J. exp. Biol. **165**, 121–136 (1992)
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THE POTENTIATING EFFECT OF PRESTRETCH ON THE CONTRACTILE PERFORMANCE OF RAT GASTROCNEMIUS MEDIALIS MUSCLE DURING SUBSEQUENT SHORTENING AND ISOMETRIC CONTRACTIONS

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Accepted 3 December 1991

Summary

The aim of the present study was to investigate the effect of an active stretch during the onset of a muscle contraction on subsequent active behaviour of the contractile machinery within an intact mammalian muscle–tendon complex. Muscle length and shortening velocity were studied because they may be important variables affecting this so-called prestretch effect. Seven gastrocnemius medialis (GM) muscles of the rat were examined. Tetanic, isovelocity shortening contractions from 3 mm above muscle optimum length (l_0) to l_0-2 mm, at velocities of 10–50 mm s^{-1} (dynamic experiments), were preceded by either an isometric contraction (PI) or an active stretch (PS). By imposing quick length decreases between the prephase and the concentric phase, all excess force generated in the prephase was instantaneously eliminated. This procedure only allowed small force changes during subsequent shortening (caused by the intrinsic properties of the contractile machinery). In this way, the influence of series elastic structures on subsequent muscle performance was minimized. Experiments were also performed at lengths ranging from $l_0+2.5$ mm to $l_0-1.5$ mm, keeping the length constant after the initial quick length changes (isometric experiments). For the dynamic experiments, enhancement of the performance of the contractile machinery (potentiation) was calculated as the ratio of the average force level over each millimetre of shortening during PS to that during PI conditions (PS/PI). For

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Key words: prestretch, mammalian skeletal muscle, isometric contraction, isovelocity contraction, potentiation.

the isometric experiments, the PS/PI force ratio after 300 ms of stimulation was used.

The main result of the present study confirmed results reported in the literature and experiments on isolated muscle fibres. For all conditions, a potentiation effect was found, ranging from about 2 to 16 %. Muscle length appeared to have a large positive effect on the degree of potentiation. At the greatest lengths potentiation was largest, but at lengths below optimum a small effect was also found. A negative influence of shortening velocity was mainly present at increased muscle lengths (l_0+2.5 mm and l_0+1.5 mm). For the dynamic experiments, no interaction was found between the effects of muscle length and shortening velocity on potentiation. However, there was a clear difference between the isometric and dynamic responses: the dependence of potentiation on muscle length was significantly greater for the isometric contractions than for the dynamic ones. These isometric–dynamic differences indicate that the processes underlying prestretch effects operate differently under isometric and dynamic conditions. The conditions under which the experiments were performed allow us to suggest that the potentiation effects described here might play an important role in ballistic movements containing active stretch periods (e.g. countermovement jumping).

Introduction

The enhancement of performance of skeletal muscle caused by active stretching is a well-known phenomenon (Abbott and Aubert, 1952; Cavagna and Citterio, 1974; Edman *et al.* 1978, 1982; van Atteveldt and Crowe, 1980; Sugi and Tsuchiya, 1981, 1988; Cavagna *et al.* 1985; de Haan *et al.* 1989*b*; Ettema *et al.* 1990*a*,*b*). The positive influence of active stretch on the work performance of the contractile machinery (i.e. cross-bridges) during subsequent shortening has been clearly demonstrated in studies on single muscle fibres (e.g. Edman *et al.* 1978; Sugi and Tsuchiya, 1981; Cavagna *et al.* 1985, 1986). Active stretch has a so-called potentiating effect on the contractile machinery (i.e. the force–velocity curve is shifted towards higher force values for a given velocity); this is probably caused by enhanced force production per cross-bridge rather than by an increase in the number of attached cross-bridges (Cavagna *et al.* 1985; Sugi and Tsuchiya, 1988). In addition to this potentiation effect on the contractile machinery, two other mechanisms cause a stretch-induced enhancement of work performance in whole muscle–tendon complexes. First, because of enhancement of muscle force, extra energy is released from the series elastic component (i.e. all elastic structures connected in series with the contractile machinery) (Cavagna, 1977; Ettema *et al.* 1990*a*,*b*). Second, there is an interaction between the series elastic component and the contractile machinery. That is, the behaviour of the series elastic structures will, for a great part, determine the loading conditions under which the contractile machinery is active. For example, during the onset of an isometric muscle contraction, the muscle fibres shorten as a result of the elongation of the tendinous structures (Ettema *et al.* 1990*b*; Griffiths, 1991). This influence of series elastic structures plays a particularly important role during stretch-shortening contrac-

tions because the large fluctuations in muscle force cause considerable changes in the length of the elastic structures (Avis *et al.* 1986; Ettema *et al.* 1990*b*). A large part of the difference in muscle performance following an active stretch or an isometric contraction can be explained by this mechanism: at a given muscle length, active muscle force is maximised when this muscle length is reached by lengthening. The tendinous structures are elongated more under these conditions and therefore the muscle fibres act at a shorter length than they do after an isometric contraction at the same muscle length.

