Energetics of lengthening in mouse and toad skeletal muscles

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1. The energetics of lengthening were studied in amphibian and mammalian skeletal muscle. The aims were to determine whether energy absorption during stretch is a general property of skeletal muscle and to investigate the influence of lengthening velocity on energy absorption.

2. Experiments were performed *in vitro* (21 °C) using bundles of muscle fibres from fast-twitch extensor digitorum longus and slow-twitch soleus muscles of the mouse and tibialis anterior muscles of a toad, *Bufo marinus*. Initial heat production and mechanical work done on muscles were measured during isovelocity lengthening. Enthalpy output during lengthening was calculated as the difference between the amount of heat produced and the work done.

3. For all three muscle types, more energy was put into muscles as work than was produced as heat. Thus, part of the energy put into muscles to stretch them must have been absorbed.

4. For all three muscle types, the amount of energy absorbed was constant at velocities exceeding $\sim 0{\cdot}5\,V_{max}$ (V_{max} is the maximum shortening velocity), but was significantly lower at slow velocities of lengthening. The same amount of energy was absorbed by all three muscles when lengthened at $\geqslant 0{\cdot}5\,V_{max}$.

5. It was concluded that absorption of energy during lengthening occurs in mammalian as well as amphibian muscle and that lengthening velocity has only a small effect on the amount of energy absorbed.

Skeletal muscles that power movement during locomotion undergo cyclic changes in length. Locomotor muscles generate power during active shortening and are then lengthened (by antagonist muscles) back to the length from which shortening starts. For most of the lengthening, the muscle is relaxed and provides little resistance to being stretched. However, in many instances, locomotor muscles start to contract and develop force before lengthening is complete (e.g. Griffiths, 1989; Altringham & Johnston, 1990; Johnston, 1991). This may be important energetically. For example, it has been suggested that energy is absorbed during the lengthening and that this energy can be converted into mechanical work during a subsequent shortening (for a review see Alexander & Bennet-Clark, 1977). However, this type of analysis is hindered by a lack of knowledge about the energetics of lengthening muscle.

The energetic characteristics of skeletal muscles during shortening are strikingly different from those during lengthening. For instance, when a contracting skeletal muscle is shortened, its force output decreases, its rate of ATP usage increases and it performs mechanical work (e.g. Kushmerick & Davies, 1969; Barclay, Constable & Gibbs, 1993). In contrast, when a muscle is lengthened, its force output increases, its rate of ATP hydrolysis decreases and the muscle has work done on it (by whatever does the lengthening) (e.g. Curtin & Davies, 1973; Flitney & Hirst, 1978). In accord with the principle of energy conservation, work done on a muscle during lengthening is ultimately transformed into heat. However, in amphibian muscle, not all the work done on the muscle is converted into heat within the time course of a brief contraction (Abbott, Aubert & Hill, 1951; Hill & Howarth, 1959). Thus, amphibian muscles absorb energy during lengthening. Surprisingly, the characteristics of this energy absorption have not been extensively studied. In particular, there have been no studies using mammalian muscle, so it is not known whether energy absorption during lengthening is a general characteristic of skeletal muscle or peculiar to amphibian muscle. Also, the effects of stretch velocity on energy absorption have not been systematically studied. Thus, the aims of these experiments were (1) to investigate the energetics of lengthening in mammalian skeletal muscle, comparing fast- and slow-twitch muscles, and (2) to investigate the effects of lengthening velocity on muscle energetics.

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In the present study, the energetics of lengthening were investigated using measurements of work input and muscle heat output. The net energy output of the muscle (or enthalpy change, ΔH) during lengthening is the difference between the energy liberated as heat and that put into the muscle as work. For example, if during lengthening the heat output is less than the work input, then the energy content of the muscle has increased and thus ΔH is negative. The heat output would be expected to include a component due to initial biochemical breakdown (i.e. phosphocreatine (PCr) hydrolysis) and a component arising from the conversion of work done on the muscle into heat. Although it is not possible to differentiate between these two sources of heat, it is possible to determine whether or not a significant fraction of the work done on the muscle is absorbed. To facilitate this analysis, the protocol used in the current study was designed so that the amount of energy used to stretch muscles was large compared with that expected to be produced as heat by the biochemical reactions underlying contraction. Thus, any change in the heat output from biochemical reactions during lengthening would have a minimal effect on the net enthalpy change. For example, in the current study, the amount of energy put into muscles as work at $0{\cdot}5\,V_{max}$ was typically 10 times greater than the amount of energy expected to have been produced by PCr splitting under isometric conditions for a time equal to the time spent stretching. In frog muscle, the rate of PCr splitting is reduced during lengthening to ~50% of the rate during an isometric contraction (Curtin & Davies, 1973). Assuming that PCr-related heat output from muscles used in the current study also decreased by 50% during lengthening, then at $0{\cdot}5\,V_{max}$, the amount of energy put into muscles to stretch them was at least 20-fold greater than would be expected to be produced by PCr splitting.

The current experiments showed energy was absorbed during lengthening not only by amphibian muscle but also by mammalian muscle. The magnitude of the absorption was independent of muscle type and was also relatively insensitive to lengthening velocity. Some of these results have been presented before in a preliminary form (Constable, Barclay & Gibbs, 1997).

