Low-frequency fatigue, post-tetanic potentiation and their interaction at different muscle lengths following eccentric exercise

J. M. Rijkelijkhuizen[1,*], C. J. de Ruiter[1], P. A. Huijing[1,2] and A. de Haan[1,3]

[1]*Institute for Fundamental and Clinical Human Movement Sciences, Vrije Universiteit, Van der Boechorststraat 9, 1081 BT Amsterdam, The Netherlands*, [2]*Integrated Biomedical Engineering for Restoration of Human Function, Instituut voor Biomedische Technologie, Faculteit Construerende Wetenschappen, Universiteit Twente, Postbus 217, 7500 AE Enschede, The Netherlands* and [3]*Institute for Biophysical and Clinical Research into Human Movement, Manchester Metropolitan University, Crewe & Alsager Faculty, Cheshire ST7 2HL, UK*

*Author for correspondence (e-mail: j.rijkelijkhuizen@vumc.nl)

Summary

Low-frequency fatigue (LFF) and post-tetanic potentiation (PTP) were quantified at different muscle lengths in rat medial gastrocnemius (GM) muscle. *In situ* experiments were performed on GM muscle–tendon complexes of anaesthetised (urethane, $1.5\,\mathrm{g\,kg^{-1}}$ i.p.) Wistar rats (N=8). Force–length characteristics were determined at maximal (200 Hz) and submaximal (60 Hz) stimulation. Data for submaximally stimulated muscle were obtained in a non-potentiated and in a potentiated condition. LFF was induced by a series of 40 eccentric contractions. Post-exercise (40–80 min), data for the force–length relationships were obtained once more. Whereas force loss at 200 Hz-stimulation was least at optimum muscle length, $L_{0,200Hz}$, (17.0±1.4%, mean ± S.E.M.), force loss at 60 Hz-stimulation was maximal near $L_{0,200Hz}$ (55.1±4.3% at $L_{0,200Hz}$–1 mm). When the muscle was potentiated, force loss at 60 Hz-stimulation was maximal at short muscle length: $L_{0,200Hz}$–4 mm (53.5±3.8%). The extent of LFF, quantified by a decrease in the 60:200 Hz force ratio, varied with muscle length: LFF increased with decreasing muscle lengths when muscles were potentiated. However, in the non-potentiated condition, LFF was maximal at a length just below $L_{0,200Hz}$; the 60:200 Hz force ratio had decreased to 54.6±5.9% of the pre-exercise ratio at $L_{0,200Hz}$–1 mm. Compared with the non-potentiated condition, LFF was less pronounced in the potentiated condition. PTP counteracted LFF particularly at long muscle lengths. However, at short muscle lengths, LFF was still observed in potentiated muscles.

Key words: force–length characteristics, maximal stimulation, submaximal stimulation.

Introduction

Repeated activation of muscle induces processes resulting in decreased performance (fatigue) as well as enhanced performance (potentiation). Fatigue is described as the failure to maintain the required or expected force or power output (Edwards, 1983). Potentiation is known as the increase of force at submaximal levels of activation as a result of repeated low-frequency stimulation (staircase potentiation) or of a previous tetanic contraction (post-tetanic potentiation, PTP; Krarup, 1981). Thus, both fatigue and/or potentiation change characteristics of skeletal muscle. The stimulation frequency–force relationship is one of the characteristics of skeletal muscle that may change as a result of previous activity. For example, short-term fatigue causes a shift in the stimulation frequency–force curve to lower frequencies (e.g. Thompson et al., 1992). Short-term fatigue is generally related to metabolic changes within the muscle and recovers relatively quickly (e.g. Edwards et al., 1975). By contrast, low-frequency fatigue (LFF) is a long-lasting type of fatigue characterised by

reduced tetanic force at low frequencies of stimulation whereas force is not, or only to a minor extent, reduced at high frequencies (Edwards et al., 1977; Westerblad et al., 1993). Thus, in contrast with short-term/metabolic fatigue, LFF is known to cause a shift of the stimulation frequency–force relationship to higher frequencies (Edwards et al., 1977) or, more precisely, causes lower forces at low stimulation frequencies. It is most likely that LFF is the result of an impairment of the process of E–C coupling (Edwards et al., 1977) leading to a reduced Ca^{2+} release from the sarcoplasmic reticulum (SR) per action potential (Hill et al., 2001; Westerblad et al., 1993). Similar changes in Ca^{2+} concentrations result in larger changes in force at low stimulation frequencies than at high frequencies (Jones, 1996; Westerblad et al., 1993).

Up to now, LFF has been studied mainly at one particular muscle length (for instance optimum length) to understand the mechanisms. However, there is a prominent influence of

muscle length on the stimulation frequency–force curve with a shift to higher frequencies at shorter muscle lengths (De Haan et al., 2003; Rack and Westbury, 1969; Roszek et al., 1994). This means that at shorter muscle lengths, higher stimulation frequencies are necessary to obtain the same percentage of the maximum force. Because of the length-dependence of the stimulation frequency–force relationship, it is conceivable that the extent of LFF differs with muscle length. Thus, the first aim of the study was to investigate the manifestation of LFF at different muscle lengths. Based on length-dependent differences in Ca^{2+} sensitivity, it was expected that the effects of LFF would be more pronounced at shorter muscle lengths.

LFF is present after different types of exercise, but is most pronounced after eccentric exercise (Edwards et al., 1981; Rijkelijkhuizen et al., 2003). Therefore, the effects of a series of eccentric contractions on the force–length characteristics for maximal (stimulation frequency of 200 Hz) and submaximal (60 Hz) stimulation were investigated in this study. Any variation of LFF with muscle length can be deduced from these relations. Consequently, by comparing the pre-exercise 60:200 Hz force ratios with the post-exercise values, the extent of LFF can be quantified.