Because of the occurrence of these different mechanisms during stretch-shortening contractions, little is known about the extent of the actual potentiation effect in whole muscle and about its dependence on factors such as muscle length and the shortening velocity of the muscle after it has been stretched. To our knowledge, only Cavagna *et al.* (1968) and Bergel *et al.* (1972) have reported measurements in which the effects of active prestretch of a muscle on subsequent shortening performance were caused exclusively by potentiation of the contractile machinery. Their protocol ensured that neither an enhanced release of elastic energy nor a difference in loading of the contractile element (caused by different series elastic behaviour) occurred. Bergel *et al.* (1972) found little or no enhancement in the performance of the contractile machinery caused by potentiation, whereas Cavagna *et al.* (1968) did find an enhanced contractile performance (see their Fig. 13). This discrepancy could have been caused, for example, by a difference in relative muscle length or shortening velocity used in the two sets of experiments.

The aim of the present study was to examine the influence of muscle length and shortening velocity on potentiation *per se*, which was induced by an active prestretch. In this way, we could examine whether the potentiation effect was a general feature, independent of muscle loading. An additional purpose was to study these effects in an intact mammalian muscle–tendon complex instead of on an isolated fibre preparation, thus mimicking some conditions found *in vivo*. This made it possible to see whether potentiation is important for muscle mechanics in real-life movements. This approach, however, demanded some compromises between fundamental research and the *in vivo* approach. One rather unnatural aspect was the quick release between the prephase and subsequent contraction, as was used by Bergel *et al.* (1972). This was necessary to minimize the influence of the series elastic component on the enhancement of muscle performance. A second artificial aspect was the maximal tetanic excitation of the muscle. This method allowed us to exclude any kind of neurological effects (reflexes) caused by mechanical stretch. Thus, we were able to restrict our study to the local stretch mechanisms within the muscle itself. It should be noted, however, that as a consequence the results may only be applicable to ballistic movements, in which muscle activation reaches the maximum level possible under *in vivo* conditions. We induced the potentiation using a more or less natural method of stretching. That is, the stretch was applied at the onset of activation instead of during the isometric force plateau (de Haan *et al.* 1989*b*; Ettema *et al.* 1990*a*,*b*). Care was

taken to ensure that the stretches produced high peak forces such as those found during ballistic movements *in vivo* (Biewener *et al.* 1988).

Materials and methods

Surgery and experimental protocol

The experiments were performed *in situ* on the gastrocnemius medialis muscle (GM) of the rat. Seven young adult Wistar rats (body mass 240–284 g) were anaesthetized with pentobarbital (initial dose 10 mg per 100 g body mass, intraperitoneally). The GM muscle–tendon complex was freed from its surrounding tissues, and the calcaneus was cut loose from the foot. The following morphological and physiological variables were measured for the seven muscles (mean, S.D.): muscle mass 849±72 mg; muscle–tendon complex optimum length 40.1±1.3 mm; muscle fibre optimum length 12.9±1.0 mm; length of the tendinous structures 28.1±0.8 mm; maximal isometric force 11.27±0.75 N. The distal tendon was tightly knotted and glued (Histoacryl Blau) to a steel wire. The calcaneus, which was left attached to the tendon, acted as an anchoring point, preventing slippage. The wire was connected to a strain gauge force transducer. The origin of the muscle was fixed by clamping the femur in a metal clamp. All measurements were made using a muscle ergometer described by Woittiez *et al.* (1987). The muscle was activated by supramaximal electrical stimulation of the severed nerve (60 Hz square-wave pulses; 0.4 ms, 3 mA). This frequency caused an almost, but not completely, fused tetanic contraction. The temperature of the muscle surface was controlled at 27±0.1 °C by means of infrared light, the intensity of which was regulated electronically. This temperature was chosen as a compromise between minimizing force transients induced by the 60 Hz stimulation frequency and working at physiological temperatures. Muscle optimum length (l_0), determined with an accuracy of 0.5 mm, was defined as the length at which isometric tetanic force (F_0) was greatest. The lengths of the muscle–tendon complex components were measured at l_0, after termination of the experiments (see above).

Stretch-shortening contractions (henceforth called prestretch or PS contractions) and shortening contractions preceded by an isometric phase (pre-isometric or PI contractions) were imposed on each muscle. The experimental protocol is shown schematically for these contractions in Fig. 1. The timing of all events during the experiments was controlled by computer. In each PS experiment the muscle was stretched at a velocity of 20 mm s^{-1} for 250 ms, whereas in the PI experiments the muscle was kept at constant length for this initial period. Stimulation started during muscle lengthening, 120 ms prior to the onset of shortening (i.e. at the end of the prephase). The stretch applied during activation was 2.4 mm. At the end of the prephase, a quick length decrease was applied, reducing muscle force instantaneously. This release was followed by a steady shortening, henceforth called the isovelocity phase. After the quick length decrease (i.e. at the onset of the isovelocity shortening), the muscle–tendon complex length was always l_0+3 mm. During the isovelocity shortening phase the

