shortening (Heglund and Cavagna, 1982; de Haan *et al.* 1989). In these experiments, however, the stretch and shortening were still performed during prolonged, tetanic contractions. Furthermore, when the work done on the muscles during the stretch was subtracted from the work done by the muscle shortening, the increase in efficiency was eliminated (de Haan *et al.* 1989). Recently, the efficiency of isolated muscles from insects and fish has been measured using much more realistic contraction protocols involving repeated, sinusoidal changes in muscle length with brief periods of activation during each length cycle (Josephson and Stevenson, 1991; Moon *et al.* 1991; Curtin and Woledge, 1993*a*,*b*). When this type of protocol was used on fish muscle, the maximum efficiency, even when incorporating the work done on muscles during stretch, was greater than that measured using isovelocity shortening during a tetanus (Curtin and Woledge, 1993*a*). In the current study, the more realistic sinusoidal length cycles have been used to study the efficiency of mammalian muscle. Both fast- and slow-twitch muscles from the mouse were used. The efficiency of these preparations has previously been measured using a protocol consisting of isovelocity shortening initiated during the force plateau of a tetanus (Barclay *et al.* 1993). In that case, the maximum efficiencies, defined as power output divided by the sum of rate of initial heat production and power output, of the two muscle types were the same (approximately 0.3). In contrast, when cyclic, sinusoidal contractions were used in the present study, the maximum efficiency of slow-twitch muscle (0.52) was 1.6-fold greater than that of fast-twitch muscle (0.34).

Materials and methods

Experiments were performed using bundles of muscle fibres dissected from soleus and extensor digitorum longus (EDL) muscles of adult, female mice (Swiss strain). Mice were anaesthetised using chloroform and then killed by cervical dislocation. All animal handling procedures conformed to local animal ethical requirements. The mean wet mass of soleus fibre bundles was 2.86±0.33 mg (mean ±1 S.E.M.; N=10) and that of EDL fibre bundles was 2.99±0.38 mg (N=10). The total lengths of soleus and EDL fibre bundles were 9.91±0.23 mm (N=10) and 9.83±0.35 mm (N=10) respectively. During dissection and experiments, muscles were bathed in oxygenated (95 % O_2, 5 % CO_2) Krebs–Henseleit solution of the following composition (in mmol l^{-1}): NaCl, 118; KCl, 4.75; $MgSO_4$, 1.18; $NaHCO_3$, 24.8; KH_2PO_4, 1.18; $CaCl_2$, 2.54; glucose, 10.0. During experiments, the temperature of the bathing solution was maintained at 31 °C.

Mounting and stimulation

Fibre bundles were mounted between a clamp and the arm of an ergometer (model 300H, Cambridge Technologies, Watertown, MA, USA). The fibre bundles lay along the

mica surface of a thermopile. The ergometer was used both to control and to measure muscle length and also to measure muscle force production.

Muscles were stimulated using fine platinum wire electrodes placed on either end of the preparation. Stimuli, consisting of supramaximal (typically 4–5 V) pulses of 0.5 ms duration, were delivered to the muscle at a frequency sufficient to give a fully fused tetanus. Stimulus frequencies were 83 Hz (corresponding to an interval of 12 ms between pulses) for soleus muscles and 166 Hz (6 ms pulse interval) for EDL muscles.

A microcomputer (IBM 486 compatible), multi-function interface board (Labmaster DMA, Scientific Solutions, Solon, Ohio, USA) and software were used to control stimulation, muscle length changes and data acquisition. Force, length and temperature signals were sampled at 500 Hz, digitized, displayed and stored on disk.

Length changes and stimulus timing

Measurements of energy output were made while muscles performed cyclic contractions. These involved sinusoidal alterations in muscle length, with stimulation occupying a fraction of each length cycle. Cycles were defined as starting and ending when the length was mid-way between the extreme lengths (Josephson, 1985). The length of fibre bundles at this point was that at which tetanic force was maximal (l_0). This length was determined at the start of each experiment using a series of brief (50 ms for EDL and 100 ms for soleus) tetanic contractions.

