

Passive mechanical properties of the medial gastrocnemius muscle of the cat

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1. This is a report on the history dependence of the passive mechanical properties of the medial gastrocnemius muscle of the anaesthetised cat.
2. The muscle was conditioned with an isometric contraction at the test length, or at 3 mm longer than the test length and then returned to the test length, where the level of resting tension was measured, as well as tension changes during a slow stretch.
3. The level of resting tension depended on the form of conditioning and, at the optimum length for active tension, the history-dependent component was 9% of the total passive tension.
4. During a slow stretch, tension initially rose steeply up to a yield point, beyond which it rose more gradually. The shape of the tension rise depended on the form of conditioning. The level of tension at the yield point consisted of a stretch-dependent component, the 'short-range tension' plus the resting tension for that length.
5. The short-range tension increased with muscle length to peak close to the optimum for active tension. The slope of the tension rise during a stretch, the short-range stiffness, peaked at 2 mm beyond the optimum.
6. The short-range tension was small immediately after a conditioning contraction but grew in size as the interval was increased up to 60 s, with a time constant of 9.9 ± 0.6 s. After a series of conditioning movements, it recovered more rapidly, with a time constant of 6.6 ± 0.5 s.
7. The history-dependent changes in passive tension and the response to stretch are interpreted in terms of the presence, in sarcomeres of resting muscle fibres, of crossbridges between actin and myosin which have very slow formation rates, both at rest and during movements.

In most experiments that investigate the mechanical properties of skeletal muscle, attention is focused on aspects of the active muscle. Passive properties are thought to be less important functionally, and, in any case, are believed to be largely understood. While that may be so, it is nevertheless worthwhile giving careful consideration to all aspects of passive properties. When a muscle, acting as a prime mover, contracts and shortens, the movement stretches its passive antagonists. So the passive tension generated in a muscle, particularly under dynamic conditions, is an important component of normal limb movements.

In the study of passive properties of muscle, there are currently two main areas of controversy. One concerns the contribution to passive tension by the elastic filament, titin (for review see Horowitz, 1999). The second, which is the subject of this paper, is the question of a contribution to passive tension by crossbridges between actin and myosin. It had been suggested by

D. K. Hill (1968) that in passive frog muscle, a component of resting tension, which he called 'filamentary resting tension', and the response to a slow stretch were the result of the presence of long-lasting, stable crossbridges in the muscle. Subsequently it was shown that this property was dependent on the muscle's previous history of contraction and length changes, called thixotropy (Herbst, 1976; Lakie & Robson, 1988a; Proske *et al.* 1993; Campbell & Lakie, 1998). More recently, alternative explanations based on properties of titin have been proposed (Mutungi & Ranatunga, 1996). It is perhaps surprising that a property of skeletal muscle as fundamental as the passive response to stretch remains the subject of debate. The most likely reason for this is that the conditions of the experiments quoted in support of each point of view are very different. Here we present observations on passive mammalian muscle which we have interpreted in terms of crossbridge action. We propose very slow attachment rates, which distinguish these crossbridges from actively cycling bridges.

METHODS

The experiments were carried out on a total of eight cats of both sexes weighing between 2.5 and 6 kg. All experiments carried out conformed with the guidelines and received the approval of the Monash University Committee for Ethics in Animal Experimentation. Anaesthesia was induced with an intraperitoneal dose of sodium pentobarbitone (40 mg kg^{-1}) and maintained during the course of the experiment with additional doses given when necessary into the cephalic vein. The trachea was cannulated and end-tidal CO_2 concentration was monitored. Expired CO_2 levels provided an indication of adequacy of ventilation and general condition of the animal. Rectal temperature was measured and body temperature maintained at 38°C by the use of a feedback-regulated heating blanket. At the end of the experiment, animals were killed with an overdose of anaesthetic.

Five experiments were carried out using the medial gastrocnemius (MG) muscle, and three using the soleus muscle. A laminectomy was carried out to expose ventral roots L6–S2. These were cut at their point of entry into the spinal cord and deflected onto a dissection plate. Electrical stimulation was used to establish where motor axons to MG or soleus ran in the ventral roots, typically L7–S1. The left hindlimb was dissected to expose the muscle. For this it was necessary to free medial gastrocnemius or soleus and to separate their tendons from the Achilles' tendon, leaving just the tendon of the chosen muscle attached to the calcaneum. All hindlimb nerves other than that supplying the muscle were cut, including those to hip muscles. The hindlimb was fixed to a rigid metal frame by means of steel pins in the pelvis and at each end of the tibia. To prevent heat and moisture loss from exposed tissues a mineral paraffin oil pool was formed which covered the site. The temperature of the paraffin pool was maintained within 2°C of core body temperature by the use of heat lamps.

At the start of each experiment the maximum physiological length of the muscle (L_{max}) was determined before the muscle was dissected free of surrounding tissue. The ankle was flexed maximally, with the knee and hip in the approximate positions they would adopt during the experiment and the distance noted between markers placed on the Achilles' tendon and on the adjacent tibia.

The calcaneum was severed and the piece attached to the tendon had a 2 mm diameter hole drilled through it. A threaded rod was passed through the hole and the calcaneum clamped between a pair of nuts and washers. This meant that the MG or soleus tendon and its attachment to the calcaneum were left essentially undisturbed. Tension was measured with a strain gauge attached to the threaded rod. The rod and supporting strain gauge screwed into the shaft of a servo-regulated muscle stretcher. The compliance of the system was $5 \mu\text{m N}^{-1}$.

The muscle's optimum length for active tension (L_{opt}) was determined by stimulating the muscle nerve at 80 pulses s^{-1} using 0.25 s duration tetani at each of a range of lengths up to L_{max} . Once the optimum length had been determined, all subsequent measurements of passive tension were related to their value at L_{opt} .

One of the important findings of this study was that the maintained level of passive tension, called here the 'resting tension', depended on the muscle's previous history of contraction and length changes. It was therefore necessary, whenever making measurements, to put the muscle into a defined state. For this two forms of conditioning were used (Fig. 1). These were based on similar methods used previously (Proske *et al.* 1993; Gregory *et al.* 1998; Proske & Morgan, 1999).