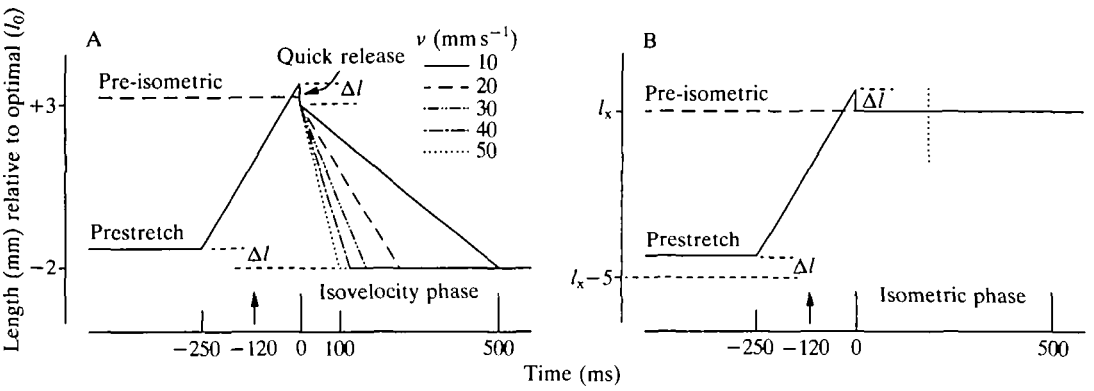

Fig. 1. Schematic representation of the experimental protocol for the dynamic (A) and isometric (B) experiments. Lines indicate length changes imposed on the muscle (shortening velocities, v, are given). The upward arrow indicates the onset of activation. Δl is the amplitude of the quick release (imposed at time zero) needed to reduce the force level. For the isometric experiments, muscle length (l_x) ranged from l_0+2.5 mm to l_0−1.5 mm. The vertical dotted line in B indicates the moment of measurement.

muscle shortened by 5 mm at a velocity of 10, 20, 30, 40 or 50 mm s^{-1}. Stimulation ceased about 30 ms after the end of muscle shortening. The prestretch experiments were also performed using an isometric period subsequent to the prestretch and quick length decrease (Fig. 1B). This isometric period was induced at muscle lengths of l_0+2.5 mm, l_0+1.5 mm, l_0+0.5 mm, l_0−0.5 mm and l_0−1.5 mm. The PI contractions for these experiments consisted of standard isometric contractions performed at the same final muscle length.

The protocol was designed in this a way to cover a large part of the length–force and force–velocity curves. Thus, we were able to examine not only the general features of prestretch effects but also specific potentiation features that depended on muscle load.

The quick release was applied to prevent a slow, long-lasting decline in force, starting from the force level at the end of the prephase to another, more or less steady, force level during the isovelocity shortening or isometric contraction period (Fig. 2). We aimed to attain the force level that would have been found by extrapolating back in time the subsequent force trajectory. This eliminated all excess force almost instantly, limiting force transients during the subsequent contraction to a minimum. Therefore, very little elastic energy release (or take up) occurred during the contraction period of interest. Furthermore, length changes of the series elastic structures were kept to a minimum, excluding the influence of the interaction between series elastic structures and the contractile machinery. The influence of the series elastic structures on work performance was thus reduced to a minimum. As a consequence, force enhancement was a good estimate of the potentiation of the contractile machinery alone. The amplitude of the quick length

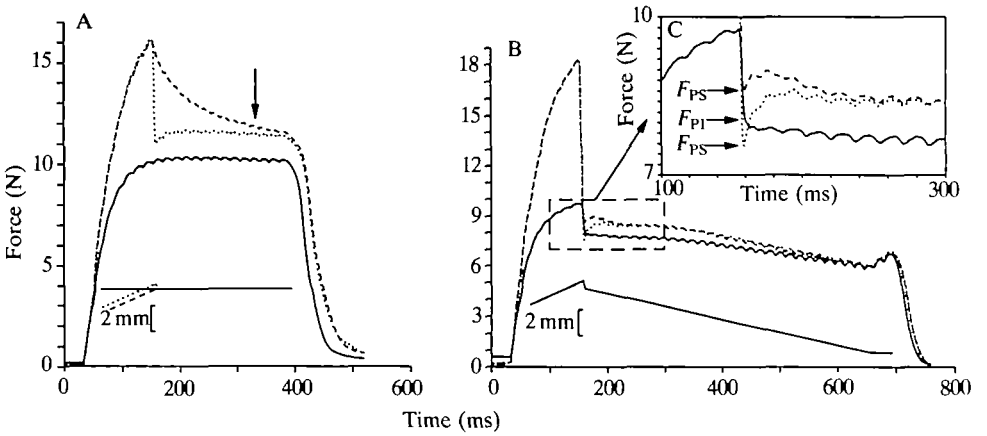

Fig. 2. (A) An example of the main effect of the use of a quick release directly after a prestretch on muscle force. The solid, dotted and dashed curves represent the isometric control, the prestretch with a quick release and the prestretch without a quick release, respectively. The lower traces indicate the corresponding length changes. The vertical arrow indicates the moment of force measurement. (B) An example of the influence of the amplitude of quick release on force levels after prestretch (dashed and dotted lines) and pre-isometric (solid lines) contractions for a shortening velocity of $10\,mm\,s^{-1}$. The lower trace indicates the length changes for one of the prestretch contractions. (C) Enlargement of the outlined part of B. Arrows indicate the force level at the onset of steady isovelocity shortening for prestretch (F_{PS}) and pre-isometric (F_{PI}) contractions.

decrease necessary for a correct force reduction was estimated roughly in a pilot experiment and was determined exactly for each individual muscle by trial and error (see Results for the strategy used for determining the 'correct' force reduction). Edman *et al.* (1982) showed that such length decreases following an active stretch did not change the final force level reached during isometric contractions and, thus, did not interfere with the effects of an active prestretch (see also the Results section).