Power output during cyclic contractions depends on the cycle frequency, the amplitude of length changes and the timing of stimulation within each cycle (Josephson, 1985). The timing of the start of stimulation relative to the length change cycle (i.e. the stimulus phase) was defined as the time between the start of stimulation and the start of shortening, expressed as a percentage of the duration of the cycle (Curtin and Woledge, 1993*a*). The timing of the end of stimulation was adjusted to allow just sufficient time for the muscle to relax (i.e. for force to return to pre-stimulation levels) before the commencement of the next stretch phase. A preliminary series of experiments, in which only mechanical recordings (i.e. muscle length and force production) were made, was performed to establish a range of cycle frequencies spanning that at which power output was maximal and to determine the stimulus variables that elicited the maximum power output at each cycle frequency. The results of these experiments are summarised in Table 1. Net work output per cycle was maximal when the length cycle amplitude was between ±4 and ±6 % l_0. The results shown in Table 1, and those from all subsequent experiments, were obtained using a cycle amplitude of ±5 % l_0. The experiments in which energy output was measured were performed using the cycle frequencies and stimulus variables shown in Table 1.

Measurement of energy output

The mechanical energy produced by a muscle is derived from energy liberated by the hydrolysis of adenosine triphosphate (ATP). However, some fraction of the energy obtained from ATP is not converted into work, but appears as heat. The amount of ATP used is therefore proportional to the sum of the mechanical and thermal energy outputs. Calculation of the total energy output therefore required measurements of muscle force and length (from which work was calculated) and of muscle heat production.

Table 1. *Maximum power output and stimulus variables used to achieve that power output at different cycle frequencies*

Cycle frequency (Hz)	Stimulus phase (%)	Number of pulses	Peak force (% P_0)	Maximum power (W kg^{-1})
Soleus				
0.5	–2.4 (2)	79	0.89±0.03	6.3±0.3
1.0	–4.8 (2)	37	0.79±0.02	10.1±0.5
1.5	–7.2 (3)	23	0.71±0.03	12.7±0.6
2.0	–7.2 (4)	16	0.65±0.04	14.9±0.7
3.0	–7.2 (4)	9	0.55±0.01	14.1±1.1
4.0	–9.6 (4)	6	0.46±0.03	14.2±1.3
Extensor digitorum longus				
2	–1.2 (1)	39	0.88±0.02	22.0±2.5
4	–4.8 (2)	20	0.78±0.03	36.8±3.6
6	–7.2 (2)	12	0.73±0.06	46.5±4.7
8	–9.6 (2)	8	0.65±0.05	49.2±4.7
10	–12.0 (2)	6	0.62±0.02	45.9±4.2
12	–7.2 (1)	4	0.50±0.05	42.9±4.4
14	–8.4 (1)	3	0.21±0.05	34.8±2.7

Values with errors are means ±1 S.E.M. (N=6 for both muscles).

P_0 is the maximum isometric force determined at l_0. P_0 for soleus preparations was 194.2±9.7 mN mm^{-2}; for EDL preparations it was 202.6±15.6 mN mm^{-2} (N=6 for both muscles).

Numbers in parentheses are the number of stimulus pulses delivered before the start of shortening (i.e. at stimulus phase less than or equal to 0 %). Stimulation frequencies were 83 Hz (12 ms between pulses) and 166 Hz (6 ms between pulses) for soleus and EDL preparations, respectively.

The net work produced during a cycle of length change was calculated by integrating force with respect to length change over the whole cycle. This is equivalent to calculating the area enclosed by a 'work loop' generated by plotting force as a function of muscle length (Josephson, 1985). The average power output over one complete cycle of length oscillation was calculated by multiplying the net work performed during the cycle by the cycle frequency.

The time course of heat production by muscles was calculated from the time course of change in temperature of preparations when they contracted. Muscle temperature was measured using a thermopile consisting of 20 antimony–bismuth thermocouples (Mulieri *et al.* 1977), each of which produced 96.8 μV °C^{-1}. Heat was recorded from a 5 mm segment in the centre of each preparation. The thermopile included additional thermocouples, not connected in the recording circuit, at either end. These ensured that heat loss, and hence muscle temperature, was uniform along the length of the preparation. Non-uniform temperature distribution can cause spurious results when muscles shorten and regions of different temperature move onto the recording section of the thermopile (Hill, 1937). Filtered temperature signals (low-pass filter, cut-off frequency 100 Hz) were amplified using a low-noise amplifier (Dijekma *et al.* 1985). Heat production is the product of the temperature rise and the heat capacities of the preparation (including any adhering saline) and of the thermopile under the preparation. Heat capacity was estimated

from the steady-state temperature reached when the preparation was heated at a constant rate by the Peltier effect (for a detailed description, see Woledge *et al.* 1985, p. 187).

Temperature signals were corrected for heat lost from the preparation during recording. The rate of heat loss was determined by recording the time course of cooling of fibre bundles following a period in which muscles were heated using the Peltier effect (Kretzschmar and Wilkie, 1972). The time course of cooling was described well by a single exponential.