METHODS

Preparations and solutions

Experiments were performed *in vitro* using small bundles of muscle fibres obtained from adult female mice (Swiss) and from cane toads (*Bufo marinus*). Mice were rendered unconscious by inhalation of chloroform then killed by cervical dislocation. Toads were stunned by a blow to the head then pithed. All animal handling procedures met local ethical requirements. Fibre bundles were dissected from extensor digitorum longus (EDL) and soleus muscles of mice and from tibialis anterior muscles of the cane toad. During both dissection and experiments, mouse muscles were bathed in Krebs–Henseleit solution containing (mM): NaCl, 118; KCl, 4·75; $MgSO_4$, 1·18; $NaHCO_3$, 24·8; KH_2PO_4, 1·18; $CaCl_2$, 2·54; and glucose, 10. Toad muscles were bathed in Ringer solution containing (mM): NaCl, 111; KCl, 2·5; $NaHCO_3$, 2·4; KH_2PO_4, 0·1; $CaCl_2$, 1·8; and glucose, 11. Solutions were continuously aerated during experiments (95% O_2–5% CO_2 for mouse muscles, 100% O_2 for toad muscles) and maintained at 21 °C.

Fibre bundles were connected to the recording apparatus using silk threads (0·075 mm diameter) tied around the tendons at either end of the preparation. To minimize compliance, the length of silk was < 5 mm and threads were tied close to the point where the muscle fibres attached to the tendon. Fibre bundles were mounted vertically on a thermopile and enclosed by a chamber containing ~80 ml of aerated bathing solution. One thread was attached to a tungsten rod (0·14 mm diameter, 7 cm length) connected to the lever arm of an ergometer, the other thread was held in a fixed-position clamp. The position of the lever, and hence muscle length, was controlled by an ergometer (model 300H; Cambridge Technologies, Watertown, MA, USA). Fibre bundles were stimulated via two platinum wire electrodes that lightly touched either side of the muscle preparation midway along its length. Supramaximal stimulus pulses were of 0·5 ms duration and ~5 V in amplitude. The minimum frequency of stimulation required to achieve a fused tetanus was used. This was 50 Hz for soleus, 80 Hz for EDL and 140 Hz for toad tibialis anterior muscle. Characteristics of the fibre bundles used are summarized in Table 1. Fibre lengths of muscles were calculated using fibre length to muscle length ratios. For mouse muscles, previously reported ratios were used (Barclay *et al.* 1993). For toad tibialis anterior muscles, the fibre length to muscle length ratio was determined using a method described previously (Barclay *et al.* 1993). From twenty-four observations, on six toad tibialis anterior fibre bundles, the mean length of fibres was approximately the same as the muscle length.

Experimental recordings

The basic mechanical and thermal measurement techniques have been described in detail previously (Barclay *et al.* 1993) and only a brief description is presented here.

Mechanical measurements. Muscle force production and length change were measured using the ergometer. Force and length signals were sampled at 500 Hz, digitized, displayed on a monitor and stored on disk.

Thermal measurements. During experiments, fibre bundles lay on a strip of platinum (14 mm × 2 mm × 0·05 mm). The temperature of the strip was measured using twenty antimony–bismuth thermocouples connected in series and attached to the underside of the platinum strip. The Peltier effect (Kretzschmar & Wilkie, 1972) was used to determine that the output of the thermopile was 1·39 mV $°C^{-1}$. Temperature signals were filtered (low-pass filter; cut-off frequency, 20 Hz) and amplified using a low-noise amplifier (model 15c-3a; Ancom Instruments, Cheltenham, UK).

The strip of platinum was longer than any fibre bundle used and thus its temperature reflected the average temperature along the length of the muscle. During experiments, heat was lost from the metal strip along the thermocouple wires to the frame of the thermopile. The amount of heat lost was calculated using the procedure of Woledge, Curtin & Homsher (1985, pp. 184–187) and heat signals were corrected accordingly. Although the metal strip averaged the temperature along the length of the muscle, it also distorted the time course of the thermal records (Wilkie, 1968). The time lag between a change in muscle temperature and detection of the change in temperature of the metal strip was ~200 ms. Thus, no information regarding the time course of energy production during lengthening was obtained. However, the primary purpose of these experiments was to measure total amounts of heat produced rather than the time course of heat evolution.

Table 1. Characteristics of fibre bundles from mouse soleus and EDL muscles and from toad tibialis anterior muscles

	Overall length (mm)	Fibre length (mm)	Wet muscle mass (mg)	Cross-sectional area (mm^2)
Soleus ($n = 6$)	10·4 ± 0·2	9·2 ± 0·2	2·59 ± 0·16	0·25 ± 0·02
EDL ($n = 6$)	9·2 ± 0·6	7·3 ± 0·5	3·18 ± 0·41	0·34 ± 0·03
Tibialis anterior ($n = 4$)	12·8 ± 0·7	12·7 ± 0·7	3·75 ± 0·56	0·29 ± 0·04

Experimental protocol

At the start of each experiment, a series of brief (0·3 s) tetani was used to determine the isometric force–length relation. The length of the fibre bundle was then set to a length corresponding to the lower (i.e. shorter) end of the plateau region of the force–length relation (initial muscle length, L_i).

To study the energetics of muscles during lengthening, fibre bundles were lengthened at constant velocity during the force plateau of an isometric tetanus. The amplitude of the stretch was 10% L_i, which corresponded approximately to the length of the plateau of the force–length relation. This length range was used in an attempt to match the isometric forces developed before and after stretch and hence to minimize the influence of any tension-dependent energy changes (Homsher, 1987). Soleus fibre bundles contracted isometrically for 1 s before being stretched, whereas both EDL and tibialis anterior fibre bundles contracted for 0·5 s prior to stretch. Following lengthening, muscles were again held at constant length and stimulation continued for at least 0·25 s. This time was sufficient to establish steady levels of both force and rate of heat production in the post-stretch phase of contraction.

Fibre bundles were lengthened at five velocities between zero and the maximum shortening velocity (V_{max}). The velocities were presented in both ascending and descending order so that each fibre bundle performed each velocity twice. The order of presentation (i.e. ascending or descending velocities first) was varied amongst preparations and an interval of at least 15 min separated the two runs. For each fibre bundle, results from the two runs were averaged.