The average force level during isovelocity shortening was calculated for each millimetre of shortening, i.e. at mean muscle lengths of $l_0+2.5\,mm$, $l_0+1.5\,mm$, $l_0+0.5\,mm$, $l_0-0.5\,mm$ and $l_0-1.5\,mm$. The ratio of these mean force levels (PS/PI) was taken as a measure of the potentiation effect. For the isometric experiments, the PS/PI ratio was calculated following 300 ms of activation (few force transients or none at all occurred at that time) (Fig. 2A). Note that, for the shortening contractions, the PS/PI ratio also reflects work performance, because the shortening range is similar for PS and PI contractions. Furthermore, for these shortening contractions, muscle length coincides with the amount of shortening done by the muscle at the point of measurement (both muscle length and the amount of shortening may affect muscle performance because of the deactivation mechanism described by Edman, 1975).

Statistics

The significance of influences of the investigated variables on the potentiation effect was tested using least-squares linear regression. For each muscle length and shortening velocity, potentiation (PS–PI difference) was tested for significance against zero using the Student's *t*-test (one-tailed, $P<0.05$). Differences between the isometric and the dynamic experiments were tested for significance using the Student's *t*-test (two-tailed, $P<0.05$). The significance of interaction effects between shortening velocity and muscle length on potentiation in the dynamic experiments was tested using a two-way analysis of variance (ANOVA, $P<0.05$).

Results

Influence of the amplitude of quick release

Fig. 2B shows an example of experimental length and force tracings of prestretch and pre-isometric contractions in which the amplitude of the quick release was varied. These tracings show that only during the first period of isovelocity shortening (in most cases less than 40 ms) was the force level clearly affected by the amplitude of the quick release. During subsequent shortening, the force patterns of contractions with a similar prephase but different amplitudes of quick release had similar values. During isovelocity shortening, a dynamic equilibrium force level was apparently reached, its value depending on the muscle length and shortening velocity and on the prephase condition. As stated above, the effects of series elastic interaction were reduced to a minimum. Therefore, the different effects of the PS and PI prephases indicate the existence of the potentiation effect at the level of the contractile machinery. The force tracings shown in Fig. 2B confirm the existence of this potentiation: even though the force at the onset of isovelocity shortening is somewhat lower for one of the prestretch contractions (dotted line), the force subsequently increases above the level for the pre-isometric contraction.

In contrast to the findings of Edman *et al.* (1982), for some of the experiments, relatively small force differences induced by different amplitudes of quick release appeared to be sustained during the entire contraction. This small influence was not taken into account when comparing prestretch and pre-isometric contractions in this study. Thus, they should be considered as a source of error for the estimate of potentiation.

A badly chosen quick release amplitude would have resulted in a considerable force transient during the first part of the isovelocity shortening. Under these conditions, a clear influence of the series elastic structures would have occurred. This would have resulted in a serious error in the estimation of the actual potentiation. It was not possible to determine exactly the correct force level for the onset of the subsequent isovelocity shortening phase. However, because of the length–force characteristics, a small rise in force was expected during the first period of the isovelocity shortening (from l_0+3 mm towards l_0). Furthermore, pilot experiments showed that a steady force level was established in a much

shorter time when the force had to increase rather than decrease to reach the equilibrium level. For these reasons, we decided to use amplitudes of quick releases such that a small increase in force (about 0.1–0.2 N) occurred in the first part of the isovelocity shortening. As a result, the force level immediately after the quick release was always somewhat higher in the prestretch experiments than in the pre-isometric experiments. For the isometric experiments we used a quick release amplitude (also resulting in a small force redevelopment, <0.3 N, rather than a force decrease) subsequent to the release (Fig. 2A). This enhanced a rapid establishment of the equilibrium force level.

Effects of muscle length and the amplitude and velocity of muscle shortening on potentiation

The PS/PI force ratios were plotted against both shortening velocity (Fig. 3) and muscle length (Fig. 4). A positive potentiation caused by prestretch was found for all except two of the 30 conditions used in this study (Student's t-test, one-tailed, $P<0.05$). At a muscle length of $l_0-1.5$ mm, there was no significant potentiation at velocities of 0 and 30 mm s^{-1}. Potentiation varied, depending on the contraction conditions, from about +2% to +16%. Even at lengths below optimal muscle length, small but significant potentiation effects were found (Fig. 3E).