The muscle length was set to a desired value and the muscle contracted isometrically for 0.5 s at 15 pulses s^{-1} at that length. This was called hold-test conditioning. Alternatively, the muscle was

stretched by 3 mm at a rate of 10 mm s^{-1} , then contracted, and held there for a further 4 s before being returned to the test length, called hold-long conditioning. The stimulation rate was kept at 15 pulses s^{-1} because, from experience, it was sufficient to adequately condition the muscle. Repeated stimulation at 80 pulses s^{-1} led to rapid fatigue. Measurements made after muscle conditioning were the subsequent level of resting tension and the tension response to a slow stretch, 3 mm at 1 mm s^{-1} . This corresponds approximately to a stretch rate of 0.05 fibre lengths per second (see Lannergren, 1971; Campell & Laskie, 1998). Test stretches were usually given 20 s after return to the test length (Fig. 1). Resting tension values were measured just before onset of the stretch. At the end of each sequence, zero tension was determined by shortening the muscle until the tendon was visibly slack.

The tension response to the test stretch consisted of two components, an initial steep rise followed by a more gradual change. The initial peak, here termed the 'yield point', at short lengths consisted of the prevailing level of resting tension plus the component generated by the stretch, the 'short-range tension' (Fig. 2). The slope of the initial tension rise was called the 'short-range stiffness'. Resting and short-range tensions, as well as short-range stiffness, were measured at each of a range of muscle lengths from $L_{\text{opt}} - 10 \text{ mm}$ to $L_{\text{opt}} + 6 \text{ mm}$. At longer lengths measurements were complicated by the fact that during the stretch an additional component of tension, due to elastic properties, the 'elastic tension', was present in the response of the muscle to stretch.

We hypothesised that the initial steep rise in tension up to the yield point was due to the presence of stable crossbridges between actin and myosin (Hill, 1968). For measurements made at lengths greater than $L_{\text{opt}} + 6 \text{ mm}$, once the short-range tension had been exceeded any further rise in tension was considered not to be of crossbridge origin, but rather to be due to other, elastic structures. To measure the component attributable to crossbridges, the difference was calculated between the total measured tension during a stretch and that estimated to be due to elastic properties. This was done by drawing a line parallel to the second slope of the tension rise, which intersected the tension trace at the start of the stretch (Fig. 2). Values along the line were considered to represent the elastic tension generated by a given length change. The tension at the yield point, after subtraction of the elastic component, was taken to be the short-range tension.

The value of short-range tension depended on how long after muscle conditioning the test stretch was applied. Most of the measurements were made 20 s after the muscle had been returned to the test length following conditioning. To obtain a measure of the rate of development of the short-range tension, test stretches were applied at various times after conditioning. For this the muscle was conditioned by an isometric contraction, 0.5 s duration, at 15 pulses s^{-1} given at the test length at intervals between 1 and 60 s before the test stretch. A second method of conditioning used a series of rapid stretch-shortening movements of 5 mm amplitude at 50 mm s^{-1} (Fig. 6). In each case the test stretch was 3 mm at 1 mm s^{-1} . All measurements were made at L_{opt} .

In two experiments the effect of grading the size of the conditioning isometric contraction was explored. The ventral root supply to MG was subdivided into five near-equal portions and these were stimulated sequentially, that is, using distributed stimulation, with supramaximal stimuli given over the range 7–100 pulses s^{-1} for 1.0 s. A test stretch was given 60 s later.

Statistical analysis

For all parameters measured, the mean and standard error of the mean (S.E.M.) was calculated. The significance level for all experiments was set at $P < 0.05$. A three-factor ANOVA with

interactions was used to test for significant differences between the two types of conditioning. The factors were conditioning, length and animal. Where the interactions of conditioning and length were significant, a least significant difference (LSD) *post hoc* test was used to identify individual lengths at which conditioning effects were significant. The relationship between short-range tension/stiffness and active tension was determined by a linear regression analysis. The statistical program used was Data Desk (Ithaca, NY, USA).

RESULTS

In a preliminary series, three experiments were carried out on the soleus muscle. A number of measurements were made, but eventually work on soleus was discontinued. This was because it was considered important to measure passive properties over as wide a range of muscle lengths as possible, including the descending limb of the muscle's active length-tension relation. Soleus has rather long muscle fibres and consequently a very broad length-tension curve, with very little of the descending limb within the physiological range of lengths (Walmsley & Proske, 1981). MG, on the other hand, has a third of its length range on the descending limb (Fig. 3). Consequently, an additional five experiments were carried out, all on MG. Apart from differences in length dependence, the passive properties of the two muscles appeared to be similar.

Resting tension

The level of resting tension measured in the muscle depended on how it had been conditioned beforehand (Fig. 1). Notice in Fig. 1 that after conditioning, before the test stretch was applied, the two tension traces were offset from one another. This represents the difference in resting tension generated under the two conditions. Notice, too, that during the stretch the two traces converge, so that at the end of the length change there is no difference.

A plot of resting tension after the two forms of conditioning and measured at different muscle lengths is

shown in Fig. 3. The differences are fairly small at short muscle lengths with, at optimum length, approximately 9% of the measured resting tension dependent on the form of conditioning. Beyond the optimum the history-dependent component increased, up to a value of about 30% at $L_{opt} + 6$ mm. There was a significant interaction between conditioning and length ($P < 0.01$) and at some, but not all, lengths hold-long and hold-test values were significantly different ($P < 0.05$).

Resting tension levels were highest when measured after hold-test conditioning. When the length to which the muscle was stretched during hold-long conditioning was increased from 0 to 5 mm in 1 mm steps, it was found that conditioning stretches of 1 mm or more produced a fall in passive tension. At L_{opt} the size of the fall was little different with stretches bigger than 1 mm.