The proportion of the total recorded heat contributed by heat produced by the passage of the stimulus current through fibre bundles was measured by stimulating muscles that had been rendered unresponsive by prolonged high-frequency (10 kHz) stimulation. Stimulus heat contributed less than 2 % to the overall heat production, so no corrections were made for it.

The net heat produced in any given cycle of length change was calculated by subtracting the total heat produced up to the start of that cycle from that produced at the end of the cycle. The average rate of heat production over a whole cycle was calculated as the product of net heat produced and cycle frequency. To accommodate lag in the heat recording apparatus (which was significant during the first 50–100 ms of recording), muscles performed a series of cycles at each cycle frequency. At cycle frequencies of up to 1 Hz, only two cycles were performed in each series and heat production and work performed were measured in the second cycle. At frequencies of greater than 1 Hz, four cycles were performed and heat was measured in either the third or fourth cycle. The work output per cycle altered little between the first and fourth cycles of such series. Each series of cycles was followed by 3 min of recovery. The order of presentation of cycle frequencies was varied amongst muscles so that equal numbers received the range of cycle frequencies in ascending order as received it in descending order.

The heat measured did not include basal heat production, only increases in heat production above the basal rate. As a consequence of the relatively short duration of activity, there was little contribution to recorded heat from processes associated with resynthesis of ATP (i.e. ‘recovery heat’). The rate of production of recovery heat, measured 1 s after a series of cyclic contractions, was less than 5 % of the average rate of initial heat production (i.e. heat arising mainly from the hydrolysis of ATP during contraction) during the contractions. This is consistent with other reports of the time course of recovery heat production by mouse muscle (Leijendekker and Elzinga, 1990). The heat measured during contractions could therefore be attributed almost entirely to initial energetic processes.

Efficiency calculation

In this study, efficiency refers to the ratio of power output to the rate of total energy output. This is usually called the mechanical efficiency (for a review of conventions regarding use of the term ‘efficiency’, see Woledge *et al.* 1985, pp. 163–167). The rate of total energy output is the sum of the mechanical power and the rate of initial heat production. Therefore, as only initial energy output was measured, a more accurate description of the calculated value is the ‘initial mechanical efficiency’.

Normalization of energy expenditure

At the end of each experiment, the total length of fibre bundles was measured. The tendons were then removed and the preparations were dried for at least 48 h. Muscle dry mass was measured using an electronic balance. The dry mass of these small preparations could be more reliably determined than their wet mass. To facilitate comparison with previous data, muscle masses were corrected to an equivalent wet mass assuming a wet mass:dry mass ratio of 5 (Leijendekker *et al.* 1987). Power output, heat production and total energy output were normalized for the calculated wet mass of fibre bundles.

Data presentation and statistical analysis

All results are presented as the mean value ±1 S.E.M. In the first series of experiments, in which only mechanical measurements were made, the statistical significance of variations in mechanical work and power output by each muscle type with cycle frequency were determined for each muscle type (N=6 for each type) using one-way analysis of variance. In the experiments in which heat production was measured (where N=4 for each muscle), a non-parametric, one-way analysis of variance, the Kruskal–Wallis test, was used. When *post hoc* comparisons between pairs of mean values were performed, the Wilcoxon rank-sum test was used, with appropriate control of the error rate for individual comparisons (see Lehmann, 1975, pp. 238–245). All statistical decisions were made with respect to the 95 % level of confidence.

Results

Examples of recordings of muscle length, force production and heat output from bundles of both EDL muscle fibres and soleus fibres are shown in Fig. 1. Also shown are the time courses of the calculated work and total energy (heat + work) outputs. In both preparations, the rates of work performance (power), total energy output and, to a lesser extent, heat output were high when the fibres were shortening. However, at the start of stimulation, when the preparation was actively developing force and yet still being stretched, the total energy output decreased slightly. This reflected work being done *on* the muscle (by the ergometer). Between periods of contraction, the rate of total energy liberation was low. Two features that distinguished the two muscle types can also be seen in these records. First, the rates of both work performance and of initial heat production, normalized for tissue mass, from fast-twitch fibres were much greater than those from slow-twitch fibres. Second, the relative contribution of mechanical work to the total energy output was much lower in fast muscle fibres than in slow fibres.