At the end of each experiment, tendons were removed and muscles were dried for at least 24 h. Dry mass was measured using an electronic balance (Cahn 25; Cahn Instruments, Cerritos, CA, USA). Wet mass was estimated using a wet : dry mass ratio of 5·0 (Leijendekker, Hardeveld & Elzinga, 1987).

Analysis of records

Isometric force was measured immediately before the start of lengthening. The work done on muscles during lengthening (W) was calculated by integrating muscle force output with respect to the distance lengthened.

For mouse muscles, the amount of heat produced during lengthening (Q) was calculated using a method described previously (Homsher, 1987; Barclay *et al.* 1993). This method (see Fig. 1) accommodated the temporal distortion arising from use of the integrating thermopile by calculating the difference between the total amount of heat produced during both lengthening and the subsequent isometric phase of the contraction (Q_t) and the estimated amount of heat produced during the post-stretch isometric contraction (Q_i). Q_i was determined by extrapolating the heat signal recorded during the post-stretch isometric tetanus back to the time at which lengthening ended (Fig. 1). Assuming that the isometric force was similar before and after lengthening, this protocol accommodated any transient changes in heat production that may arise from purely force-dependent energy changes. For example, two processes which could have had transient effects on heat records were muscle thermoelasticity (Woledge, 1961; Gilbert & Matsumoto, 1976) and extension of elastic components of muscle. It should be noted that this analysis did not include heat produced during relaxation after stimulation ended.

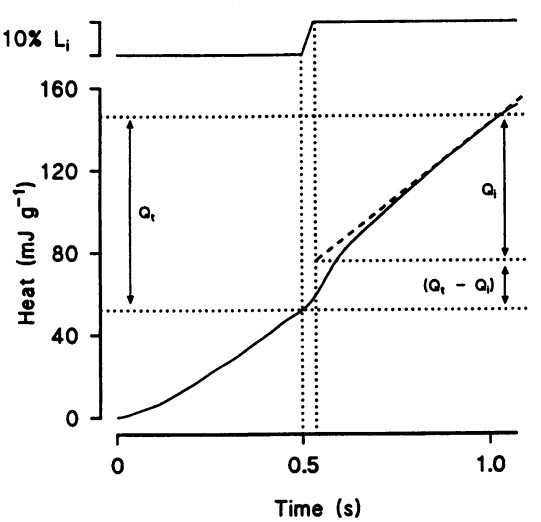

Figure 1. Method for calculating the amount of heat produced during lengthening in mouse muscle

Records of muscle length (upper panel) and heat production (lower panel) from an EDL fibre bundle. The records were made during a 1 s tetanus, with lengthening at ~0·2 V_{max} after 0·5 s. The duration of lengthening is indicated by the vertical dotted lines. Q_i is the amount of heat produced during the post-stretch isometric phase of the contraction and was determined by extrapolating (dashed line) from the heat produced during the post-stretch isometric contraction back to the time at which lengthening ended. Q_t is the total heat produced both during lengthening and the isometric phase of contraction following lengthening. The amount of heat produced due to lengthening was the difference between Q_t and Q_i.

The assumption that heat was produced at a constant rate during the pre- and post-stretch isometric phases of contraction was not met by toad fibre bundles (see Fig. 2). Thus, the amount of heat produced by toad fibre bundles during lengthening was calculated by determining the difference between the total amount of heat produced during a contraction which included a period of stretch ($Q_{stretch}$) and the total amount of heat produced during an isometric tetanus (Q_{isom}) (Fig. 2). $Q_{stretch}$ and Q_{isom} were the mean values of the heat signals measured over a 200 ms section of the records starting 100 ms before the end of stimulation. It should be noted that this method of analysis determined the heat produced in excess of the isometric heat during lengthening. To be consistent with the data for mouse muscles, it was necessary to add an amount of heat equivalent to the heat produced under isometric conditions in the lengthening duration. Thus, both methods of analysis calculated the total heat output during lengthening.

For all muscles, the mean enthalpy change (ΔH) was calculated from measurements of the mean heat output and the mean work done. That is:

$$\Delta H = Q + W.$$

During an isometric contraction, no external work is performed and hence enthalpy production is equal to heat production (i.e. $\Delta H = Q$). However, when a muscle is lengthened, work is done on the muscle by the apparatus. By convention, the work done on the muscle during lengthening is assigned a negative value (i.e. $W < 0$). Thus, during lengthening, the enthalpy output is the difference between the amount of heat produced by the muscle and the amount of work done on the muscle (i.e. $\Delta H = Q - W$).

During stimulation, some heat is produced by stimulus current passing through the muscle. The amount of stimulus heat was estimated by stimulating fibre bundles after they had been rendered inexcitable by prolonged depolarization. This accounted for ~14, 12 and 19% of the isometric heat output in mouse soleus, mouse EDL and toad tibialis anterior muscles, respectively. Stimulus heat was subtracted from records of muscle heat production prior to analysis.

Data presentation and statistical analysis

Data are presented as means ± S.E.M. Where data are presented as a function of velocity, lengthening velocity is expressed as a fraction of V_{max}. For mouse muscles, V_{max} values reported previously for mouse soleus and EDL muscles were used (Barclay *et al.* 1993). For toad muscles, V_{max} was estimated during each experiment by determining the shortening velocity required to just reduce force to zero. Shortening velocity estimated in this manner is, in fact, ~108% of V_{max} estimated from the force–velocity relation (Edman, 1979). However, this difference had minimal effect on the relationship between energy output and normalized shortening velocity and therefore no adjustment has been made. For mouse muscles, the statistical significance of variations in amount of energy production with velocity were determined using one-way analysis of variance. When required, *post hoc* analysis was performed using the Tukey–Kramer test for multiple comparisons. The non-parametric Freidman test for repeated measures was used to determine the statistical significance of changes in energy production with lengthening velocity in toad muscles. All statistical decisions were made with respect to the 95% level of confidence.