When examining of the influence of shortening velocity on potentiation, the isometric experiments (i.e. $v=0$) were not taken into account because of the difference in the protocol (see below for further explanation). An effect of shortening velocity is clearly present in the first part of the shortening phase (i.e. at $l_0+2.5$ mm and $l_0+1.5$ mm, Fig. 3A,B): a higher shortening velocity results in a smaller potentiation. Later in the period of shortening the velocity effect was smaller (Fig. 3E) or not present (Fig. 3C,D). Note that, at short lengths, during the last 2 mm of shortening the potentiation is rather low, so an actual dependence of potentiation on velocity would have been difficult to detect. Fig. 5 shows the relative force enhancement calculated for the entire shortening of 5 mm. For the entire range of velocities measured in this study, an active prestretch moved the force–velocity curve towards higher force levels.

In the shortening experiments, the dependence of the potentiation effect on muscle length (and thus on the amount of shortening as well as on contraction time) is very clear: at shorter lengths the potentiation effect is greater at all shortening velocities (Fig. 4B–F: significant PS/PI–length regression slopes for all velocities). A similar but stronger dependence of potentiation on muscle length was found for isometric contractions. The PS/PI–length regression slope was significantly greater for the isometric experiments, compared to the shortening experiments (Fig. 6, Student's t-test, paired comparison, two-tailed, $P<0.05$). In contrast to the shortening experiments, only a single contraction was performed for each length in the isometric condition. Therefore, for the isometric experiments, the difference in muscle length was independent of the amount and

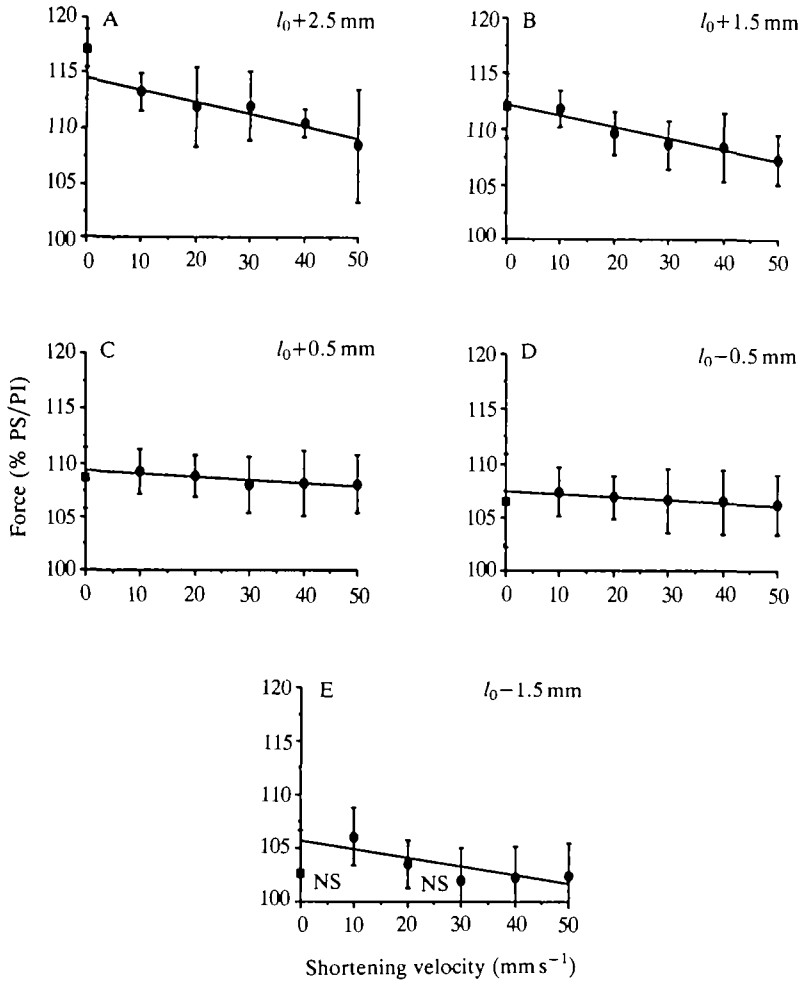

Fig. 3. Force potentiation as a function of shortening velocity at different muscle lengths. Solid lines are linear regressions. Mean values (N=7) ±S.D. (bars) are shown. Isometric data (■) (not used in the regression analysis) are shown for comparison. All mean values are significantly greater than 100 % (Student's t-test, one-tailed, P<0.05), except where marked not significant (NS). Results of linear regressions: (A) y=114.5–0.112x, r=0.460***; (B) y=112.1–0.103x, r=0.560***; (C) y=109.3–0.030x, r=0.179, NS; (D) y=107.5–0.027x, r=0.157, NS; (E) y=105.7–0.084x, r=0.402*; slopes differ from zero: *P<0.05, ***P<0.01, NS, not significant.

duration of shortening. Thus, the stronger length dependence for the isometric condition suggests that the amplitude of shortening has a positive effect on the potentiation mechanism. In contrast, the fact that the muscle length has itself become smaller has a negative effect on potentiation.

Results of the two-way analysis of variance (ANOVA) showed no interaction effect of length and velocity on the potentiation for the dynamic experiments.