Energy output and cycle frequency

The distinctive energetic characteristics of the two muscle types during cyclic contractions are further illustrated in Fig. 2. Measurements of heat and work production were made from four preparations of each type of muscle. For both soleus and EDL, the rate of total initial energy output (i.e. power + heat rate) varied significantly (P<0.05) with cycle frequency. The maximum rate of total energy output from fast-twitch muscle

was sixfold higher than that of slow-twitch muscle. The mean maximum rate of energy liberation from soleus muscles was 35.3±5.2 W kg^{-1} (*N*=4; Fig. 2A), which occurred at a cycle frequency of 2 Hz. For EDL muscles, the total rate of energy output was maximal at the 6 Hz cycle frequency, where it had a value of 211.3±29.8 W kg^{-1} (*N*=4; Fig. 2B).

The rate of total energy output is the sum of the power output and the rate of heat production. There was a twofold variation in the power output of soleus muscles across the range of cycle frequencies used (Fig. 2A, filled circles). Despite this, there was no significant variation in the rate of heat production over this range of frequencies (Fig. 2A, open circles). Thus, the variation in the rate of total energy output with cycle frequency reflected variations in power output only. Furthermore, at cycle frequencies between 1.5 and 4 Hz, the rate of heat output was almost identical to the power output. In contrast, both power output and the rate of heat production by EDL fibres varied significantly with cycle frequency. At all cycle frequencies tested, the rate of heat production by EDL muscle exceeded the power output.

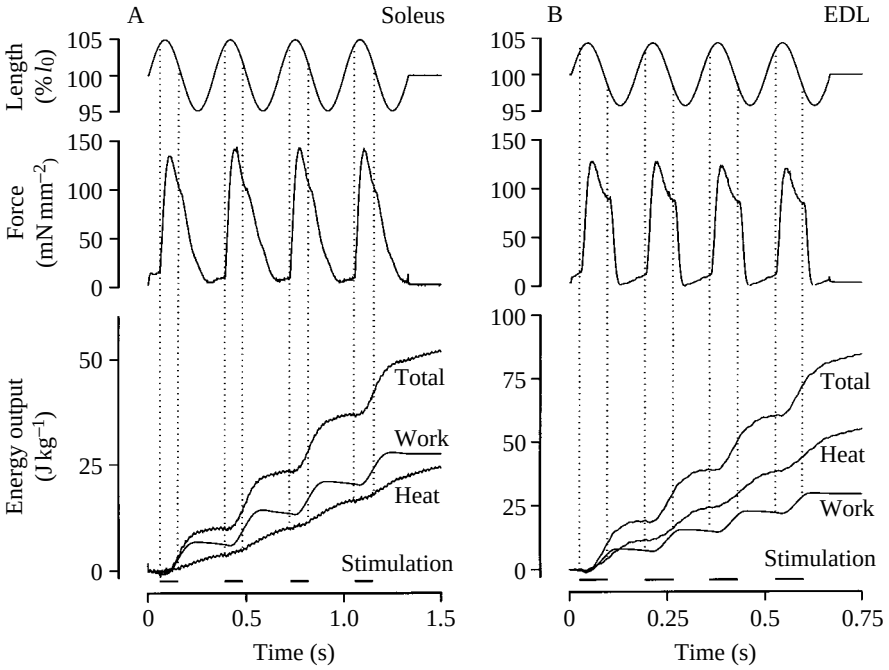

Fig. 1. Records showing the time course of changes in muscle length, force production and energy output for soleus (A) and EDL (B) muscle fibre bundles. The energy output traces show the measured heat production and work output, and the calculated total energy (work + heat) output. Note that the scales used to display energy output differ for the two preparations. The vertical dotted lines and solid horizontal bars mark the times during which the muscles were stimulated. The cycle frequencies were 3 Hz for the soleus fibre bundle and 6 Hz for the EDL fibre bundle. The stimulus variables were those given for these cycle frequencies in Table 1. The characteristics of the two preparations (soleus and EDL, respectively) were: wet mass (mg), 2.31, 4.90; total length (mm), 11.5, 10.3; maximum isometric force (mN mm^{-2}), 251.0, 248.9.