RESULTS

Energetics of lengthening in toad muscle

Previous myothermic experiments that have examined the energetics of lengthening have been performed using amphibian muscle (Abbott & Aubert, 1951; Abbott *et al.* 1951; Hill & Howarth, 1959). These experiments have shown that, during lengthening, the amount of heat produced by frog muscle is often considerably less than the amount of work done on the muscle to lengthen it. In the current study, the heat-measuring device and the procedure for heat calibration were different to those used previously, at least in their details. Thus, it was decided to repeat the early experiments using an amphibian muscle. The tibialis anterior muscle of *Bufo marinus* was used.

Figure 3 shows typical records of force and heat production by toad tibialis anterior fibre bundles. In response to lengthening, there was an initial, rapid rise in force that gave way to a slower, more sustained increase that continued throughout the lengthening. Force production decreased rapidly at the end of lengthening, to a new isometric level that was between 12 and 20% greater than

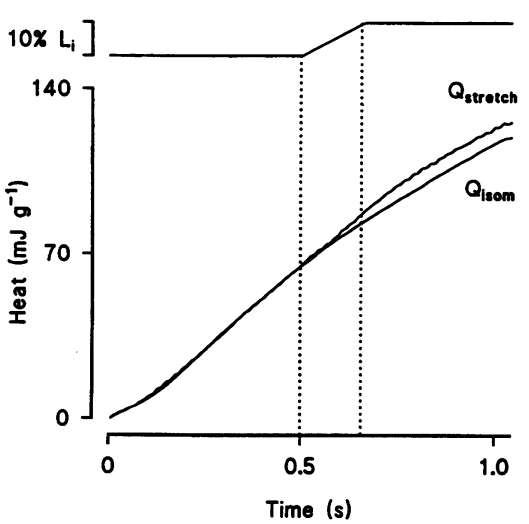

Figure 2. Method for calculating the amount of heat produced during lengthening in toad muscle

Records of muscle length (upper panel) and heat production (lower panel) from a toad tibialis anterior fibre bundle. Record of muscle lengthening was made during a 1 s tetanus with lengthening at ~0·2 V_{max} after 0·5 s (enclosed by vertical dotted lines). Two records of heat production from the same fibre bundle are superimposed; the lower trace was recorded during an isometric contraction (at the pre-stretch muscle length), the upper trace during an isovelocity contraction. Q_{isom} is the amount of heat produced during an isometric contraction, $Q_{stretch}$ is the total amount of heat produced during an isovelocity lengthening (see text for details). The heat due to lengthening was determined as the difference between $Q_{stretch}$ and Q_{isom}.

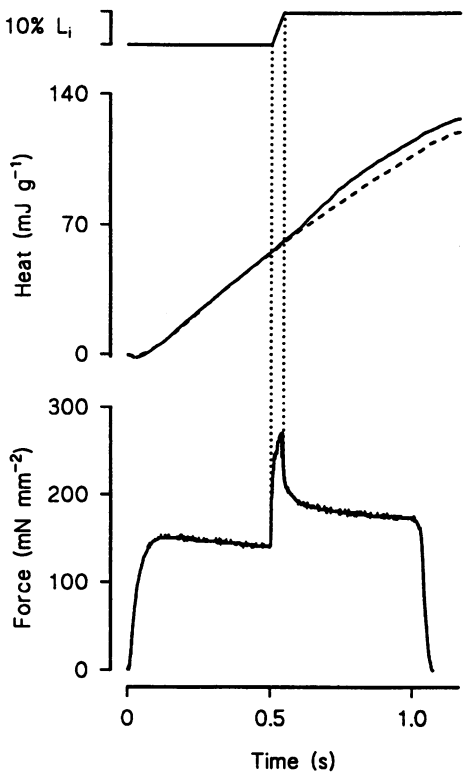

Figure 3. Examples of force, heat and length records from toad muscle

Records of force production (lower panel), heat production (middle panel) and muscle length change (upper panel) from a bundle of toad tibialis anterior muscle fibres (wet muscle mass, 3·94 mg; fibre length, 12·55 mm). Records were made during a 1 s isovelocity contraction. The period of $\sim 0{\cdot}7\ V_{max}$ lengthening commenced at 0·5 s; the duration of lengthening is enclosed by the vertical, dotted lines. The dashed line on the middle panel is a record of heat production from the same fibre bundle during an isometric contraction.

the pre-stretch isometric force. The changes in the force response are typical of muscles during lengthening and have often been described (e.g. Flitney & Hirst, 1978; Lombardi & Piazzesi, 1990). In contrast, the changes in the heat response were much more subtle. During the pre-stretch isometric phase of the contraction, the rate of heat production was constant (mean rate, $80{\cdot}7 \pm 16{\cdot}2$ mW g^{-1}; $n = 4$). When the muscle was lengthened, there was a small, transient increase in the rate of heat production. The heat rate then decreased gradually throughout the post-stretch isometric phase of contraction. A progressive reduction in the heat rate was also evident during isometric contractions (see both Figs 2 and 3) and is consistent with records from frog muscles which contain a labile heat component (e.g. Curtin & Woledge, 1977). The procedure used to determine heat production during lengthening accommodated any contribution from this component of the thermal records.