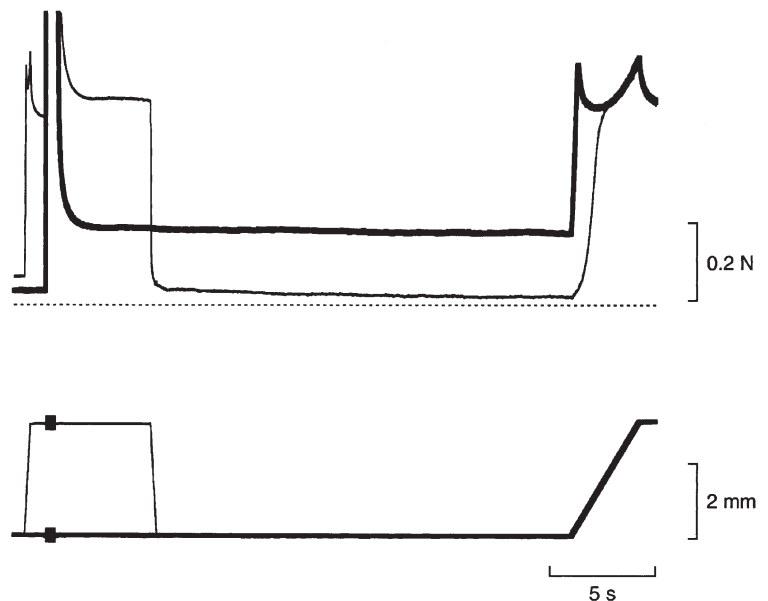
Short-range tension

Our working hypothesis was that the history-dependent passive tension in the muscle was due to stable cross-bridges between actin and myosin in myofibrils (Hill, 1968; Herbst, 1976). This tension consisted of two components, the resting tension plus the tension generated during a stretch, the short-range tension (see Fig. 2).

A point to which we assign importance is the fact that at all muscle lengths there were measurable differences in the tension response to stretch after the two forms of conditioning. In response to a stretch at lengths $L_{opt} - 6$ mm or longer, after hold-test conditioning, tension rose steeply, then fell slightly before continuing to rise more gradually. After hold-long conditioning, the tension rise was less steep and the yield point much more rounded (Fig. 1). At $L_{opt} - 4$ mm, after hold-test conditioning the yield point occurred after 0.2 mm of length change, compared with 0.3 mm after hold-long (Fig. 4). At L_{opt} there was little difference in stretch responses after the two forms of conditioning while

Figure 1. The method of muscle conditioning

Tension responses to stretch of the passive medial gastrocnemius muscle of the cat. Tension (upper traces) and length (lower traces) are shown superimposed, in response to two conditioning test sequences. The thick traces show hold-test conditioning, where the muscle was contracted isometrically (for 0.5 s at 15 pulses s^{-1}) and then a slow test stretch (3 mm at 1 mm s^{-1}) was applied 24 s later. For hold-long conditioning (thin traces), the muscle was stretched by 3 mm, held at that length for 1 s then contracted isometrically as before. After 4 s it was shortened back to its original length and a test stretch was applied 20 s later. Note, peak tension during the isometric contractions is not shown. The periods of stimulation are indicated on the length traces. The dotted line below the tension traces indicates zero tension. Muscle length, $L_{opt} - 6$ mm.



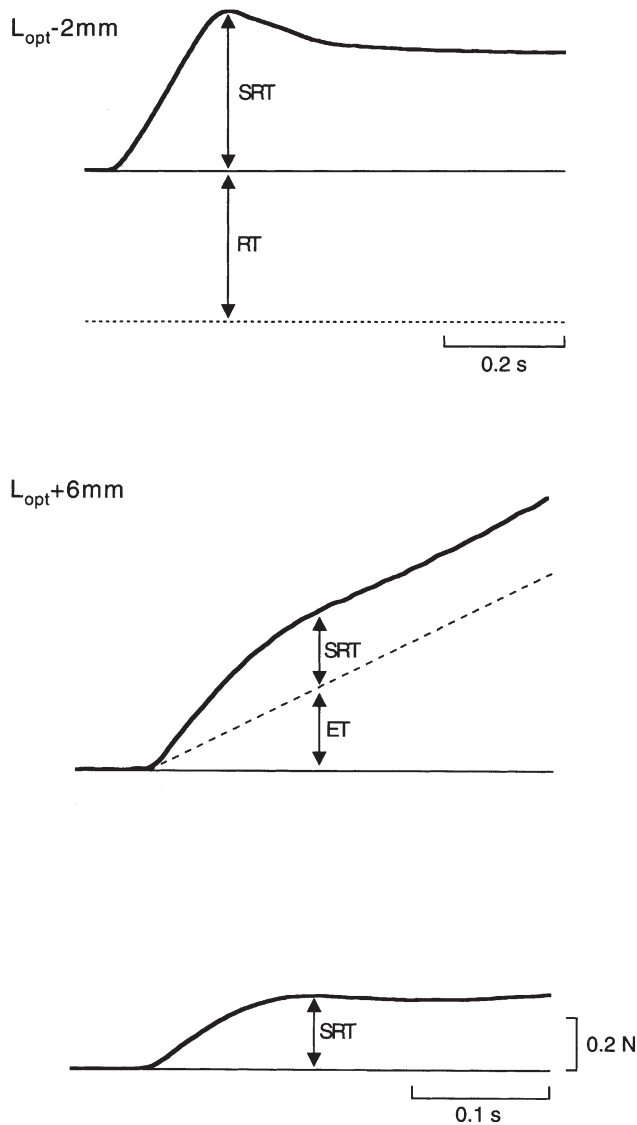


Figure 2. Measurement of short-range tension

At short muscle lengths, up to L_{opt} (upper panel), there were two components of tension, the level of resting tension (RT) before onset of stretch and the tension during a slow stretch (3 mm at 1 mm s^{-1}) up to a maximum, called the short-range tension (SRT). The dotted line below the record indicates zero tension, the thin continuous line the level of resting tension. At longer lengths ($L_{opt} + 6 \text{ mm}$) an additional component of the tension rise during stretch was attributed to elastic structures, not associated with short-range properties. To extract this component, the elastic tension (ET), a line was drawn parallel to the second, more gradual tension rise which intersected the tension trace at the start of the stretch (dashed line). It represented the amount by which tension increased with length, independent of short-range properties. For each record with a significant component of elastic tension this was subtracted to derive the short-range tension. The bottom panel shows the record at $L_{opt} + 6 \text{ mm}$ after subtraction of the elastic tension. The tension calibration applies to all three panels.

resting tension had increased considerably. However, the short-range tension was larger and the length change required to reach the corner had fallen further to 0.13–0.14 mm. Notice that after the stretch the two tension traces no longer fully converged. At $L_{opt} + 2 \text{ mm}$ the resting tension had increased further and, more importantly, the difference in resting tension after the two forms of conditioning was maintained throughout