Stimulation frequency–force relationships are highly affected by potentiation, which progressively enhances force at lower stimulation frequencies (e.g. MacIntosh and Willis, 2000). Since the submaximal forces are affected in LFF, we were interested to find out whether PTP could counteract the effects of LFF. Potentiation of force by previous activation is caused by increased rates of phosphorylation of the myosin light chains (MLCs) (e.g. Manning and Stull, 1979; Moore and Stull, 1984), leading to an increased sensitivity to Ca^{2+}. Therefore, it might be expected that potentiation can counteract the effects of the reduced Ca^{2+} release found during LFF. Thus, the second aim of this study was to compare LFF in non-potentiated muscles with LFF in muscles potentiated by a previous tetanic contraction. This is particularly interesting because muscles *in vivo* are likely to be active more often in a (more or less) potentiated state than in a non-potentiated state. It was hypothesised that PTP can (partly) compensate for LFF. Because potentiation (when expressed as potentiated force relative to non-potentiated force) is muscle length dependent, being higher at short muscle lengths (Rassier and MacIntosh, 2002; Roszek et al., 1994; Wallinga-de Jonge et al., 1980), we investigated how the extent of LFF was expressed across different muscle lengths both in a potentiated and a non-potentiated condition.

Materials and methods

In situ experiments were performed on medial gastrocnemius (GM) muscle–tendon complexes of eight male Wistar rats (*Rattus norvegicus albinus* Berkenhaut 1769; body mass 273–285 g). The rats were anaesthetised with urethane (1.5 g kg^{-1} body mass, injected intraperitoneally). Supplementary doses of 0.63 g kg^{-1} body mass were given if necessary. Experiments were approved by the Committee on Animal Experimentation of the Vrije Universiteit Amsterdam and complied with Dutch law.

Muscle preparation and experimental set-up

During surgery as well as during the experiment, the animal was placed prone on a heated pad of 35°C to prevent hypothermia. The GM muscle–tendon complex of the right leg (*N*=8) was dissected free of surrounding skin, connective tissue and other muscles. This means that most, if not all, effects of extra- and intermuscular force transmission (e.g. Huijing, 1999) were excluded. The muscle origin and the blood supply remained intact. The sciatic nerve was cut as proximally as possible within the upper leg. All distal branches of this nerve were cut except the branch innervating the GM.

The femur was clamped vertically past the edge of the heated pad and the muscle was positioned horizontally (see also De Haan et al., 1989a). The distal tendon with a piece of the calcaneal bone was connected to a force transducer. The sciatic nerve was placed on a bipolar electrode used for stimulation. Muscle temperature was controlled by a water-saturated airflow of 33°C around the muscle. The force transducer (custom made, compliance 8 µm N^{-1}, resolution 0.005 N) used was part of an isovelocity measuring system. The force transducer was mounted on the lever arm of a servomotor. Acceleration, velocity, start length, (onset of) movement, (onset of) stimulation, stimulation frequency and duration of the muscle contractions were computer controlled. Stimulation current was 1 mA with a pulse width of 0.05 ms for maximal stimulation of all fibres. The data (force, length and stimulation pattern) were AD converted with a sample frequency of 1000 Hz and stored on disc. After the experiments, the rats were killed by cervical dislocation.

Experimental muscle length

Tetanic optimum muscle length for maximal force ($L_{0,200Hz}$) was determined using 200 ms tetani with a stimulation frequency of 200 Hz. Other muscle lengths were expressed relative to $L_{0,200Hz}$. After lengthening or shortening the muscle to the desired length, the measurements started with recording of passive muscle force during 100 ms in which time the muscle–tendon complex adjusted to the new length. The passive force measured after the contraction was subtracted from recorded force to obtain active force.

Pre-exercise force–length characteristics, potentiation and 60:200 Hz force ratios

Following determination of $L_{0,200Hz}$, force–length data for maximal stimulation were obtained by imposing tetani at a stimulation frequency of 200 Hz. The duration of each contraction was 200 ms, which was sufficient to reach a force plateau. Contractions were performed at nine lengths in random order (range: 4 mm below to 4 mm above $L_{0,200Hz}$ with 1.0 mm increments). Time between contractions was 2 min, which was enough time to avoid fatigue. After this set of contractions, 20 min of rest (duration determined in pilot

experiments) was included to make sure that no potentiation of the previous contractions was left. Subsequently, force–length data for submaximal stimulation were obtained at the same nine muscle lengths but with a stimulation frequency of 60 Hz and a pulse train of 500 ms, which time was needed to reach a force plateau at this frequency of stimulation.

To study the effect of PTP, the muscle was potentiated with an isometric tetanus (duration 800 ms, stimulation frequency 200 Hz). Pilot experiments showed that such tetanic contractions yield a high level of potentiation without significant fatigue (see also Abbate et al., 2000). Potentiation remained constant for more than 20 s and had vanished after 15 min. Directly after the potentiating tetanus, the force–length relationship was determined again for a stimulation frequency of 60 Hz to study the effects of potentiation on the (submaximal) 60 Hz force. Time between the nine contractions was 2 s to maintain potentiation. The contractions were applied in random order to minimise effects due to changes in potentiation and/or fatigue. Using the measurements at stimulation frequencies of 60 Hz and 200 Hz, the 60:200 Hz force ratio was calculated at each muscle length (both in the non-potentiated and in the potentiated condition).

Eccentric exercise

After 10 min rest, a series of 40 eccentric contractions was performed within 14 s. Each contraction was performed with a velocity of 20 mm s^{-1}, lasted 70 ms and was induced by 10 stimulation pulses applied at a frequency of 150 Hz. Pilot experiments had indicated that a stimulation frequency of 150 Hz was sufficient to obtain maximal activation for eccentric contractions and that a stimulation duration of 70 ms resulted in an eccentric force that did not exceed maximal isometric force of the preparation. During the eccentric contractions, the stimulation started simultaneously with lengthening of the muscle (i.e. without any prior isometric phase). In these dynamic conditions, the range of movement was from –3.5 to +0.5 mm, where 0 mm indicates $L_{0,200Hz}$. The peak force during the stretch was reached at approximately $L_{0,200Hz}$–2 mm and relaxation was completed during the stretch phase. Fig. 1 shows typical examples of eccentric contractions as performed in this protocol. By avoiding stretching to non-physiologically long muscle lengths we aimed to prevent severe force loss due to the eccentric exercise.