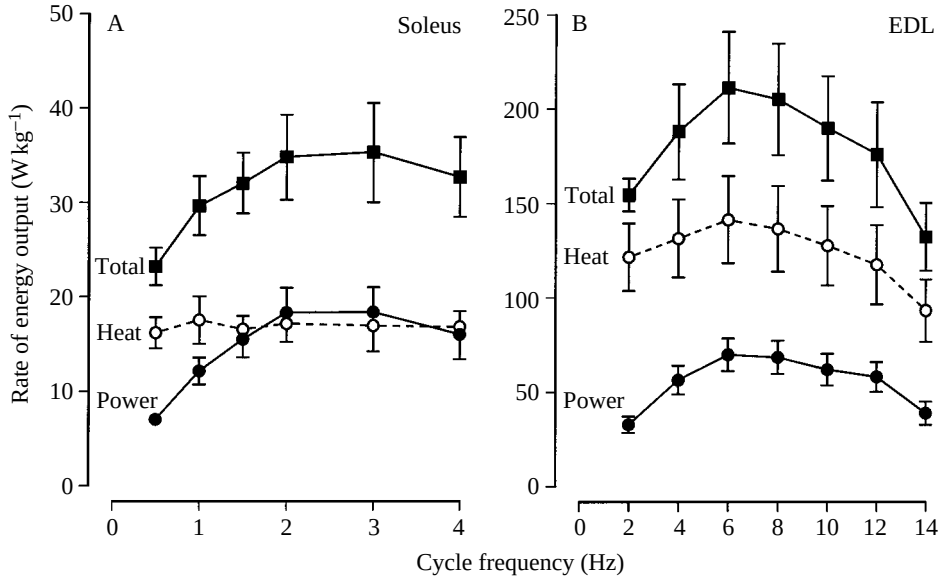

Fig. 2. The dependence on cycle frequency of power output, the rate of initial heat production and rate of total initial energy output for soleus (A) and EDL (B) muscle (N=4 for both muscles). Total initial energy output is the sum of the rate of initial heat production and the mechanical power output. Both power and heat rate were averaged over whole cycles. Note that the scales are different for the two muscles. Values are means ±1 S.E.M. Where the error bars were smaller than the radius of the symbols, they have been omitted.

Efficiency

The relationship between power output and the rate of total initial energy output is quantified by the initial mechanical efficiency. Fig. 3 shows the dependence of mechanical efficiency on cycle frequency for the two muscles. The initial mechanical efficiency of soleus muscles varied greatly with cycle frequency (Fig. 3A). For example, at a cycle frequency of 0.5 Hz, efficiency was 0.30±0.03 (N=4) whereas at 3 Hz, efficiency had increased to its maximum value of 0.52±0.01 (N=4). Efficiency was within 10 % of the peak value for frequencies from 1.5 to 4 Hz. Thus, over this range of frequencies, about half the total energy output of slow-twitch muscle appeared as mechanical work. In contrast, work constituted, at most, about one-third of the energy liberated from fast-twitch muscle (Fig. 3B). Peak efficiency of fast-twitch muscle was 0.34±0.03 (N=4) calculated at a cycle frequency of 12 Hz. However, efficiency was within 10 % of this value for cycle frequencies from 4 to 14 Hz.

Discussion

The aim of these experiments was to determine the initial mechanical efficiency of isolated mammalian skeletal muscle contracting in a realistic manner. Although the contraction protocol used may not exactly simulate the patterns of activity of mouse

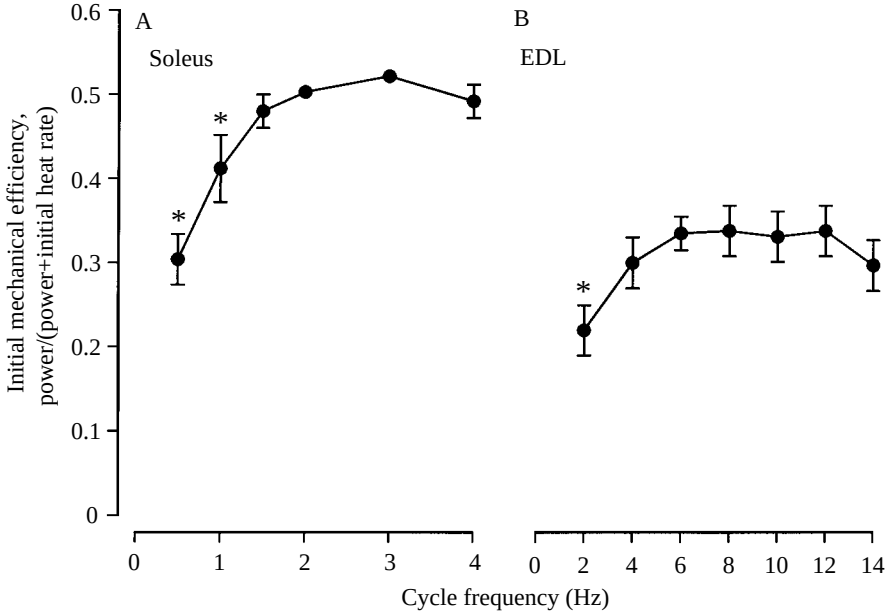

Fig. 3. The dependence of initial mechanical efficiency on cycle frequency for soleus (A) and EDL (B) muscle (N=4 of each muscle type). At each cycle frequency, efficiency was measured using the stimulus variables that gave the maximum power output at that cycle frequency. Asterisks indicate values that differ significantly (P<0.05) from the maximum value. Values are means ±1 S.E.M.