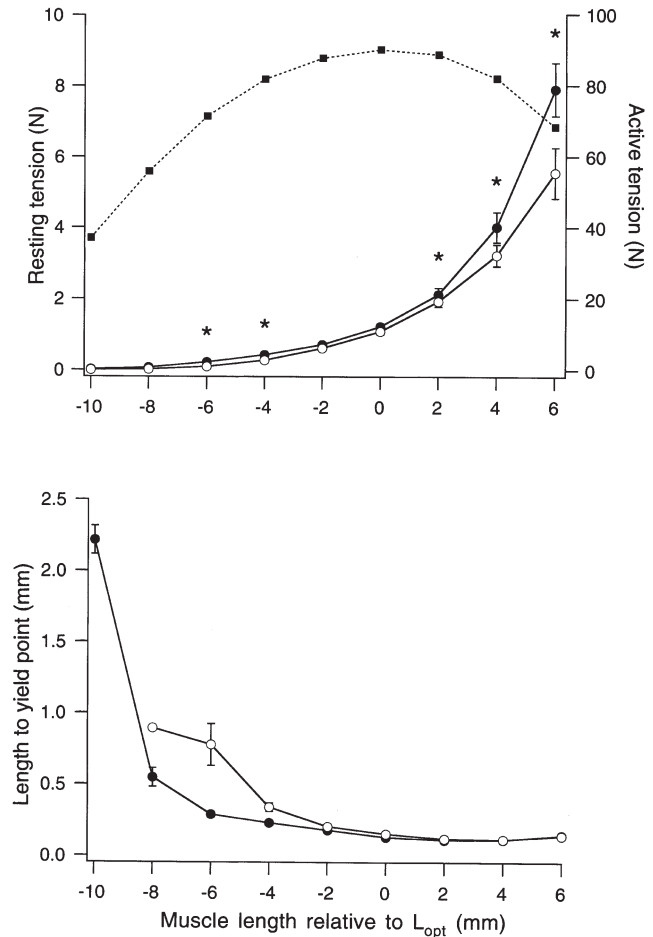


Figure 3. Length dependence of passive tension and length to yield point

Upper panel, measured level of resting tension after hold-long conditioning (O) or hold-test conditioning (●). Values are means \pm S.E.M. from five experiments. The squares joined by a dotted line indicate, for one experiment, the muscle's active length–tension relation. Asterisks indicate lengths where hold-long and hold-test values were significantly different ($P < 0.05$). Lower panel, plot of length to yield point against muscle length. ●, values after hold-test conditioning; ○, values after hold-long conditioning. Values are means \pm S.E.M. At $L_{opt} - 10 \text{ mm}$, after hold-long conditioning there was no measurable yield point. After hold-test, only values from three experiments could be used. At $L_{opt} - 8 \text{ mm}$ for hold-long, only values from two experiments could be used. In both upper and lower panels length has been shown relative to the optimum for active tension (L_{opt}).

the stretch response. That is, the two traces remained well separated after the yield point had been reached. Notice, too, that the short-range tension had fallen a little (Fig. 4).

To demonstrate the history dependence of the length change required to reach the yield point, this was plotted for each form of conditioning against muscle length (Fig. 3, lower panel). The most important point of this figure is that from $L_{\text{opt}} - 2$ mm onwards, in the direction of longer lengths, there is little difference in the length to yield point after the two forms of conditioning. Furthermore, the yield point value remains low, despite the very large rise in passive tension in this length range (Fig. 3, upper panel). In other words, at longer lengths, where passive tension is high, and where tendon stretch might have been expected to play a role, length to the yield point remains low. It suggested that the stretch was being taken up by muscle fibres and not by the tendon.

We propose that at short muscle lengths, slack is introduced in the muscle after hold-long conditioning, the slack arising largely in the tendon (see Discussion). Once tendon slack has been taken up by a stretch, the muscle fibres begin to stretch, as they are less stiff than the tendon. It means that values for the length to the yield point were very much larger after hold-long conditioning since the stretch first had to take up the slack. At lengths shorter than $L_{\text{opt}} - 8$ mm, after hold-long conditioning,

there was no yield point at all while after hold-test it was ill defined and could only be measured in some records. Over the range $L_{\text{opt}} - 8$ mm to L_{opt} , the length to the yield point became progressively less, reaching a minimum value of 0.11 ± 0.01 mm at $L_{\text{opt}} + 2$ mm. The point emphasises the importance of having a known previous history when measuring the yield point in passive muscle, particularly at lengths below the optimum.

Assuming an average muscle fibre length of 20 mm for MG (Walmsley & Proske, 1981), a length to the yield point of 0.11 mm represents a fibre length change of 0.55%.

The peak of the short-range tension was measured after the two forms of conditioning, at different muscle lengths. The length dependence is shown in Fig. 5. For lengths $L_{\text{opt}} + 2$ mm and longer, both the short-range tension and the sum of short-range and elastic tensions have been plotted (see Fig. 2). The curve for the average value for short-range tension from the five experiments is approximately bell shaped, reaching a peak of 0.74 ± 0.08 N at L_{opt} after hold-long conditioning. Analysis showed that for lengths $L_{\text{opt}} - 6$ mm or longer, there was a significant effect of conditioning on the value of the short-range tension ($P < 0.05$).

Another measurement made was the slope of the tension rise during a stretch, up to the yield point, here called the