Comparison of heat output and work input

Figure 4 shows the mean amounts of heat output, work input and enthalpy change when toad fibre bundles were lengthened. The amount of work put into muscles during lengthening was clearly greater than the amount of heat produced by muscles. Consequently, ΔH was negative. For example, when fibre bundles were lengthened at $\sim 0{\cdot}5\ V_{max}$, ~ 27 mJ g^{-1} of energy was put into muscles as work, yet only about 14 mJ g^{-1} was produced as heat, so ΔH was -13 mJ g^{-1}. This indicates that energy was absorbed and is

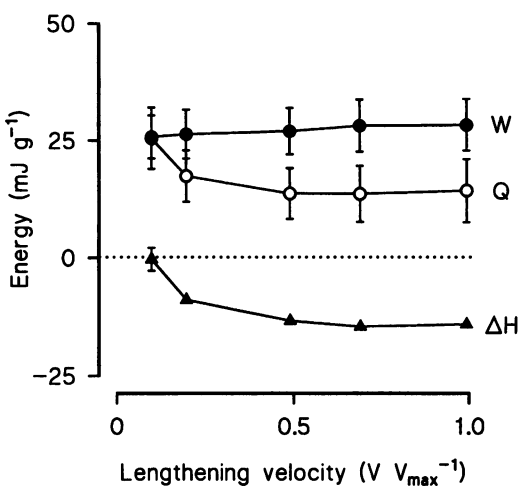

Figure 4. Effect of lengthening velocity on the mean amounts of work input, heat output and enthalpy change in toad muscle

Mean amounts of work input (●), heat output (○) and enthalpy change (▲) are expressed as functions of the relative lengthening velocity. Symbols represent mean values ($n = 4$) and error bars are S.E.M.

consistent with previous experiments on frog muscles during lengthening at ~0·5 V_{max} (Hill & Howarth, 1959).

It is not clear from previous experiments whether energy is also absorbed during slow increases in muscle length (Abbott & Aubert, 1951). Therefore in this study, the amphibian muscle was also used to examine the effect of lengthening velocity on the heat output, work input and enthalpy change (Fig. 4). The amount of heat produced by muscles was significantly affected by lengthening velocity ($P < 0·05$, $n = 4$), but the amount of work put into muscles was not. Furthermore, at velocities of lengthening $\geqslant 0·2\,V_{max}$, the work input clearly exceeded the heat output and consequently, ΔH was negative. Above ~0·5 V_{max}, ΔH was independent of lengthening velocity, but at lower velocities, its magnitude was smaller. Thus, energy was absorbed by toad muscles at all velocities other than the lowest used.

Energetics of lengthening in mouse muscle

The main purpose of this study was to determine whether the ability to absorb energy during lengthening is a general characteristic of skeletal muscle or peculiar to amphibian muscle. In particular, the energetics of lengthening were examined using fast- and slow-twitch mouse muscles.

Figure 5 shows typical records of force and heat production by soleus and EDL fibre bundles. The force response during lengthening was similar in both types of muscle and was also similar to that of the toad muscle. However, the force records of the mouse and toad muscles did differ during the post-stretch isometric phase of contraction. After lengthening, the isometric force produced by toad muscles was ~15% greater than the pre-stretch isometric force. Yet in both types of mouse muscle, the enhancement was not so marked; the isometric force after lengthening was always less than 10% greater than the force produced before lengthening. The heat response to lengthening was also similar in all three types of muscle. There was a slight difference between the records from mouse muscles and those from the toad muscle in that, for both types of mouse muscle, the rates of heat production before and after lengthening were similar.

Do mouse muscles absorb energy?

To determine whether mouse muscles absorb energy in response to lengthening, the amounts of work input and heat output were compared. Figure 6 shows work input, heat output and enthalpy change, for both soleus and EDL fibre bundles, when lengthened at ~0·5 V_{max}. In both types

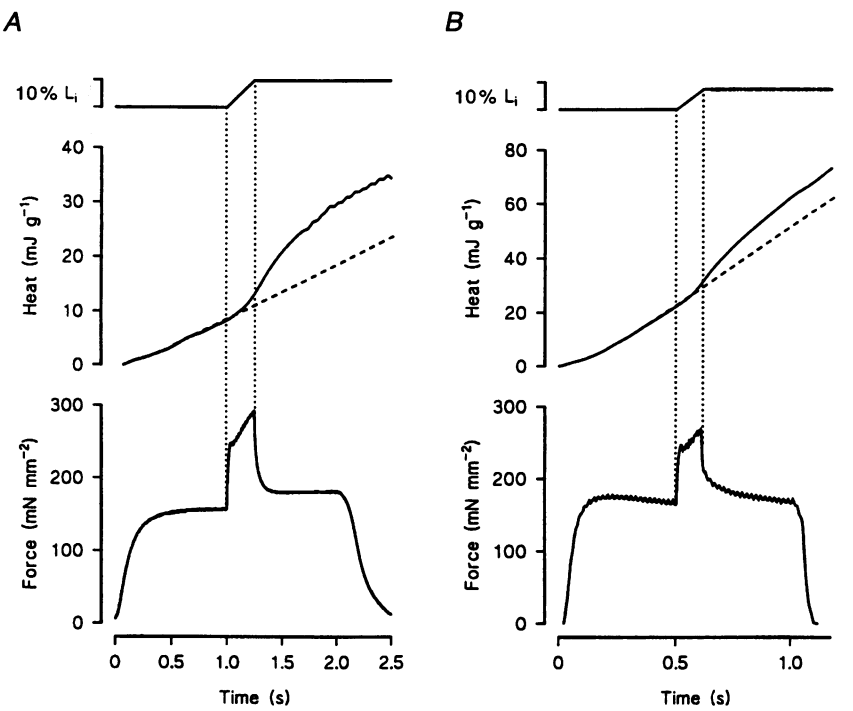

Figure 5. Examples of force, heat and length records from mouse muscles

Records are shown for soleus (*A*) and EDL (*B*) fibre bundles during isovelocity lengthening at ~0·2 V_{max}. For the soleus fibre bundle (wet mass, 2·72 mg; fibre length, 10·3 mm), lengthening commenced after 1 s of isometric contraction. For the EDL fibre bundle (wet mass, 2·96 mg; fibre length, 9·35 mm), lengthening began after 0·5 s of isometric contraction. The lengthening duration is indicated by the vertical dotted lines. The dashed lines drawn on the heat records are extrapolations of the isometric heat rate recorded before lengthening. Note that the scales for heat and time differ for the two preparations.