muscles during locomotion, it must resemble them more closely than protocols previously used in studies of the efficiency of mammalian muscle. Initial mechanical efficiency quantifies the fraction of the energy liberated from high-energy phosphates during contraction that appears as useful mechanical work. The source of most of this energy is ultimately ATP. In unfatigued muscle, ATP is rapidly resynthesised by the breakdown of creatine phosphate, and it is the enthalpy of this process that is actually measured. Energy from ATP is converted into mechanical work, both external and internal, and is also used by various ion pumps, particularly the Ca^{2+} pump of the sarcoplasmic reticulum.

These processes can be categorised as either force-generating processes or non-force-generating processes. The partitioning of energy output between force-generating and non-force-generating processes affects the efficiency of muscle. If a greater fraction of total energy output were due to non-force-generating processes, then the mechanical efficiency would be lower. This has been suggested to account for some of the difference in efficiency of fast and slow muscles of dogfish during performance of cyclic contractions (Curtin and Woledge, 1993*b*). The relative energy expenditure on force-generating and non-force-generating processes in cyclically contracting muscles is not yet known, so the possible contribution it may make to the relative efficiencies of EDL and soleus muscles is difficult to assess. However, to take an extreme case, even if non-force-generating processes contributed 25 % of the total energy output of EDL muscles

during cyclic contractions, as they do during isometric contractions (Wendt and Barclay, 1980), and made no contribution at all in soleus muscles, then only half of the difference in efficiency would be accounted for. So, although differences in the relative energetic cost of non-force-generating processes may contribute to the difference in mechanical efficiency of the two muscles, it is very unlikely to be the sole source of the difference.

Another factor that could account for the different efficiencies of the two muscle types is energy used to perform internal work. Internal work is that done by the contractile apparatus in stretching series elastic elements within the muscle. Internal work, like external work, uses chemical energy and produces heat. The amount of internal work performed is not included in the measurement of external work. However, when the internal work is reversed, for example during relaxation, the energy used for internal work would be expected to appear as heat. In the protocol used in this study, heat measurements encompassed both the period of force development, when internal work would have been performed, and the period of force relaxation, when the internal work would have been dissipated as heat. Thus, any internal work performed was included in the net heat output measured over a cycle of length change. Performance of internal work would increase the total initial energy output (heat + work) but would not increase external work: initial mechanical efficiency would thus be decreased by internal work. The EDL fibre bundles used in this study have a smaller fibre length:muscle length ratio than do soleus preparations (0.79 *versus* 0.89; Barclay *et al.* 1993) and hence the relative length of tendon was greater in EDL fibre bundles than in soleus fibre bundles. Tendon is a major component of the series elastic elements of muscle (for a review, see Ettema and Huijing, 1990), so it seems reasonable to suppose that more internal work occurred in EDL preparations. In the absence of data concerning the series compliance and time course of changes in the lengths of compliant components, it is difficult to make a quantitative assessment of the relative internal work, and of the energetic cost of that work, in each muscle. Further experiments should be performed to assess the contribution that differences in internal work make to mechanical efficiency of fast- and slow-twitch mouse muscle. Internal work will most probably occur not only in isolated muscles but also *in vivo* and is therefore one of the energy-using processes of functioning skeletal muscle.

Heat liberation from parvalbumin–Ca^{2+} interaction

Calculations of efficiency involving heat measurement can also be affected by heat liberated by processes not associated with high-energy phosphate use. Net production of heat from non-metabolic sources would cause heat measurements to overestimate the amount of ATP (or creatine phosphate) hydrolysed and therefore to underestimate mechanical efficiency. One potential source of such heat is the exothermic binding of Ca^{2+} to parvalbumin, which makes a significant contribution to initial heat production in some skeletal muscles (Woledge *et al.* 1985, pp. 257–260). This protein is present in mouse EDL muscle, but not in soleus muscle (Heizmann *et al.* 1982). Parvalbumin binds Ca^{2+} rapidly during contraction, but the subsequent dissociation occurs relatively slowly (Hou *et al.* 1991). If, in EDL preparations, there was a net increase in the saturation of parvalbumin during a contraction cycle, then there would also have been net heat production.