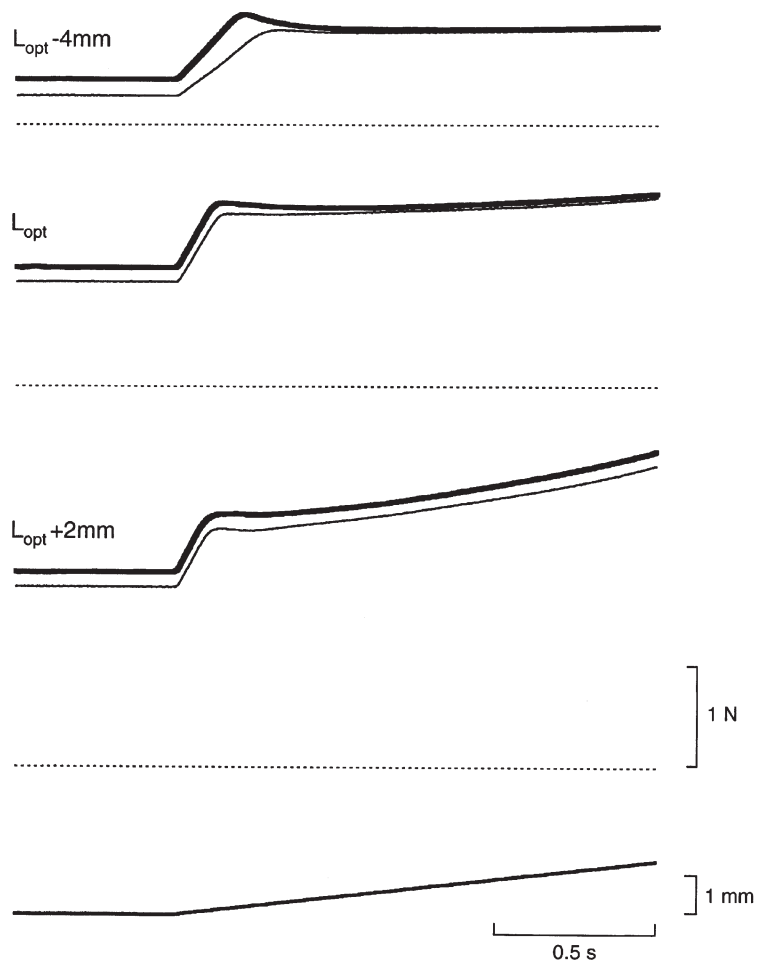


Figure 4. Tension changes during a slow stretch

Tension changes in the passive MG muscle during a slow stretch (3 mm at 1 mm s^{-1}), starting 20 s after muscle conditioning. Upper three pairs of traces, tension; bottom trace, length change. Each pair of tension traces consists of two records superimposed. Thin traces, after hold-long conditioning; thick traces, after hold-test conditioning. The stretches were carried out at three different muscle lengths, $L_{\text{opt}} - 4$ mm, L_{opt} and $L_{\text{opt}} + 2$ mm. Dotted lines indicate zero tension.

short-range stiffness (Fig. 5). This, like the short-range tension, showed a length dependence, peaking at $L_{\text{opt}} + 2$ mm for hold-test conditioning (6.3 ± 0.42 N mm^{-1}), provided it was calculated after the elastic tension had been subtracted. Analysis showed a significant interaction between conditioning and length ($P < 0.01$).

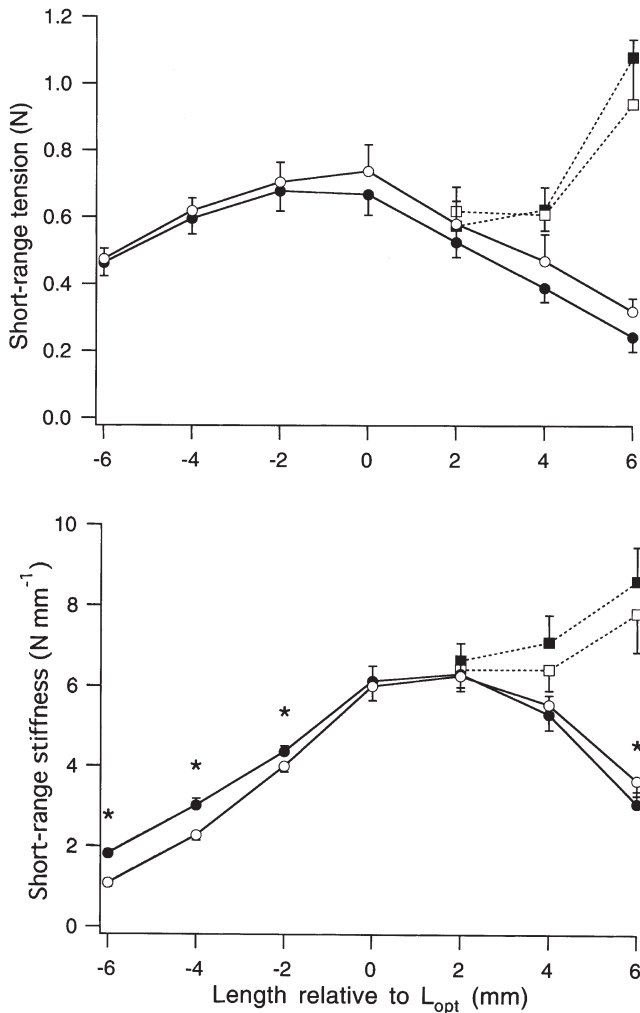


Figure 5. Length dependence of short-range tension and stiffness

Upper panel, length dependence of the short-range tension (circles, continuous lines) measured in response to a slow stretch (3 mm at 1 mm s^{-1}) after hold-long conditioning (open symbols) or hold-test conditioning (filled symbols). For values at $L_{\text{opt}} + 2$ mm or longer, the sum of elastic and short-range tensions have also been shown (squares, dotted lines). Lower panel, plot of short-range stiffness against muscle length. Stiffness was calculated from the slope of the tension rise during stretch. Symbols as in upper panel. Asterisks show lengths where values after hold-test conditioning were significantly different from hold-long values ($P < 0.05$). Values in both panels have been shown only down to a length of $L_{\text{opt}} - 6$ mm because measurements at shorter lengths were unreliable.

The finding of a correspondence between the peak of the short-range tension and the optimum length for active tension was considered to support the view that the short-range tension was of crossbridge origin. To further emphasise the point, for each experiment the short-range tension and stiffness, measured at different lengths and using different forms of conditioning, were normalised and plotted against the corresponding whole muscle active tension. There was a significant linear relationship between short-range tension and active tension. Regression analysis gave a slope of 1.76 ($r^2 = 0.63$; $P < 0.001$) for values between $L_{\text{opt}} - 2$ mm and $L_{\text{opt}} + 4$ mm. It meant that over this length range a unit increase in active tension led to a 1.76 times increase in short-range tension.

Notice in Fig. 4 that at each length, before stretch onset, there were differences in resting tension after the two forms of conditioning. However, at the shorter lengths, $L_{\text{opt}} - 4$ mm and L_{opt} , after the end of stretch, the traces converged. It implied that a larger resting tension was associated with a smaller short-range tension. This relation no longer held at longer lengths where the resting tension difference persisted throughout the stretch.