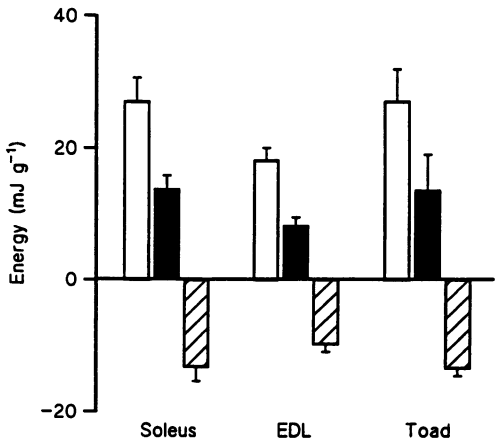

Figure 6. Work input, heat output and enthalpy change when lengthened at ~0·5 V_{max}

The mean values ($n = 6$) of the amounts of work input (□), heat output (■) and enthalpy change (▨) are shown for mouse soleus, mouse EDL and toad tibialis anterior fibre bundles when lengthened at ~0·5 V_{max}. The mean ΔH values were $-13{\cdot}2 \pm 2{\cdot}2$ mJ g^{-1} ($n = 6$), $-9{\cdot}8 \pm 2{\cdot}9$ mJ g^{-1} ($n = 6$) and $-13{\cdot}4 \pm 1{\cdot}2$ mJ g^{-1} ($n = 4$) for soleus, EDL and tibialis anterior fibre bundles, respectively.

of muscle, more work was put into muscles during lengthening ($27{\cdot}0 \pm 3{\cdot}6$ mJ g^{-1} for soleus; $18{\cdot}1 \pm 1{\cdot}9$ mJ g^{-1} for EDL), than was produced by the muscles as heat ($13{\cdot}8 \pm 2{\cdot}0$ mJ g^{-1} for soleus; $8{\cdot}3 \pm 1{\cdot}1$ mJ g^{-1} for EDL). Consequently, ΔH was negative ($-13{\cdot}2 \pm 2{\cdot}2$ mJ g^{-1} and $-9{\cdot}8 \pm 2{\cdot}9$ mJ g^{-1} for soleus and EDL, respectively) and there was no significant difference between the two types of muscle. This indicates that both fast- and slow-twitch muscles absorbed energy when lengthened at 0·5 V_{max}, and that both absorbed the same amount of energy. Furthermore, Fig. 6 also shows that the magnitude of ΔH in toad tibialis anterior muscle ($-13{\cdot}4 \pm 1{\cdot}2$ mJ g^{-1}) was the same as in the mouse muscles.

Does energy absorption in mouse muscle depend on lengthening velocity?

These results clearly show that both fast-and slow-twitch mouse muscles have the ability to absorb energy when lengthened at ~0·5 V_{max}. To determine whether energy absorption is affected by the rate of work input, muscles were lengthened at a range of speeds. The effect of lengthening velocity on the mean amounts of work input, heat output and enthalpy change are summarized in Fig. 7. In both types of muscle and at all velocities used, the mean amount of work done during stretch exceeded the mean amount of heat produced. Hence, ΔH was always negative, indicating that both types of mouse muscle absorbed energy at all velocities of lengthening.

The same amount of energy was not absorbed at each lengthening velocity. At velocities greater than ~0·5 V_{max}, in both types of muscle, the mean amounts of work input and heat output were constant (Fig. 7). For instance, when soleus muscles were lengthened at ~0·5, 0·7 or 1·2 V_{max}, there was no significant difference in either the amount of work done on muscles (~27 mJ g^{-1}), or the amount of heat produced by muscles (~13 mJ g^{-1}) (Fig. 7*A*). The results were similar for EDL muscle lengthened at the same relative

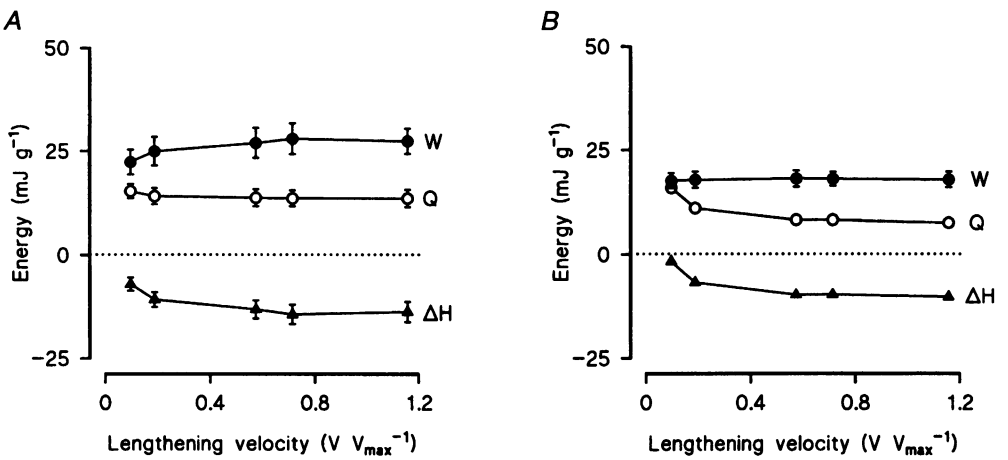

Figure 7. Effect of lengthening velocity on the mean amounts of work input, heat output and enthalpy change in mouse muscle

Mean amounts of work input, heat output and enthalpy change are shown as a function of relative lengthening velocity for soleus (*A*) and EDL (*B*) fibre bundles. Symbols represent mean values ($n = 6$): ●, work input; ○, heat output; and ▲, enthalpy change.