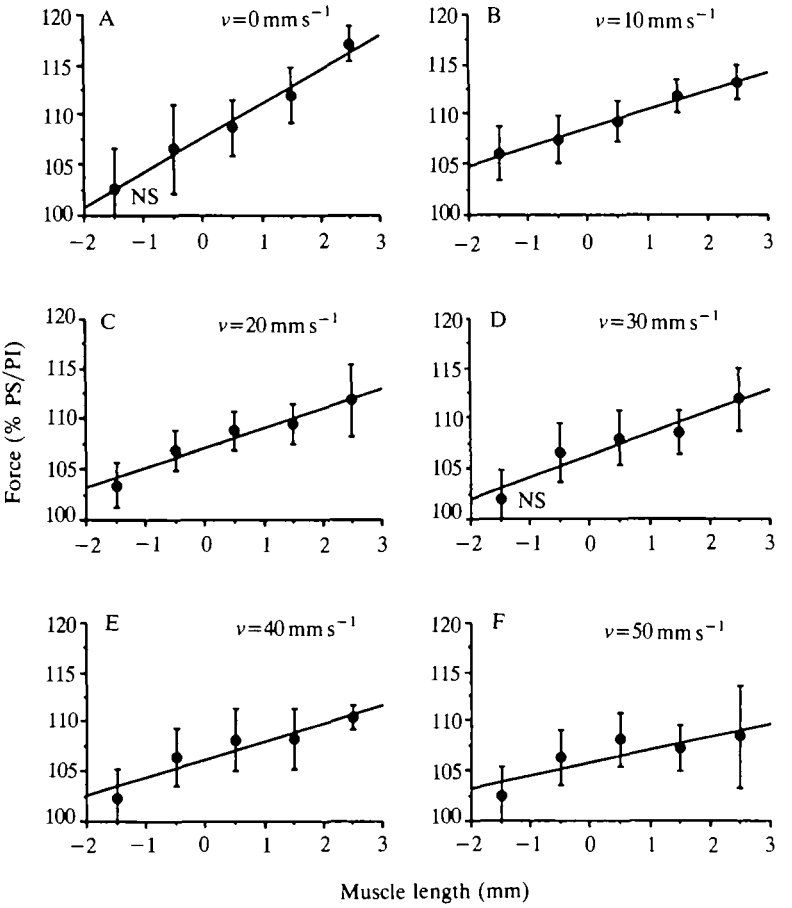

Fig. 4. Force potentiation as a function of muscle length at different shortening velocities (v). Solid lines are linear regressions. Mean values (N=7) ±S.D. (bars) are shown. All mean values are significantly greater than 100% (Student's t-test, one-tailed, $P<0.05$), except where marked not significant (NS). Results of linear regression: (A) $y=107.7+3.44x$, $r=0.842$; (B) $y=108.6+1.88x$, $r=0.799$; (C) $y=107.1+1.95x$, $r=0.766$; (D) $y=106.3+2.17x$, $r=0.748$; (E) $y=106.2+1.81x$, $r=0.688$; (F) $y=105.8+1.29x$, $r=0.488$. All slopes differ significantly from zero ($P<0.01$).

Discussion

The results of the present study show that an active prestretch causes a substantial enhancement of the performance of the contractile machinery during a subsequent period of contraction (for at least 500 ms). In addition to parameters of the prestretch period (see Ettema *et al.* 1990*a*), the type of load applied to the muscle after the prestretch period seems to play a crucial role in this potentiation effect. In accordance with Edman *et al.* (1978), Ettema *et al.* (1990*b*) and de Haan *et al.* (1991), muscle length was shown to be the dominant factor regulating the amount of potentiation. Furthermore, particularly at muscle lengths greater than

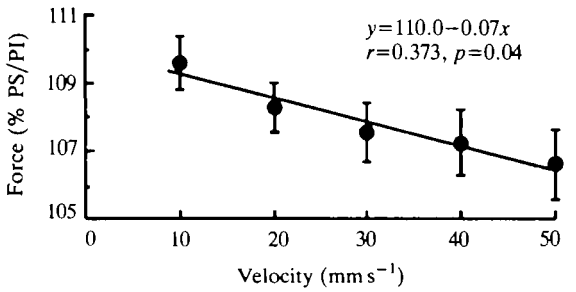

Fig. 5. Relative force enhancement (% PS/PI) as a function of shortening velocity caused by prestretch (mean ±S.D., $N=7$) calculated for the entire shortening of 5 mm.

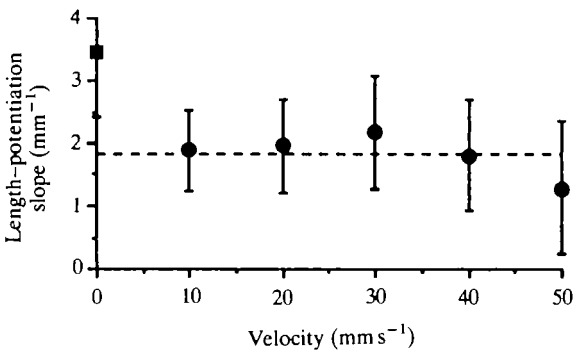

Fig. 6. The slope of the muscle length–potentiation regressions as a function of shortening velocity. The bars indicate ±S.D. ($N=7$) and the dashed line indicates the mean value for the dynamic experiments. ●, isovelocity (dynamic); ■, isometric.