Post-exercise force–length characteristics, potentiation and 60:200 Hz force ratios

Because of possible changes in optimum length and their effects on the 60:200 Hz force ratio, $L_{0,200Hz}$ was determined again after the eccentric series of contractions with a few isometric contractions at a stimulation frequency of 200 Hz. Due to the eccentric series of contractions, $L_{0,200Hz}$ had changed and therefore the muscle length was set at the new (i.e. 1.0 mm longer) length. In the period between 40 and 60 min after the exercise, force–length data were obtained using a stimulation frequency of 200 Hz. After an additional 20 min of rest (i.e. 80 min after the exercise), force–length data
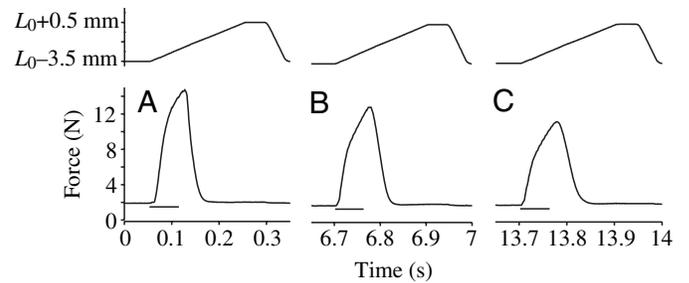

Fig. 1. Typical example of length and force traces of eccentric contractions as imposed during the eccentric exercise. Length (top panels) and force (bottom panels) traces during eccentric contractions of a medial gastrocnemius (GM) muscle. The first (A), 20th (B) and last (C) contraction of the eccentric fatiguing protocol are shown. The length of the muscle–tendon complex is expressed relative to the isometric optimum length for maximal stimulation ($L_{0,200Hz}$). The bold line below the force trace indicates stimulation.

were collected using a stimulation frequency of 60 Hz with 2 min rest in between contractions. Subsequently, the muscle was potentiated with a potentiating tetanus similar as in the pre-fatigue condition. Tubman et al. (1996) showed that in *in situ* rat GM muscle, MLC phosphorylation was significantly lower in fatigued muscles than in fresh muscles after the same tetanic contraction, while the extent of PTP was similar. Directly after the potentiating tetanus, the force–length data were collected at a stimulation frequency of 60 Hz, with 2 s in between contractions.

To quantify LFF, 60:200 Hz force ratios were calculated at all muscle lengths studied and compared with the pre-exercise values both in the potentiated and the non-potentiated condition. A decrease of the 60:200 Hz force ratio indicated the presence of LFF. LFF was quantified in a period of 40–80 min after the cessation of the fatiguing exercise because muscle metabolites causing short-term fatigue were expected to have returned to their pre-exercise values (De Haan et al., 1989b) and pilot experiments had indicated that LFF had fully developed by that time.

Statistics

All values are described as mean ± standard error of the mean (S.E.M.). Analyses of variance (ANOVA) for repeated measures on one or two factors ('condition' and/or muscle length) were used to determine statistical differences in force or 60:200 Hz ratio. The factor 'condition' consisted of fur levels: pre-exercise, pre-exercise-PTP, post-exercise, post-exercise-PTP. If significant main effects or interaction effects were observed, Bonferroni *post-hoc* tests were performed. The level of significance was 0.05.

Results

Pre- and post-exercise force–length characteristics
Isometric force at maximal stimulation (200 Hz)
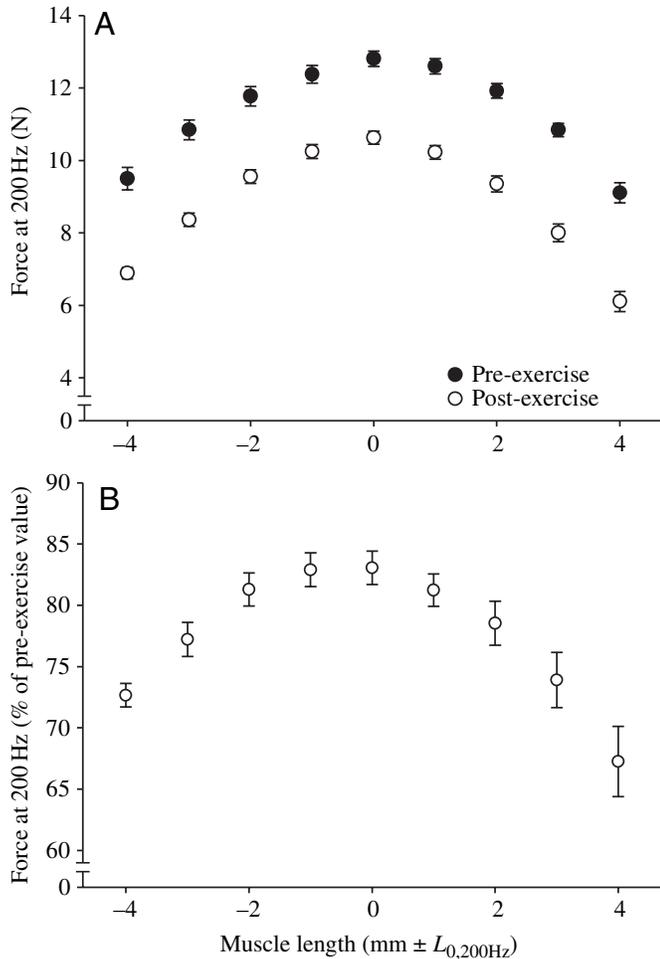
The effect of a series of eccentric contractions on the

Fig. 2. Sustained effect of eccentric exercise on isometric force–length characteristics for maximally stimulated muscle. (A) Maximal isometric force–length relationships before (pre-exercise, closed circles) and 40–60 min after a series of eccentric contractions (post-exercise, open circles). Maximal force, obtained at a stimulation frequency of 200 Hz, was significantly reduced post-exercise at all muscle lengths. (B) Maximal isometric force–length relationship post-exercise, expressed relative to the pre-exercise values. Force loss was less pronounced at $L_{0,200Hz}$ compared with both shorter and longer lengths. Note that post-exercise (open circles in Fig. 1A and 1B), the muscle was set on the new (1 mm longer) $L_{0,200Hz}$. Data are presented as mean ± S.E.M.