In the following estimate of the amount of heat liberated from this source, and its effect on the calculated efficiency, it has been assumed that the Ca^{2+}-binding kinetics of frog muscle parvalbumin, determined *in vitro* (Hou *et al.* 1991), are applicable to mouse EDL muscle. Adjusting the Ca^{2+}-binding and dissociation rates to those expected at 31 °C, using Q_{10} values of 1.9 and 2.3, respectively (Hou *et al.* 1990), gives a Ca^{2+}-binding rate constant of $6.8\,s^{-1}$ and a dissociation rate constant of $2.7\,s^{-1}$. Mouse EDL contains approximately $0.4\,mmol\,l^{-1}$ parvalbumin, which binds $0.8\,mmol\,l^{-1}$ Ca^{2+}, liberating $25\,kJ\,mol^{-1}$ of Ca^{2+} bound (Woledge *et al.* 1985, p. 258). Assuming that Ca^{2+} occupies 40 % of the binding sites in resting muscle (Hou *et al.* 1991), and that Ca^{2+} binding occurs for 50 % of a cycle and dissociation for the other 50 %, then at a 6 Hz cycle frequency, the net rate of heat production in the fourth cycle of a series resulting from binding of Ca^{2+} by parvalbumin would have been approximately $1\,W\,kg^{-1}$, or 0.5 %, of the total energy output. This extra heat production would result in an underestimate of the efficiency of EDL muscle of only 0.2 % (i.e. 0.34 *versus* 0.342). At a 12 Hz cycle frequency, this source of heat would account for approximately 4 % of the total energy output and would result in a 1.3 % underestimate of efficiency. Heat arising from net binding of Ca^{2+} by parvalbumin therefore probably does make a small contribution to initial heat production, but the effect on calculated efficiency is also small and accounts for little of the difference between soleus and EDL muscles. This means that the different efficiencies of fast- and slow-twitch muscles are not fully accounted for either by a relatively low energetic cost of Ca^{2+} cycling in soleus or by extra, non-metabolic heat production due to parvalbumin–Ca^{2+} interactions in EDL muscles. The possible contribution of internal work production to the calculated efficiencies is unclear. However, it seems very likely that part, perhaps the major part, of the difference reflects a real difference in the efficiency of myofibrillar energy conversion in the two muscle types. This would be consistent with the inverse relationship between myofibrillar power output and mechanical efficiency seen in many other types of muscle (Josephson and Stevenson, 1991; Curtin and Woledge, 1991).

Efficiency in isovelocity contractions and cyclic contractions

It is of interest to compare the initial mechanical efficiencies determined using cyclic contractions (this study) with those determined previously using isovelocity contractions (Barclay *et al.* 1993). The previous experiments were performed at a lower temperature (21 °C) than were the current experiments. However, mechanical efficiency varies little with temperature (Rall and Woledge, 1990) so, assuming this holds for a comparison of efficiency values determined using quite distinct mechanical protocols, a comparison can still be made. The peak efficiency of mouse soleus muscle was approximately 20 % higher during cyclic contractions than during isovelocity contractions (0.52 *versus* 0.31). In contrast, the peak efficiency of fast-twitch EDL muscle was only 6 % higher during cyclic contractions than during isovelocity contractions (0.34 *versus* 0.28). Thus, the initial mechanical efficiency determined during cyclic contractions was greater than that determined during isovelocity shortening and, furthermore, this effect was most striking in the slow-twitch muscle. What might account for such results?

The protocols used to determine efficiency during isovelocity contractions (Barclay *et al.* 1993) and cyclic contractions (this study) were distinguished not only by the

temporal pattern of stimulation (prolonged, maximal contractions *versus* brief, submaximal contractions) and changes in muscle length, but also by the activities of muscles during the time over which energy output was measured. In the isovelocity contractions, energy output was measured while the muscle was both stimulated and shortening. In the present study, the energy output was calculated over a full cycle that included both stretch and shortening during stimulation, shortening during relaxation (i.e. after the end of stimulation but before active force production had ceased), and stretch with no active force production. Several of these characteristics, for example stretch at the start of contraction, work performance during relaxation and the relatively brief contraction duration, could contribute to the higher efficiency of cyclic contractions compared with isovelocity contractions.