the optimal length, potentiation is negatively related to the shortening velocity of the muscle. The results suggest that the muscle potentiation effects described here probably play an important role in ballistic movements containing active stretch periods (e.g. countermovement jumping). Furthermore, the amount of this potentiation will clearly depend on the dynamics of the muscles involved in the movements. Ettema *et al.* (1990*a*) concluded from *in situ* experiments on rat GM that potentiation only plays a minor role during movement *in vivo* (when compared to the enhanced release of series elastic energy). Similar conclusions were drawn by de Graaf *et al.* (1987) and Avis *et al.* (1986), based on *in vivo* experiments concerning, respectively, jumping and isovelocity leg extension in humans. All of the prestretch-induced enhancement of muscle performance reported by those authors could be explained by enhancement of elastic energy release or by interaction between series elastic structures and the contractile machinery. Our present results show that such conclusions should not be

generalized to all types of countermovement in which prestretch plays a role. The static and dynamic properties of the muscles involved must be considered when studying the role of muscle potentiation in different movements.

One could argue that the potentiation found in this study occurred only in a part of the muscle which, in real-life situations, may never be active while it is stretched. However, the literature indicates that, for ballistic movements such as jumping, it is most likely that a large part of the gastrocnemius is active during the stretch period. For example, Biewener *et al.* (1988) found peak forces of about 175 % of F_0 for gastrocnemius and plantaris muscles during jumping of the kangaroo rat. These force levels indicate approximately maximal recruitment of those muscles (Biewener *et al.* 1988). The results of Sullivan and Armstrong (1978) also point in this direction: for the rat, fast galloping induced glycogen depletion in nearly 100 % of the gastrocnemius muscle fibres. In voluntary eccentric contractions of the human triceps surae, most large motor units are probably active (Nardone *et al.* 1989). Therefore, if in real-life situations potentiation only occurs in a fibre population that is not active during stretch, it must be restricted to only a small portion of the total muscle fibres. The potentiation values found in the present study, however, are of such a level that they cannot be attributed to just a small population of fibres. If this were so, relative potentiation would yield unrealistically high levels for the affected fibres. (If, for example, 10 % of the fibre population is responsible for a total muscle potentiation of 10 %, then the potentiation would have to be 100 % for that fibre population.) Thus, we conclude that potentiation of the contractile machinery probably plays an important role during *in vivo* stretch-shortening contractions.

Influence of muscle length on potentiation

The literature indicates that potentiation occurs at long muscle lengths, but not below optimum length. For example, Edman *et al.* (1978) found no isometric force enhancement after prestretch at sarcomere lengths below 1.8 μm, i.e. about 14 % below optimum length (2.1 μm). Ettema *et al.* (1990*b*) found a slightly negative effect of prestretch on muscle performance just below muscle optimum length (about 1 mm) during isotonic shortening. However, these negative effects appeared to be of a transient character and could not be demonstrated for a period of 140 ms of isotonic shortening (Ettema *et al.* 1990*b*). In contrast, we obtained significant potentiation at lengths below muscle optimum length (Fig. 3D,E). For instance, at $l_0-1.5$ mm, muscle fibre length was estimated to be about 12 % below fibre optimum length, but some potentiation still occurred.

There can be no doubt about the qualitative influence of muscle length on the effects of prestretch, but it is not yet clear what length results in no potentiation effect at all. Our present results show, however, that this critical length lies well below optimum length. It may be that the discrepancy between whole-muscle and single-fibre studies reflects the effect of a diversity of relative fibre lengths within the intact muscle (Herzog and ter Keurs, 1988; Huijing, 1988; Bobbert *et al.* 1990).

Effects of potentiation on the force–velocity curve

For isolated fibres, Edman *et al.* (1978) found a shift in the isotonically measured force–velocity curve only at lengths above the optimum length. In the present study, such a shift occurred at both long and short muscle lengths (i.e. at, and just below, muscle optimum length) for the range of slow shortening velocities. Cavagna and Citterio (1974), Edman *et al.* (1978) and Sugi and Tsuchiya (1981) showed that maximal unloaded shortening velocity is not affected by active stretch. Most of our present findings are in agreement with this. Assuming that a linear extrapolation of our data towards maximum shortening velocities (i.e. $>100\,mm\,s^{-1}$; de Haan, 1988; de Haan *et al.* 1989*a*) is justified, virtually no potentiation effect would be left at maximum shortening velocity. According to the regression shown in Fig. 5, zero potentiation would be reached at a shortening velocity of about $140\,mm\,s^{-1}$. However, the range of velocities over which the data were obtained does not justify a linear extrapolation over such a wide range. For example, a hyperbolic curve fit may be more suitable in this case. It should be noted, therefore, that the linear extrapolation only gives a rough approximation of the velocity yielding zero potentiation. The overall negative influence of shortening velocity on the potentiation effect indicates (as proposed by Edman *et al.* 1978) that active stretch does not alter the kinetics of cross-bridge function. Instead, it is more likely that, at a given and unaltered cross-bridge cycling rate, higher forces can be generated after active stretch.