force–length characteristics at 200 Hz is shown in Fig. 2A. Post-exercise data were collected in a period of 40–60 min after exercise. Note that post-exercise, the muscle was set at its new 1 mm longer $L_{0,200Hz}$ (see Materials and methods). A significant reduction in absolute force at maximal stimulation occurred post-exercise and this effect was not equal for all muscle lengths measured: ANOVA showed significant effects of exercise and muscle length as well as an interaction effect (all $P<0.01$). The relative force loss with pre-exercise force set at 100% at each length was different for the measured muscle lengths 40–60 min after the exercise (Fig. 2B); statistical analysis revealed a significant effect of muscle length ($P<0.01$). Force loss was least near $L_{0,200Hz}$ (17.0±1.4%),
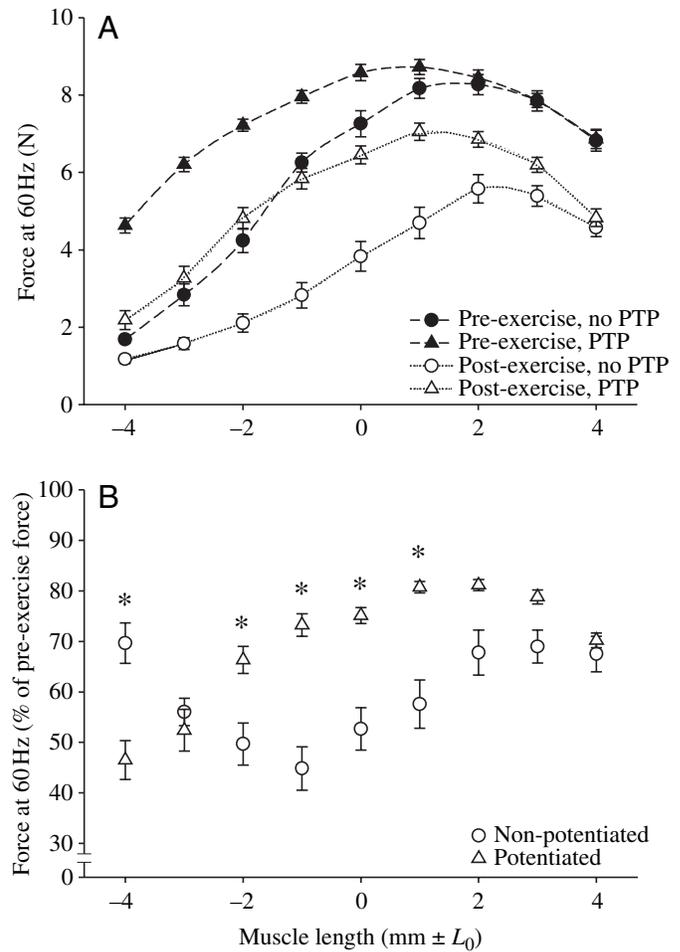
Fig. 3. Sustained effect of eccentric exercise on isometric force–length characteristics for submaximally stimulated muscle. (A) Submaximal isometric force–length characteristics before (pre-exercise, closed symbols) and 80 min after a series of eccentric contractions (post-exercise, open symbols) (mean ± S.E.M.). Data were collected before (circles) and after (triangles) a potentiating tetanus, which caused post-tetanic potentiation (PTP). Exercise reduced submaximal force (obtained with a stimulation frequency of 60 Hz), whereas the potentiating tetanus raised force at most muscle lengths. (B) Submaximal isometric force–length relationship post-exercise, expressed relative to the pre-exercise values. Different effects of muscle length were found in the potentiated (triangles) compared with the non-potentiated (circles) condition. *Significant difference between non-potentiated and potentiated condition.

whereas at shorter and longer muscle lengths, force depression was more pronounced (>25%).

Submaximal isometric force (60 Hz)

Force–length characteristics at 60 Hz (with and without potentiation) are illustrated for the pre- and post-exercise conditions (Fig. 3A). Note the different shape of the force–length relationship (levelling off of force at shorter lengths) at 60 Hz in the non-potentiated muscle post-exercise compared with the pre-exercise condition. In the pre- and post-exercise condition, optimum muscle length for force at 60 Hz
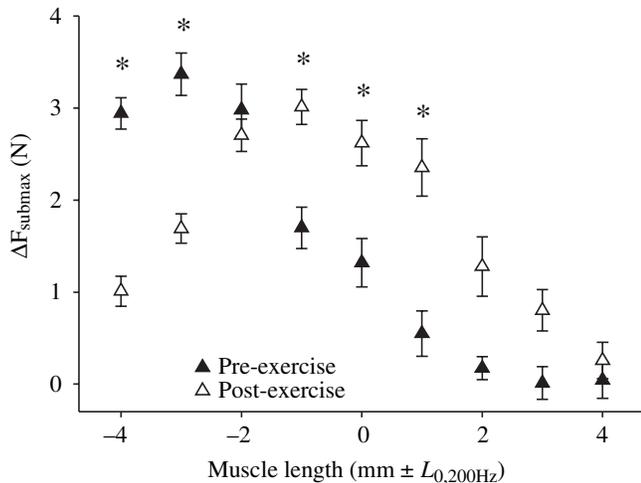
Fig. 4. Sustained effect of eccentric exercise on post-tetanic potentiation (PTP) at different muscle lengths. The increase in submaximal force (ΔF) of muscles in the potentiated compared with the non-potentiated condition was calculated before (closed triangles) and 80 min after (open triangles) eccentric exercise. Different effects of muscle length were found in the pre- compared with the post-exercise condition. On average, PTP had similar effects (*P*>0.05) pre- and post-exercise. However, post-exercise, PTP was significantly lower at short and higher at long muscle lengths than pre-exercise. Data are presented as mean ± S.E.M. *Significant difference between pre- and post-exercise condition.

without potentiation was approximately 2 mm longer than at 200 Hz ($L_{0,200Hz}$+2 mm). Potentiation shifted optimum muscle length for 60 Hz force to $L_{0,200Hz}$+1 mm. Significant main effects of condition (exercise and/or PTP) and muscle length on submaximal force were indicated, as well as an interaction effect (all *P*<0.01). *Post-hoc* comparison revealed that the curves differed significantly in each of the four conditions. Hence, the effects of exercise and PTP were different for the various muscle lengths but, in general, exercise reduced force at 60 Hz whereas PTP raised the 60 Hz force.