Stretching a contracting muscle enhances force output and increases the work that can be performed during a subsequent shortening (e.g. Heglund and Cavagna, 1987). Several experiments have addressed the possibility that stretching a muscle immediately before it shortens increases mechanical efficiency. In these experiments, frog and rat muscles performed prolonged (longer than 0.5 s) tetanic contractions, which contained isovelocity stretches, immediately before an isovelocity shortening (Heglund and Cavagna, 1987; de Haan *et al.* 1989). Efficiency in both these cases was defined as work done *by* the muscle shortening divided by energy cost, with energy cost estimated from the use of high-energy phosphate (de Haan *et al.* 1989) or from oxygen consumption (Heglund and Cavagna, 1987). Isovelocity stretches did increase the mechanical efficiency during the shortening. However, if the work done on the muscle during the stretch was also taken into account, as it was in the current study, the increases in efficiency were eliminated. The definition of mechanical efficiency used in this study is fundamentally different in that it uses net work output. It therefore includes both work done *on* the muscle during stretch and that done *by* the muscle during shortening (this study; Curtin and Woledge, 1993*a*,*b*; Josephson and Stevenson, 1991). Using this definition, the initial mechanical efficiency of fish muscle, in particular slow, red muscle, is greater when stimulation starts during stretch than when it begins at the start of the shortening phase (Curtin and Woledge, 1993*a*,*b*). Therefore, stretch may contribute to the high mechanical efficiency determined in cyclic contractions. A difference between cyclic contractions and the isovelocity stretch-and-shorten protocols that may account for these contrasting results is that the amplitude of stretches, and their effect on force, in the cyclic contractions was small relative to those used in the isovelocity stretch-and-shorten protocols (Curtin and Woledge, 1993*a*).

Part of the work performed during the cyclic contractions occurs during force relaxation. The energetic cost of force production during relaxation of frog muscle is lower than that during the plateau of an isometric contraction (Curtin and Woledge, 1974). If the energetic cost of work performance during relaxation is also lower than that during a tetanic contraction, then this could account for some of the difference between efficiencies determined in cyclic contractions and in isovelocity contractions. Two possible mechanisms by which work could be performed more efficiently during relaxation have been discussed by Curtin and Woledge (1993*a*). These possibilities were (1) that the energy expended on Ca^{2+} pumping may be much lower during relaxation when free Ca^{2+} concentrations are very low; and (2) that cross-bridges may be able to perform several

power strokes with the energy from hydrolysis of one ATP molecule (in contrast to the conventional idea that one ATP is hydrolysed per power stroke) and that this process would be favoured when fewer cross-bridges are attached, as during relaxation. If, however, a relaxation-based mechanism does account for the difference between efficiencies determined in cyclic contractions and those determined in classical isovelocity-release protocols, then there must be a differential effect on slow and fast muscles. In both muscles, relaxation (i.e. the interval between the delivery of the last stimulus pulse and the time at which force decreased to less than 10 % of the peak force) occupied approximately 30 % of the whole cycle at the cycle frequencies at which efficiency was maximal. The high efficiency of soleus muscle relative to that of EDL is not, therefore, due to a greater fraction of the work being performed during relaxation in the slow muscle.

A characteristic that distinguishes the mechanical performance of muscles during cyclic contractions at the frequencies at which efficiency is maximal from that during isovelocity releases within the force plateau of an isometric tetanus is the maximum force reached. In both soleus and EDL fibres, contracting at cycle frequencies at which initial mechanical efficiency was high, the peak forces were between 40 and 60 % of maximal isometric force. Although this must partly be a reflection of the force–velocity properties of the muscles, it also suggests that the level of activation of the myofilaments by Ca^{2+} may have been less than that required to activate the muscles fully. Sinusoidal work performance by insect muscle is affected by the degree of activation. The mechanical efficiency (net power output divided by rate of myofibrillar ATP use) of skinned insect muscle fibres was greatest when myofibrillar Ca^{2+} concentration was less than that required to activate the muscles fully (Steiger and Rüegg, 1969). Although those experiments were performed on insect muscles and under very different conditions from those of the current experiments, the idea that the efficiency of sinusoidal work performance may vary with the level of activation is worthy of further investigation.

The contrasting results from isovelocity shortening protocols, isovelocity stretch-and-shorten protocols and brief, sinusoidal cyclic contractions illustrate that the contraction protocol has a crucial influence on mechanical efficiency. Therefore, when the results of isolated muscle experiments are used to make inferences regarding locomotion, it is important that the contraction protocol matches, as closely as possible, the patterns of muscle activity occurring during locomotion.

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