Dynamic versus *isometric loading conditions*

A striking result is the clear difference in potentiation effects between isometric and dynamic (i.e. shortening) conditions following a prestretch. The influence of muscle length on the potentiation mechanism is stronger for isometric contractions than for shortening contractions [i.e. the slope of the muscle length–potentiation regression curve is greater for the isometric experiment (Fig. 6). For the isometric experiment, the slope is 3.44, whereas for the shortening experiments it ranges from 1.29 to 2.17]. This discrepancy can be explained in part by a simple interaction between the influence of muscle length and shortening velocity. The interaction works as follows. First, at short muscle lengths only minor potentiation occurs. This potentiation is probably zero at a certain length below muscle optimum length, and this length is independent of the shortening velocity. Second, at increased muscle lengths the potentiation effect is clearly negatively related to shortening velocity (Fig. 3A). A simple combination of these two phenomena results in a relationship between the length–potentiation regression coefficient and shortening velocity. Such a hypothetical relationship could not be demonstrated for the dynamic data. (A two-way ANOVA showed no interaction effect of length and velocity on the potentiation for the dynamic experiments. This means that velocity does not influence the length–potentiation slope.) Thus, this interaction mechanism cannot explain the significant differences between the isometric and dynamic PS/PI–length regression slopes (Fig. 6). Apparently some

difference between the dynamic and isometric conditions caused the potentiation effect to become less dependent on muscle length for the dynamic condition than for the isometric condition. A logical first step is to relate this feature to some aspects of the experimental design, instead of seeking a difference in principle between dynamic and isometric contractions. We discuss aspects of the experimental design below, and show that it is unlikely that they caused the discrepancy between the dynamic and isometric results.

First, the time elapsed from the end of the prephase (prestretch or isometric prephase) was exactly the same for all isometric experiments, while for the shortening experiments the duration of activation was related to the muscle length and reciprocally related to the velocity of shortening. De Haan *et al.* (1991) showed that potentiation caused by active stretch lasted for longer (about 1 s) than did the contractions in our experiments (0.5 s). Nevertheless, if any effect of prestretch changed during a subsequent contraction, it seems logical to suppose that the effects would have decreased with time. Because a given amount of shortening at a higher velocity took less time, one would expect the slope of the length–potentiation regression to decrease with increasing shortening velocity. However, as already mentioned, no differences were found between these slopes at different shortening velocities. Even more importantly, in the isometric experiments, the time after the prephase had no influence on the muscle length–potentiation relationship. Therefore, one would expect the coefficient for this relationship to be lowest at a shortening velocity of $0\,mm\,s^{-1}$ (i.e. isometric experiments), which is clearly not the case (Fig. 6).

Second, the differences in the prestretch period between the shortening and isometric experiments are a factor worth considering. For the isometric contractions, not only the isometric period following the prephase but also the prephase itself (stretch) were set at different lengths. This length was directly coupled to the isometric length after the prephase. It is possible that the length at which the stretching occurs is also of importance, thus increasing the dependence of potentiation on muscle length. If this were the case, one would have expected the potentiation at long muscle lengths to be similar for both isometric and shortening conditions and, at short muscle lengths, to be lower for the isometric condition than for the shortening condition. Our results do not support this hypothesis. Furthermore, recent experiments show that neither the length during the prestretch phase nor the amplitude of subsequent shortening affected this effect. Only the length at which the muscle was held afterwards determined the amount of potentiation (A. de Haan, unpublished results).

It is interesting to consider the discrepancy between the isometric and dynamic potentiation effects as a reflection of an essential difference of contraction dynamics under these two conditions. A possible explanation is that, instead of one, several different mechanisms induce the potentiation effect. Amemiya *et al.* (1988) proposed that the myofilament lattice was disordered by active stretch, thus enhancing muscle force: repulsion forces between the filaments would be increased, in turn being converted into enhanced fibre forces by means of the

constant-volume mechanism. This disordering behaves like an extra parallel force component. Another mechanism lies within the cross-bridges, which are pulled into a higher energy state by an active stretch (Hatze, 1981). It should be noted that this higher energy state is different from an elastic-like extension of the cross-bridges, which should be equated to storage of series elastic energy (Flitney and Hirst, 1978; Cavagna *et al.* 1985). This elastic extension only results in an enhancement of work performance during the first cross-bridge cycle of such extended cross-bridges. We propose that at least one of the potentiating mechanisms depends on muscle length and is abolished by shortening of the contractile machinery (i.e. cross-bridge cycling). For example, the amount of disordering of the myofilament lattice caused by stretch may depend on fibre length. Since the inner myofilament distance decreases with fibre length, a given displacement within the lattice will have a relatively greater effect at long fibre lengths. Suppose, for example, that active cross-bridge cycling quickly pulls the filaments back into order again, regardless of the velocity of the cross-bridge cycling. This would explain the lower dependence of potentiation on muscle length in the dynamic experiments. (The potentiation caused by this lattice-disordering mechanism would then be represented by the difference between the regression line in Fig. 4A and the average of the lines in Fig. 4B–F.) Some other potentiation mechanism must also be present, because there is still considerable potentiation left in the dynamic contractions (Fig. 4B–F). Such a hypothesis is very preliminary, of course. Clearly, research on isolated fibres is needed. Our results show that some fundamental aspects of potentiation (and muscle contraction in general) can be demonstrated even in an intact muscle–tendon complex.

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