When the post-exercise values are expressed relative to the pre-exercise values (Fig. 3B), the relative force loss as a result of the series of eccentric contractions at each muscle length is visible. Significant main effects were detected for PTP (*P*<0.01) and for muscle length as well as a significant interaction of the effects of PTP and muscle length. At most muscle lengths ($L_{0,200Hz}$−2 mm to $L_{0,200Hz}$+3 mm), the force loss in the potentiated condition was much less than in the non-potentiated condition. In the non-potentiated condition, the 60 Hz force decreased most near $L_{0,200Hz}$. At a muscle length of $L_{0,200Hz}$−1 mm, the force at 60 Hz had decreased by 55.1±4.3%. In the potentiated state, however, the influence of muscle length on 60 Hz force was similar to the influence on maximal force (at 200 Hz) (compare Figs 3B and 2B). The force loss was most pronounced at short muscle lengths (53.5±3.8% at $L_{0,200Hz}$−4 mm). Thus, length-effects were different in non-potentiated and potentiated conditions.

Post-tetanic potentiation

The effect of the potentiating tetanus (800 ms, 200 Hz) increased submaximal force at most of the studied muscle lengths (Fig. 3A). To evaluate the quantitative effects of eccentric exercise on potentiation and the force–length characteristics, we calculated the difference between the 60 Hz force–length curve in the potentiated condition and the curve in the non-potentiated condition (Fig. 4). Due to eccentric exercise, optimal potentiation was found at a longer muscle length (a shift from $L_{0,200Hz}$−3 mm to $L_{0,200Hz}$−1 mm). Significance was obtained for a main effect of muscle length, as well as for the interaction of exercise and muscle length effects (*P*<0.01), whereas the main effect of exercise was not significant (*P*>0.05). Thus, different effects of muscle length were found in the pre-exercise compared with the post-exercise condition. On average, PTP had similar absolute effects pre- and post-exercise. However, post-exercise PTP was significantly lower at short lengths but higher at long muscle lengths than pre-exercise PTP.

Pre- and post-exercise 60:200 Hz force ratios

In all four conditions (pre-exercise, pre-exercise-PTP, post-exercise, post-exercise-PTP), 60:200 Hz force ratios were calculated for all muscle lengths studied (Fig. 5A). This ratio was used to quantify LFF. A significant main effect of condition (exercise and/or PTP) (*P*<0.01) was present. Furthermore, a significant main effect of muscle length was found, and an interaction between effects of muscle length and condition (all *P*<0.01). This means that the effects of exercise and PTP depend on which length is considered. Exercise decreased the 60:200 Hz force ratio whereas PTP increased the 60:200 Hz force ratio significantly at most (see below) muscle lengths. The decrease of the ratio as a result of the eccentric exercise indicated the presence of LFF. LFF was particularly evident in the non-potentiated condition; in the potentiated condition, no significant main effect of exercise was found when the pre-exercise values were compared with the post-exercise values, but merely a significant interaction between effects of exercise and muscle length, indicating that the effect of exercise was different for shorter compared with longer muscle lengths.

Additionally, post-exercise ratios were expressed as a percentage of the pre-exercise ratios (for the non-potentiated and for the potentiated condition) (Fig. 5B). This shows the effect of PTP on the 60:200 Hz force ratios pre- and post-exercise at all muscle lengths. ANOVA indicated significant effects of PTP and muscle length as well as a significant interaction effect (all *P*<0.01). Thus, the deviation of 100% of the 60:200 Hz force ratio in the potentiated condition was smaller, indicating significantly less LFF, than in the non-potentiated condition at most muscle lengths. In the non-potentiated condition, LFF was most pronounced at muscle lengths near $L_{0,200Hz}$; the 60:200 Hz force ratio had decreased to 54.6±5.9% of the pre-exercise ratio at $L_{0,200Hz}$−1 mm. In the potentiated condition, LFF was only present at shorter muscle lengths; LFF increased with decreasing muscle lengths (the
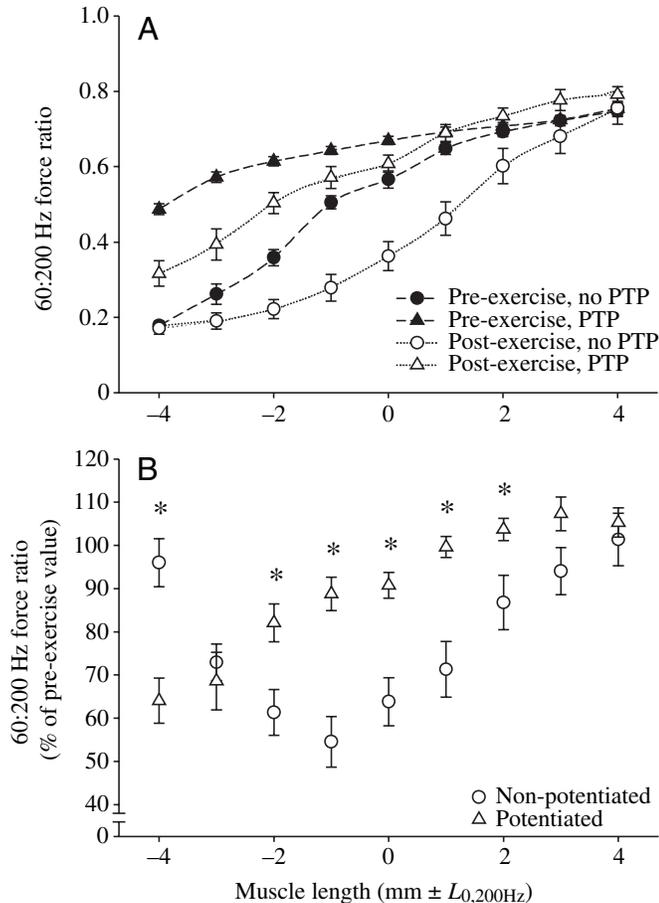
Fig. 5. Length-dependent effects of eccentric exercise and post-tetanic potentiation (PTP) on the 60:200 Hz force ratio. (A) 60:200 Hz force ratios before (pre-exercise, closed symbols) and 80 min after (post-exercise, open symbols) eccentric exercise. The ratios were calculated for the non-potentiated (circles) as well as for the potentiated (triangles) condition (mean ± S.E.M). Exercise decreased the 60:200 Hz force ratio whereas PTP increased the 60:200 Hz force ratio significantly at most muscle lengths. (B) Post-exercise 60:200 Hz force ratios expressed as a percentage of the pre-exercise ratios at different muscle lengths (circles: non-potentiated condition, triangles: potentiated condition). Significantly less LFF was present in the potentiated condition than in the non-potentiated condition at most muscle lengths. *Significant difference between non-potentiated and potentiated condition.