Time course of redevelopment of short-range tension

The working hypothesis throughout these experiments was that the yield point at the peak of the short-range tension represented detachment of crossbridges by the stretch. If stretch detaches bridges, the tension at the yield point during a second stretch, given at various times after the first, should be an indication of the re-attachment rate. This kind of experiment had been carried out by others using pairs of triangular stretches, separated by different intervals (Hufschmidt & Schwaller, 1987; Campbell & Lakie, 1998). However, it seemed likely that during the slow shortening following the stretch phase of the first triangle there would be some re-attachment of bridges, which would leave muscle fibres in a complex mechanical state, including the presence of some slack. To overcome this complication we did the experiment slightly differently.

Two methods were used. In the first, we applied a slow test stretch at various times after an isometric contraction (1.0 s duration tetanus at 15 pulses s^{-1}). The rationale was that during the contraction there would be active cycling of crossbridges and that 'stable' crossbridge formation would commence at the end of the contraction. This assumes that calcium levels had rapidly returned to resting values at the end of the contraction.

As indicated above, these stable crossbridges can be detached by stretch as well as by muscle activation. In a second experiment, to avoid possible complications from a conditioning contraction, a series of rapid stretches and shortenings was used to condition the muscle (Morgan *et al.* 1984). The movements were five stretches of 5 mm amplitude at 50 mm s^{-1} , carried out within 1 s. Short-range tension was measured in response to a slow test

stretch (3 mm at 1 mm s^{-1}) at various times after the conditioning movements (Fig. 6).

Examples of the time course of development of short-range tension after movement or contraction conditioning are shown in Fig. 6. It was found that after movement conditioning, short-range tension was always higher than after contraction conditioning. An exponential fitted to the recovery of tension after the movements had a time constant of $6.6 \pm 0.5 \text{ s}$. After contraction conditioning at 15 pulses s^{-1} the time constant was $9.9 \pm 0.6 \text{ s}$.

In one experiment, contraction conditioning had been carried out using stimulation rates that gave near maximum tension (80 pulses s^{-1}). Interestingly, short-range tension measured at all times afterwards was much lower than after conditioning using contractions at 15 pulses s^{-1} or using movements (Fig. 6). However, the conditioning at high-rate stimulation also led to a higher level of resting tension. It suggested that tendon recoil during relaxation from a larger conditioning contraction strained crossbridges more than after a smaller contraction, leading to a higher level of resting tension. This point was systematically studied in two experiments. The muscle was subjected to hold-long conditioning to introduce slack and then isometric contractions of various sizes were given, all at the same test length, in this case, L_{opt} . Resting and short-range tensions were measured 60 s later. As the size of the conditioning isometric contraction was increased, short-range tension fell while resting tension rose. In other words, there was an inverse relationship between the two. In one experiment, plotting resting tension against short-range tension showed a significant inverse relationship, with a slope of -0.31 ($r^2 = 0.48$; $P < 0.01$).

DISCUSSION

These experiments were carried out on the cat MG muscle and its tendon, *in situ*, in a whole animal preparation. Traditionally, measurements of the kind made in this study have been carried out on single fibre preparations where complications from structures such as the series elastic component can be minimised. However, we felt it was worthwhile making the measurements in this way since our long-term goal was to try to understand muscle properties in the freely moving animal.

An important consideration was the question of how the tendon might modify the tension response to a stretch. We have proposed that after hold-long conditioning the muscle fell slack. We mean by slack that on shortening the muscle, sarcomeres in muscle fibres did not follow the length change but, stiffened by the presence of stable crossbridges, retained their original length, or at least something intermediate between the original and final lengths. That is, the compressive forces from shortening the muscle may lead to crossbridge detachment in some but not all sarcomeres. Given that for MG the tendon is so

much longer than the muscle fibres (Walmsley & Proske, 1981), it is most probably the tendon that falls slack. This has indeed been observed at short lengths (Elek *et al.* 1990). In a single fibre preparation it is the fibre itself which falls slack and this can be demonstrated by the time to onset of tension in an isometric contraction (Proske *et al.* 1993, Fig. 1).

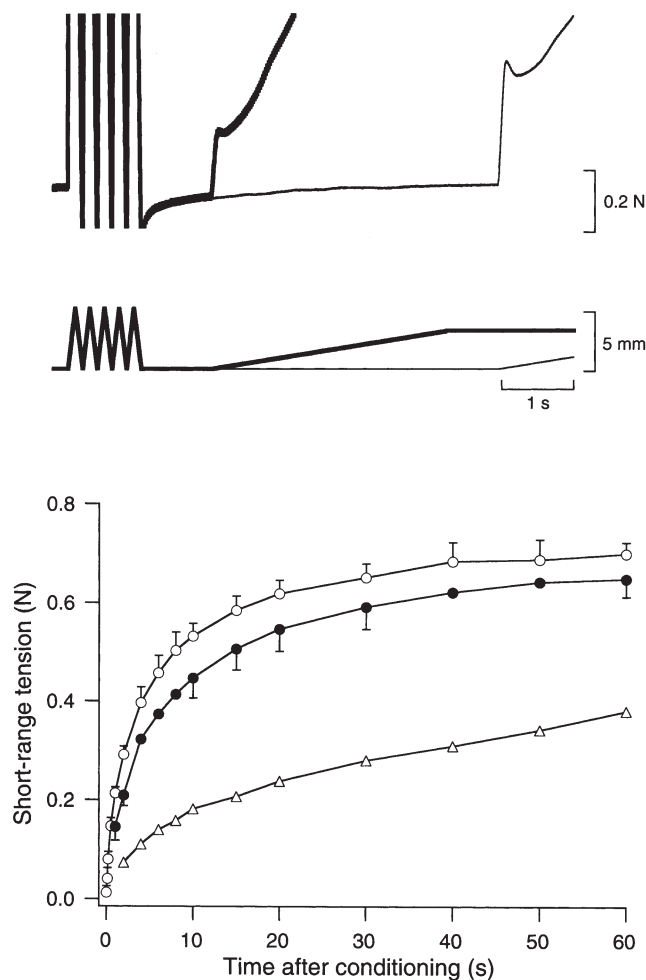


Figure 6. Time course of development of short-range tension

Upper panel, sample records of tension (upper traces) and length (lower traces) during muscle conditioning using five rapid (50 mm s^{-1}) stretch and shortening movements of 5 mm amplitude and lasting 1 s. Following conditioning, a slow test stretch of 3 mm amplitude at 1 mm s^{-1} was given at various times. Thick traces, conditioning-test interval of 1 s; thin traces, 5 s interval. Lower panel, plot of short-range tension measured at various times after muscle conditioning. O, means \pm S.E.M. from four experiments using movement conditioning. ●, values after conditioning with an isometric contraction, 1 s duration at 15 pulses s^{-1} . Δ, values after conditioning with an isometric contraction, 1 s duration at 80 pulses s^{-1} . All measurements were made at L_{opt} .