Table 2. Estimated fraction of work stored (E_S) by mouse soleus and EDL muscles and toad tibialis anterior muscles during lengthening

	W (mJ g^{-1})	Q_T (mJ g^{-1})	Q_M (mJ g^{-1})	E_S
Soleus	27	14	0·3–1	0·50–0·52
EDL	18	8	0·9–3	0·60–0·72
Tibialis anterior	27	17	1·5–5	0·54–0·66

velocities; the amount of work done was constant (~18 mJ g^{-1}) and the amount of heat produced was constant (~8 mJ g^{-1}) (Fig. 7*B*). Consequently, ΔH was also constant in both types of muscle (~−14 and ~−10 mJ g^{-1} for soleus and EDL fibre bundles, respectively), which means that both soleus and EDL fibre bundles absorbed a constant amount of energy at rapid lengthening velocities (approximately $\geqslant 0{\cdot}5\,V_{max}$).

However, at slower velocities of lengthening (i.e. ~0·1 and $0{\cdot}2\,V_{max}$), both the amount of work input and heat output varied with velocity (Fig. 7). There was a difference between soleus and EDL muscles. In soleus muscles, a constant amount of heat was produced at all velocities used, but the amount of work done was significantly lower at the two slowest lengthening velocities ($P < 0{\cdot}05$, $n = 6$). Thus, lengthening velocity affected the amount of work done. In contrast, when EDL muscles were lengthened, the amount of work done was constant at all velocities while the amount of heat produced was significantly higher at 0·1 and $0{\cdot}2\,V_{max}$ ($P < 0{\cdot}05$, $n = 6$). Hence, lengthening velocity affected the amount of heat produced. However, in both types of muscle the net effect was the same; the magnitude of ΔH was reduced at slow lengthening velocities. So less energy was absorbed by both soleus and EDL muscles during slow lengthenings.

DISCUSSION

This study investigated the energetics of lengthening in mouse and toad skeletal muscle. The main findings to emerge from these experiments were: (1) for both mouse and toad skeletal muscle, more energy was put into muscles as work than arose from them as heat and thus energy was absorbed; (2) similar amounts of energy were absorbed by all types of muscle tested; and (3) for all three types of muscle used, less energy was absorbed during slow lengthenings. Any mechanism proposed to explain how energy is absorbed during lengthening must be able to account for all of these findings.

How much mechanical energy is absorbed?

When a muscle is lengthened, heat potentially arises from two main sources. First, continued PCr breakdown by cross-bridge cycling and ion pumping would produce some heat and second, heat would arise from the conversion of work into heat. Thus, the fraction of mechanical work absorbed depends on the magnitude of the contribution of metabolic processes to heat output during lengthening. The fraction of the work stored (E_S) can be estimated as follows:

$$E_S = \frac{W - (Q_T - Q_M)}{W},$$

where W is the work done on the muscle, Q_T is the total heat produced during lengthening and Q_M is the heat arising from metabolic processes. The exact magnitude of Q_M in the current experiments is not known, but in frog muscle the rate of ATP splitting during lengthening is lower than that during isometric contraction (Curtin & Davies, 1973). Two estimates of Q_M were made covering the extreme possibilities that either cross-bridge ATP splitting was completely inhibited during lengthening or, alternatively, was unaltered from the isometric rate. It was further assumed that ATP splitting by non-cross-bridge processes was unaffected by lengthening and that the magnitude of this component was 30% of the isometric heat rate in all three muscles (Homsher, Mommaerts, Ricchiuti & Wallner, 1972; Wendt & Barclay, 1980). The results of this analysis are shown in Table 2. These estimates were made using energy input and output values recorded during lengthening at $\sim 0{\cdot}5\,V_{max}$.

The first point to note is that the relative magnitude of cross-bridge ATP splitting assumed to occur during lengthening had little influence on the estimated fraction of work absorbed. This is a consequence of designing the experiment so that the magnitude of work input was large relative to the expected metabolic heat output during lengthening. Second, there were only small differences among the muscles in the estimated fraction of the energy absorbed (Table 2). In all cases, approximately 50–60% of the work input was absorbed.

Potential mechanisms for absorbing energy

Biochemical reactions. When the negative enthalpy change was first observed, it was suggested that energy put into the muscle during stretch might drive the reaction responsible for energy production during muscle contraction (i.e. ATP hydrolysis) in the reverse direction, thereby absorbing energy (Hill & Howarth, 1959). This idea has been investigated using chemical measurements to try and detect ATP synthesis in frog muscle (e.g. Infante, Klaupiks &

Davies, 1964; Curtin & Davies, 1973). However, no evidence for the formation of ATP during lengthening has been found. A further possibility is that energy is absorbed by some other endothermic biochemical reaction but again, no such reaction has yet been identified. No comparable experiments have been performed using mammalian muscle, so the nature of biochemical changes in these muscles is unknown. Interestingly, the current experiments show that the energetics of mouse and toad muscles during lengthening were similar to each other and to those reported previously for frog muscle. If it is assumed that the previous biochemical measurements in frog muscle also apply to the mouse and toad muscles used in the present study, then it seems unlikely that energy absorption by biochemical reactions could explain the current results.