60:200 Hz force ratio had decreased to 64.1±5.2% of the pre-exercise ratio at $L_{0,200Hz}$–4 mm), indicated by the significant length effect (Fig. 5B).

Discussion

The present study aimed to discover the relationship between LFF and PTP at different muscle lengths after eccentric exercise. Therefore, the extent of LFF caused by eccentric exercise for *in situ* rat GM muscle in a potentiated condition was compared with LFF in a non-potentiated condition. The extent of LFF was determined at nine different muscle lengths. Compared with the non-potentiated condition, LFF was less pronounced in the potentiated condition. The data further showed that the extent of LFF after eccentric exercise varied when measured at different muscle lengths. The extent of LFF increased with decreasing muscle lengths in the potentiated condition, whereas in the non-potentiated condition, LFF was maximal at a length just below $L_{0,200Hz}$. Potentiation was length-dependent, both in pre-exercise- and in post-exercise conditions. Eccentric exercise shifted the length for optimal potentiation to a longer muscle length (from $L_{0,200Hz}$–3 mm to $L_{0,200Hz}$–1 mm).

Several aspects may underlie changes in muscle function following eccentric exercise: First, muscle damage (A): for instance, regions of lengthened sarcomeres associated with Z-line streaming are mentioned as a result of eccentric exercise (Armstrong et al., 1983; Friden et al., 1983). It has been suggested that the Z-lines may be disrupted due to eccentric contractions, leading to affected muscle fibres (Friden et al., 1983). Second, an impairment of the excitation–contraction coupling resulting in reduced Ca^{2+} release (B) is associated with eccentric exercise but is also marked as the main factor causing LFF (Edwards et al., 1977; Hill et al., 2001; Westerblad et al., 1993). Third, deformed series-elastic elements, such as deformed myotendinous attachments or an increased compliance of the tendon (C; Jones et al., 1989; Lieber et al., 1991), might play a role in the changed muscle function after eccentric exercise. Damaged myotendinous attachments are associated with muscle damage (A) and will therefore not be discussed separately. An increased compliance of the tendon may lead to changes in the stimulation frequency–force relationship (Jones et al., 1989) and may therefore be a reason for the force losses found in the present study. The results of the present study will be discussed below, taking these three aspects into account.

Reduction of force at maximal stimulation in relation to muscle length

The series of eccentric contractions in the present study resulted in a decrease in force at maximal as well as at submaximal stimulation with significant effects of muscle length. Force at maximal stimulation was affected less near $L_{0,200Hz}$ than at shorter and longer muscle lengths (Fig. 2). It is possible that the force loss at maximal stimulation is caused mainly by mechanical damage (A) possibly in combination with a more compliant tendon (C) and not by reduced tetanic Ca^{2+} concentrations (B), since reduced Ca^{2+} levels are known to result in large changes in force at low stimulation frequencies but only in small changes in force at maximal stimulation (Westerblad et al., 1993).

The length-dependence of the force loss may be related to a changed serial distribution of sarcomere lengths caused by muscle fibre damage (A). It has been suggested that eccentric exercise may result in regions of non-functional lengthened sarcomeres (Armstrong et al., 1983; Friden et al., 1983), which may have two consequences. First, upon activation of the muscle–tendon complex, the functional sarcomeres need

to shorten relatively more to stretch the series-elastic components. As a result, the optimum length will shift to a longer length. After the eccentric contractions in our experiment, we observed a shift of $L_{0,200Hz}$ to a 1 mm longer length, for which we have corrected. Second, the functional fibre length will be shorter, which may lead to relatively more force loss at the longest and shortest muscle lengths studied, as we observed (Fig. 2B).

Reduction of submaximal force in relation to muscle length

Force loss at maximal stimulation was minimal near $L_{0,200Hz}$. By contrast, force loss at submaximal stimulation in the non-potentiated condition was maximal near $L_{0,200Hz}$. Since the characteristic of LFF is a greater force loss at lower frequencies than at higher frequencies, and the cause is found in a disturbance of the E–C coupling (Edwards et al., 1977), it could be expected that this decrease in submaximal force is the result of a disturbance in E–C coupling (B) leading to a decreased tetanic Ca^{2+} concentration, possibly in combination with muscle damage (A) and a more compliant tendon (C). In the potentiated condition, the loss of submaximal force was much less, and the length effects were similar as for force production at maximal stimulation: minimal force loss was found near $L_{0,200Hz}$. Since PTP is thought to have a greater effect in situations of lower Ca^{2+} concentrations, the effects of a disturbance of E–C coupling by low Ca^{2+} concentrations (B) should be smaller following PTP. Mechanical damage (A) possibly in combination with a more compliant tendon (C) might be the main factors causing the submaximal force loss in the potentiated condition.