We envisaged that during a stretch once slack had been taken up in the tendon, further length changes took place in muscle fibres. This is because tendon is relatively inelastic compared with passive muscle fibres, even in its toe region (Proske & Morgan, 1987) and tension in the present experiments was never more than 1% of whole muscle tension (Fig. 5). Our view was supported by the finding that at long muscle lengths ($L_m + 2$ mm to $L_m + 6$ mm) the length change required to reach the yield point was at its lowest value and was independent of muscle length, despite large increases in resting tension (Fig. 3, lower panel).

After hold-long conditioning at short lengths, once the slack in the tendon had been taken up, the rise in tension in the short range was less steep and the yield point was more rounded than after hold-test conditioning (Fig. 1). We propose that because MG is composed of fibres of different lengths (Ounjian *et al.* 1991), the point at which each fibre begins to be stretched is distributed across a range of lengths, leading to the more rounded record. At the two shortest lengths studied ($L_{opt} - 8$ mm and $L_{opt} - 10$ mm) there was also a small increase in length to yield point after hold-test conditioning (Fig. 3, lower panel). The most probable reason for this is that at these lengths the conditioning contraction was not fully able to take up all of the slack.

The aim of the experiments was threefold. First we wanted to demonstrate the existence in mammalian muscle of a component of resting tension that was dependent on muscle history. Secondly we wanted to show that in response to a slow stretch the length and tension changes required to reach the yield point also depended on the previous history. Thirdly, we wanted to argue that such behaviour was consistent with the presence of stable crossbridges between actin and myosin in resting muscle fibres (Hill, 1968).

We propose that the stable crossbridges manifest themselves in two ways, as a component of resting tension and as the short-range tension in response to a stretch. After hold-long conditioning, slack is introduced in muscle fibres (Proske *et al.* 1993) so that the crossbridge component of the resting tension is low. During a subsequent stretch, crossbridges would not become detached until the slack had been taken up and tension levels were high enough. After hold-test conditioning, no slack would be present and stable crossbridges would be under stress, thus generating some resting tension. The stressed crossbridges would detach earlier during the stretch so that under these conditions the short-range tension would be lower. So the history-dependent level of resting tension at short lengths is an indication of both the number of stable crossbridges present in fibres and their average tension.

Inspection of Fig. 3 (upper panel) shows that up to the optimum length there was a progressive increase in a

component of resting tensions that was largely independent of the form of muscle conditioning. This component, we propose, is due to elastic structures in the muscle, perhaps titin. At lengths beyond the optimum for active tension the resting tension increased further and, more importantly, it became much more strongly history dependent. In addition, there was a difference in tension throughout the stretch response after the two forms of conditioning and the traces no longer converged (Fig. 4). We would like to propose that this history-dependent component of passive tension at long lengths is also due to elastic filaments.

The filament titin shows 'molecular fatigue' during repeated stretch and release cycles, particularly at long muscle lengths (Kellermayer *et al.* 2001). The process is characterised by a shift of the stretch-force curve towards longer lengths. We propose that after hold-long conditioning, the length adopted by titin is longer than after hold-test conditioning and this is accompanied by a lower level of resting tension. The process is reversible since during a series of repeated conditioning movements, the hold-long:hold-test difference remains about the same (J. E. Gregory, D. L. Morgan & U. Proske, unpublished observation). We propose that the history-dependent behaviour of titin is strongly length dependent. At lengths up to the optimum it shows little history dependence and any difference in resting tension after the two forms of conditioning is largely due to crossbridges. This is demonstrated by the interdependence of resting and short-range tensions. At lengths beyond the optimum the tension supported by titin becomes much more history dependent. To summarise, we propose that there are two sources of resting tension in a passive muscle which have a history dependence. One is a component due to crossbridges, which is prominent at short muscle lengths. Secondly, there is a component due to elastic filaments which becomes significant only at lengths beyond the optimum for active tension.

The presence of a history-dependent component of passive tension arising from stable crossbridges appears to be a fundamental property of muscle. It was first described by D. K. Hill (1968) for frog muscle and referred to by him as filamentary resting tension. More recently it has been demonstrated for human muscle (Gregory *et al.* 1987, Fig. 5) where a rise in passive torque is accompanied by an increase in stretch reflex size (see also Hagbarth *et al.* 1985). Indeed, much of the history-dependent behaviour of muscle spindles can be interpreted in terms of changes in passive tension in intrafusal fibres (Proske *et al.* 1993).

An important finding of this study was that the measured value of short-range tension was dependent on the form of conditioning and on the length of the muscle. After hold-long conditioning at very short lengths there is no discontinuity at all, as the stretch simply takes up

slack in muscle and tendon. At intermediate lengths the discontinuity becomes more prominent and the short-range tension is higher. Significantly, at lengths beyond the optimum the short-range tension falls, we suggest, because of reduced overlap between myofilaments (Fig. 5).

Reports in the literature suggest that such a correspondence between the length dependence of the short-range tension and the active tension is not present in amphibian muscle. Haugen & Sten-Knudsen (1981) using a similar subtraction process as we have used here to remove elastic components from the tension response to stretch (Fig. 2) showed that short-range stiffness and what they called the 'stationary plateau tension', the equivalent of our short-range tension, peaked at $3\ \mu\text{m}$, about half-way down the descending limb of the active length-tension curve. We, too, found that short-range tension and stiffness increased with length, up to lengths beyond the optimum for active tension, but only when elastic tension had not been subtracted (Fig. 5). Why the 'stationary plateau tension' of Haugen & Sten-Knudsen peaked at such long lengths remains uncertain and may represent a species difference. Perhaps there is a difference in length dependence of the sensitivity of the myofilaments to Ca^{2+} levels in resting fibres (Endo, 1973). We conclude that our finding of a correspondence between the length dependence of the short-range tension and the active length-tension relation provides important supporting evidence for a crossbridge origin of the short-range tension, evidence which was previously lacking.