Extension of tendons. A widely reported characteristic of skeletal muscle is that the isometric force produced after stretch is higher than that produced prior to stretch (e.g. Flitney & Hirst, 1978; Lombardi & Piazzesi, 1990). Recently, it has been suggested that this phenomenon is not related to cross-bridge interactions, but is due to a maintained stretch of both series and parallel elastic structures (Edman & Tsuchiya, 1996). In the present study, isometric force after stretch was elevated in all muscles used, so it is of interest to consider the potential that various elastic elements have to absorb energy. Tendons are compliant structures with a great capacity to absorb elastic strain energy (Alexander & Bennet-Clark, 1977). Thus, an estimate was made of the increase in tendon length that would have been required to account for all the observed energy absorption. This calculation was made using a tendon stiffness value determined for tendons of whole mouse EDL muscle ($0{\cdot}24 \times 10^9$ Pa; James, Young & Altringham, 1995) and assuming that the cross-sectional area of tendon in our preparation corresponded to 20% of that of a whole muscle. Tendon stiffness was estimated to be 480 N m^{-1}, and an extension of ~6·5 mm would be required to absorb 10 mJ of energy. This is more than 6 times greater than the total length change used in this study. Thus, even in the extreme situation that only the tendons were stretched and there was no increase in sarcomere length, tendon compliance could explain only ~15% of the total energy absorption.

Extension of muscle fibres. Muscle fibres themselves are also compliant, although much less so than tendons. For example, the instantaneous stiffness of active muscle can be estimated from the slope of the T1 curve determined in rapid transient experiments (e.g. Huxley, 1974). Extrapolating to preparations of the size used in the current study (cross-sectional area, 0·4 mm^2) gives a stiffness of $\sim 5 \times 10^3$ N m^{-1} or 10 times that of the tendons. An element with this elasticity would have to be stretched more than 2 mm to absorb 10 mJ, which, again, is greater than the total imposed length change. However, if a very stiff element (e.g. muscle fibre stiffness, 5×10^3 N m^{-1}) was in series with a more compliant one (e.g. tendon stiffness, 5×10^2 N m^{-1}), applying a force would extend the compliant element further (by a factor of $5 \times 10^3/5 \times 10^2 = 10$) and more energy would be absorbed by the compliant element. In the case of tendon in series with a muscle fibre, the tendon would be expected to absorb 10-fold more energy than the muscle fibre (as energy absorbed = 0·5 × stiffness × extension^2). Thus, it seems that the potential for simple elastic mechanisms to account for the observed energy absorption is not markedly increased by considering another, less compliant element in series with the tendons. Consistent with this conclusion is the observation that the energy remained absorbed, at least for the remainder of the contraction, which would not be expected from simple elastic elements.

If energy was absorbed by a structural change during lengthening, then the change must have persisted beyond the lengthening phase of contraction. Therefore, the elastic properties of tendons and fibres can account for neither the duration of energy absorption nor the magnitude of energy absorption. It has recently been demonstrated that the contractile filaments are themselves quite compliant (Huxley, Stewart, Sosa & Irving, 1994; Wakabayashi, Sugimoto, Ueno, Takezawa & Amemiya, 1994) and thus have the capacity to be stretched and absorb energy. However, unless the filaments can, in some way, remain stretched so that energy could remain stored after lengthening ended, the same arguments as those presented for other compliant elements must also apply.

Changes in cross-bridge potential energy. Another possible mechanism for absorbing energy is that lengthening changed the distribution of cross-bridges between states in such a way that the mean cross-bridge potential energy was increased. The absorbed energy would then reflect the difference in mean potential energy due to the lengthening. The energy difference per cross-bridge required to account for 10 mJ g^{-1} energy absorption can be estimated. If the concentration of cross-bridges is assumed to be 0·3 μmol (g wet muscle mass)$^{-1}$ (Ebashi, Endo & Ohtsuki, 1969) then the energy difference per cross-bridge would have to be 10×10^{-3} J g^{-1}/(0·3 μmol g^{-1} × $6{\cdot}02 \times 10^{23}$ cross-bridges mol^{-1}) = 55×10^{-21} J per cross-bridge. This value is of the correct order of magnitude for the expected differences in potential energy between different attached states (Woledge *et al.* 1985, pp. 293–298). Therefore, it may be feasible for variations in the distribution of cross-bridges between different states to account for the amount of energy absorbed during lengthening. However, this possibility cannot be assessed further from the current data as they provide no evidence concerning changes in cross-bridge populations before and after lengthening.

Effect of velocity on energy absorbed

In the current experiments, a constant amount of energy was absorbed by all three types of muscle at velocities exceeding ~0·5 V_{max}. Previous studies of muscle mechanics

have shown that the magnitude of the increase in isometric force output after stretch is also independent of velocity (e.g. Edman, Elzinga & Noble, 1978, 1982). This raises the possibility that a single mechanism could explain both responses. If the mechanism responsible for the enhanced force output after stretch is also responsible for absorbing energy, then when stimulation ends and the muscle relaxes, the absorbed energy would be expected to be liberated as heat. It would be of interest to test this idea by performing experiments to measure heat output for a duration extending beyond the end of stimulation and muscle relaxation.

Conclusion

To conclude, these experiments have demonstrated that the energetics of lengthening are similar in amphibian and mammalian muscle. Not only amphibian muscles but also mouse muscles absorb energy during stretch, supporting the idea that this is a general property of skeletal muscle. The amount of energy absorbed was relatively insensitive to lengthening velocity. Further, it was estimated that a similar fraction of the energy used to lengthen muscles was absorbed by each of the three muscles tested.

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