Low-frequency fatigue in relation to muscle length

The manifestation of LFF at different muscle lengths was deduced from the force–length relationships at maximal and submaximal stimulation. A decrease of the 60:200 Hz ratio as a result of the eccentric exercise indicated the presence of LFF. A decrease of this ratio was present mainly in the non-potentiated condition; significantly less LFF occurred in the potentiated condition. In both the non-potentiated and the potentiated condition, no LFF was present at longer muscle lengths and LFF increased with decreasing muscle length. This may be related to a reduced Ca^{2+} release (B) in combination with length-dependent Ca^{2+} sensitivity. A lower Ca^{2+} release can result in a greater loss of submaximal force at shorter muscle lengths because the Ca^{2+} sensitivity of muscle is lower at those lengths (e.g. Stienen et al., 1985). However, in non-potentiated muscle, LFF was less at the shortest muscle lengths studied (Fig. 5B). This is the result of the different shape of the force–length relationship (levelling off of force at shorter lengths) at 60 Hz in the non-potentiated muscle post-exercise (Fig. 3A) compared with the pre-exercise condition. The levelling off of force at shorter lengths in a force–length relationship is probably caused by a distribution of sarcomere lengths, which is always present in a muscle (Huijing, 1996). Therefore, force–length relationships in general show a levelling off when approaching low force levels at short lengths. In rat GM muscle, the different distribution of sarcomere lengths may be intensified by a distribution of optimum lengths of the proximal and distal fibres. The proximal muscle part of the rat GM consists predominantly of fast oxidative fibres, the distal part of fast glycolytic fibres (De Ruiter et al., 1995). In addition, it is known that optimum length of the fast glycolytic part is about 1.5 mm higher than of the fast oxidative part (De Ruiter et al., 1995). Therefore, at short muscle lengths the fast oxidative fibres are at higher relative length than the fast glycolytic fibres and will contribute more to the total force. Additionally, fast oxidative fibres are less susceptible to LFF (Rijkelijkhuizen et al., 2003) and will therefore be able to exert relatively more force than fast glycolytic fibres in the presence of LFF.

In the potentiated condition, a more linear relationship between the extent of LFF and muscle length was found, with increasing LFF occurring with decreasing muscle lengths. PTP reduced LFF significantly and the effects on maximal and potentiated submaximal force showed the same length dependence. It should be noted that the absence of LFF at certain muscle lengths does not imply that there were no effects of the eccentric exercise but merely that the force loss at maximal and submaximal stimulation was similar at those muscle lengths, resulting in an unchanged 60:200 Hz force ratio.

Counteracting effects of LFF and PTP

Potentiation led to a reduction in LFF over a wide range of muscle lengths (Fig. 5). Potentiation of force by previous activation is caused by increased levels of phosphorylation of MLCs (e.g. Manning and Stull, 1979; Moore and Stull, 1984). It has been proposed that MLC phosphorylation causes individual myosin heads to swing out from the myosin backbone (Sweeney et al., 1993), thereby bringing the actin binding site of the myosin head in close proximity to the actin filament. This is thought to permit a faster rate of engagement of cross-bridges on activation. A faster rate of engagement of cross-bridges, with no change in rate of dissociation will result in more cross-bridges in the force-generating state during contraction at a given level of activation (Ca^{2+} bound to troponin). Thus, potentiation could be explained by an increased fraction of cross-bridges in the force-generating state (Sweeney and Stull, 1990) or a prolonged force-generating state of the cross-bridges (Patel et al., 1998). Potentiation probably decreases LFF after eccentric exercise by increasing Ca^{2+} sensitivity, which would counteract the effects of the reduced Ca^{2+} release (B). Therefore, when muscles are potentiated, it may seem as if no LFF is present. However, the reduced Ca^{2+} release may still be present in the muscle but the effect (LFF) may be counteracted by an increased Ca^{2+} sensitivity induced by potentiation.

The increased Ca^{2+} sensitivity caused by PTP may be length dependent. For the descending limb of the force–length relation, the present study found a curve with a negative slope relating PTP and muscle length (Fig. 4). In the pre-exercise condition, the negative slope was present at muscle lengths of

$L_{0,200Hz}$−3 mm and longer. However, on the ascending limb of the force–length relationship in the post-exercise condition, the relationship between PTP and muscle length showed a positive slope, indicating less PTP at shorter muscle lengths. It has been suggested that the mechanism for the length dependence of potentiation may be related to length dependence of activation (Rassier, 2000). At long muscle lengths, the affinity among the actin binding site and the myosin head is higher because the interfilament spacing is small. This results in a higher rate of attachment while the rate of detachment is not affected. Thus, stretching the muscle as well as MLC phosphorylation during repetitive stimulation causes an increase in Ca^{2+} sensitivity. Therefore, muscles that are active at a longer length are already 'potentiated' due to a stretch-induced increase in Ca^{2+} sensitivity, and the effects of MLC phosphorylation are likely to be smaller. However, in the present study, at the shortest muscle lengths studied in the post-exercise condition, potentiation was less than at the muscle lengths just below $L_{0,200Hz}$. This deviation may be related to the earlier mentioned levelling off of the force–length curve at 60 Hz in the post-exercise condition.

Thus, the increasing Ca^{2+} sensitivity caused by PTP seems to be length-dependent with a small effect at long length where interfilament spacing is small, an optimal effect at lengths near optimum length, and a decreasing effect at shorter lengths where interfilament spacing is high. The present study showed that the extent of LFF increased with decreasing muscle length when muscles were potentiated, indicating that the length effect of the submaximal force loss as a consequence of the reduced Ca^{2+} release may be strong(er) than the increase of submaximal force as a result of the increase in Ca^{2+} sensitivity induced by a potentiating tetanus.

The functional relevance of LFF for in vivo functioning muscles

When LFF is present, force is considerably decreased at submaximal stimulation frequencies and recovery of this phenomenon can take up to 24 h (Edwards et al., 1977). During *in vivo* action, motoneurones usually fire at relatively low (for rat muscle: <60 Hz) frequencies (Hennig and Lømo, 1987). Therefore, LFF may have large effects on *in vivo* performance and important consequences for muscle control. The central nervous system has to increase muscle activation to prevent a loss of force output. The present study showed that PTP counteracts LFF mainly at long muscle lengths, probably by increasing the sensitivity for Ca^{2+}, and therefore compensating for the effect of the reduced Ca^{2+} release, which is causing LFF. Since muscles are potentiated quickly during repeated activity, the functional significance of LFF in a potentiated muscle may be most relevant at shorter muscle lengths during *in vivo* activities. In conclusion, this study showed that the manifestation of LFF as a result of a series of eccentric contractions varied when measured at different muscle lengths. PTP fully counteracted the effects of eccentric exercise at long muscle lengths but LFF was still observed at the shortest muscle lengths studied.