In our discussion of the data we have preferred an interpretation based on stable crossbridges. It is necessary, here, to try to justify our view. A number of arguments have already been presented (Proske & Morgan, 1999). In response to our proposal, Mutungi & Ranatunga (2000) provided a series of alternative interpretations. Here we would like to consider some of these. The argument centres on whether the tension discontinuity seen with slow stretches, referred to by these authors as the P2 component, is due to crossbridges or to elastic filaments. The phenomena described here were always associated with time constants of several seconds (Fig. 6). So we do not believe that a rapid turnover of crossbridges is involved (see Bagni *et al.* 1995; Schoenberg, 1998).

In his original experiments Hill (1968) showed that the muscle had to be stretched through a length range corresponding to 0.2% of fibre length to reach the yield tension. In more recent experiments on mammalian muscle, an 'apparent break point' was not reached until the muscle had been stretched by 1–2% of fibre length, that is, 10 times further. This was taken as behaviour inconsistent with Hill's hypothesis (Mutungi & Ranatunga, 1996). However, the observations were made on muscle with an unknown previous history of contraction and length changes. Our experiments have shown that a whole range of break point lengths ranging from 0.55 to

4.5% can be obtained, depending on the form of conditioning and length of the muscle (Fig. 3, lower panel).

Recent experiments by Campbell & Lakie (1998) gave values for the break point length of 0.4%, that is, twice that reported by Hill (1968). It means that our minimum value of 0.55% is no longer so different from that reported for frog muscle.

Mutungi & Ranatunga (2000) argue that since the break point tension increases with sarcomere length to lengths beyond optimum overlap, this represents further evidence against a crossbridge origin for the discontinuity. In the study presented here we have shown that for mammalian muscle the short-range tension shows a clear peak at the optimum length, provided that a component of elastic tension has been subtracted (Fig. 5).

Further evidence in support of a crossbridge mechanism for muscle thixotropy comes from experiments on permeabilised rabbit psoas muscle fibres (Campbell & Moss, 2000). In solutions which were essentially free of Ca^{2+} ($\text{pCa} = 9.0$), no biphasic response to stretch was present. The small rise in tension which was independent of muscle history was presumably due to passive structural elements such as titin. When Ca^{2+} concentration was increased to values similar to those likely to be present in resting intact muscle fibres ($\text{pCa} \leq 6.3$), thixotropic behaviour became apparent. These findings led to the conclusion that measured stiffness in resting muscle is due to low levels of crossbridge engagement, dependent on the prevailing levels of Ca^{2+} .

Such a conclusion raises the more general question of whether crossbridge properties in resting muscle simply represent a scaled version of crossbridge properties in actively contracting muscle or whether any distinguishing features can be identified between the two states. Here the time course of development of short-range tension (Fig. 6) can provide some comment. In discussing the point Campbell & Moss (2000) pointed out that their own measurements on actively contracting fibre segments from the rabbit psoas muscle suggested a crossbridge reformation rate of about $1\ \text{s}^{-1}$ which is 10 times faster than values reported for resting frog fibres (Lannergren, 1971; Campbell & Lakie, 1998). They proposed that crossbridges cycle more rapidly in actively contracting muscle than in relaxed muscle.

Our measured time constant for recovery of short-range tension was 6–10 s. This compares with 4 s reported by Hufschmidt & Schwaller (1987) for human ankle muscles and 30 s for rapid stiffness recovery in human finger muscles (Lakie & Robson, 1988*b*). All of these values presumably relate to crossbridge cycle rates in resting muscle. They suggest that cycle rates in resting mammalian muscle are an order of magnitude faster than for resting frog muscle. This difference may, in turn, relate to the differences in temperature at which the

measurements were made, 5°C for the amphibian muscle and body temperature for the mammalian muscle. There is, however, a suggestion that the stiffness recovery rate of relaxed frog fibres is independent of temperature, over the range 3–17°C (Lakie & Robson, 1988a).

In response to a test stretch, once the yield point for stable crossbridge detachment had been reached, tension fell slightly. It might have been expected to fall to zero since the formation rate constant was so low. In an attempt to overcome this difficulty, Campbell & Lakie (1998) proposed that the stable crossbridge formation rate was movement sensitive and it increased during a stretch so that any bridges detached by the stretch were able to rapidly reform, allowing tension levels to be maintained. This situation was modelled with a crossbridge population displacement mechanism.

We would like to propose an alternative mechanism (Proske & Morgan, 1999). Our explanation is based on the assumption that the stable crossbridge turnover rate remains low, whether or not the muscle is being stretched. This is a more satisfactory position in terms of the Huxley (1957) crossbridge theory. We have shown previously that the tension response to stretch and the subsequent level of maintained tension in an actively contracting muscle can be explained in terms of a non-uniform distribution of the length change between sarcomeres in muscle fibres (Morgan, 1990; Morgan *et al.* 2000). We propose a similar explanation for the response to passive stretch. Not all sarcomeres are likely to resist a stretch with the same strength. Some will be stretched more than others. The weakest sarcomeres will reach their yield point first and then continue to lengthen until passive tension in them, of non-crossbridge origin, rises to reach the yield point tension of the next-weakest sarcomeres. With this kind of model, tension will not fall to zero beyond the yield point, yet the reformation rate of new bridges can remain low. A comparable process is presumably taking place during stretch of actively contracting muscle, when the stimulation rates are kept very low (Joyce *et al.* 1969, Fig. 6).

To conclude, we believe that the observations we have made greatly increase the plausibility of a crossbridge explanation for stretch responses of passive muscle. We have shown that any routine measurement of passive tension in a muscle is likely to be influenced by what has happened to the muscle in the immediate period beforehand. The history-dependent component of passive tension is related to the tension response to a slow stretch. Our interpretation of the stretch response is based on a non-uniform distribution of the length change between sarcomeres, leading to the detachment of stable crossbridges and their slow reformation.

Given that at short and intermediate lengths the history-dependent component of the passive tension is only a small fraction of the total, it could be argued that it is

only of minor importance. More important, perhaps, is the stretch response. Presumably, the motor system must have to take into account the fact that during any voluntary movement, the passive resistance of the antagonist muscle is length dependent and changes significantly to the second and subsequent stretches in a series of repetitive movements (Campbell & Moss, 2000; Axelson & Hagbarth, 2001).

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