References

Abbate, F., Sargeant, A. J., Verdijk, P. W. and de Haan, A. (2000). Effects of high-frequency initial pulses and posttetanic potentiation on power output of skeletal muscle. *J. Appl. Physiol.* **88**, 35-40.

Armstrong, R. B., Ogilvie, R. W. and Schwane, J. A. (1983). Eccentric exercise-induced injury to rat skeletal muscle. *J. Appl. Physiol.* **54**, 80-93.

De Haan, A., Jones, D. A. and Sargeant, A. J. (1989a). Changes in velocity of shortening, power output and relaxation rate during fatigue of rat medial gastrocnemius muscle. *Pflügers Arch.* **413**, 422-428.

De Haan, A., Lodder, M. A. N. and Sargeant, A. J. (1989b). Age-related effects of fatigue and recovery from fatigue in rat medial gastrocnemius muscle. *Q. J. Exp. Physiol.* **74**, 715-726.

De Haan, A., Huijing, P. A. and Van Der Vliet, M. R. (2003). Rat medial gastrocnemius muscles produce maximal power at a length lower than the isometric optimum length. *Pflügers Arch.* **445**, 728-733.

De Ruiter, C. J., De Haan, A. and Sargeant, A. J. (1995). Physiological characteristics of two extreme muscle compartments in gastrocnemius medialis of the anaesthetized rat. *Acta Physiol. Scand.* **153**, 313-324.

Edwards, R. H. T. (1983). Biochemical bases of fatigue in exercise performance: catastrophe theory of muscular fatigue. In *Biochemistry of Exercise. Proceedings of the Fifth International Symposium on the Biochemistry of Exercise, June 1-5, 1982, Boston, Massachusetts*, vol. 13 (ed. H. G. Knuttgen, J. A. Vogel and J. Poortmans), pp. 3-28. Champaign, IL: Human Kinetics Publishers.

Edwards, R. H., Hill, D. K. and Jones, D. A. (1975). Metabolic changes associated with the slowing of relaxation in fatigued mouse muscle. *J. Physiol.* **251**, 287-301.

Edwards, R. H. T., Hill, D. K., Jones, D. A. and Merton, P. A. (1977). Fatigue of long duration in human skeletal muscle after exercise. *J. Physiol.* **272**, 769-778.

Edwards, R. H. T., Mills, K. R. and Newham, D. J. (1981). Greater low frequency fatigue produced by eccentric than concentric muscle contractions. *J. Physiol.* **317**, 17P.

Friden, J., Sjostrom, M. and Ekblom, B. (1983). Myofibrillar damage following intense eccentric exercise in man. *Int. J. Sports Med.* **4**, 170-176.

Hennig, R. and Lømo, T. (1987). Gradation of force output in normal fast and slow muscles of the rat. *Acta Physiol. Scand.* **130**, 133-142.

Hill, C. A., Thompson, M. W., Ruell, P. A., Thom, J. M. and White, M. J. (2001). Sarcoplasmic reticulum function and muscle contractile character following fatiguing exercise in humans. *J. Physiol.* **531**, 871-878.

Huijing, P. A. (1996). Important experimental factors for skeletal muscle modelling: non-linear changes of muscle length force characteristics as a function of degree of activity. *Eur. J. Morphol.* **34**, 47-54.

Huijing, P. A. (1999). Muscular force transmission: a unified, dual or multiple system? A review and some explorative experimental results. *Arch. Physiol. Biochem.* **107**, 292-311.

Jones, D. A. (1996). High-and low-frequency fatigue revisited. *Acta Physiol. Scand.* **156**, 265-270.

Jones, D. A., Newham, D. J. and Torgan, C. (1989). Mechanical influences on long-lasting human muscle fatigue and delayed-onset pain. *J. Physiol.* **412**, 415-427.

Krarup, C. (1981). Enhancement and diminution of mechanical tension evoked by staircase and by tetanus in rat muscle. *J. Physiol.* **311**, 355-372.

Lieber, R. L., Woodburn, T. M. and Friden, J. (1991). Muscle damage induced by eccentric contractions of 25% strain. *J. Appl. Physiol.* **70**, 2498-2507.

MacIntosh, B. R. and Willis, J. C. (2000). Force-frequency relationship and potentiation in mammalian skeletal muscle. *J. Appl. Physiol.* **88**, 2088-2096.

Manning, D. R. and Stull, J. T. (1979). Myosin light chain phosphorylation and phosphorylase A activity in rat extensor digitorum longus muscle. *Biochem. Biophys. Res. Commun.* **90**, 164-170.

Moore, R. L. and Stull, J. T. (1984). Myosin light chain phosphorylation in fast and slow skeletal muscles in situ. *Am. J. Physiol.* **247**, C462-C471.

Patel, J. R., Diffee, G. M., Huang, X. P. and Moss, R. L. (1998). Phosphorylation of myosin regulatory light chain eliminates force-dependent changes in relaxation rates in skeletal muscle. *Biophys. J.* **74**, 360-368.

Rack, P. M. H. and Westbury, D. R. (1969). The effects of length and stimulus rate on tension in the isometric cat soleus muscle. *J. Physiol.* **204**, 443-460.

Rassier, D. E. (2000). The effects of length on fatigue and twitch potentiation in human skeletal muscle. *Clin. Physiol.* **20**, 474-482.

Rassier, D. E. and MacIntosh, B. R. (2002). Sarcomere length-dependence

of activity-dependent twitch potentiation in mouse skeletal muscle. *BMC Physiol.* **2**, 19.

Rijkelijkhuizen, J. M., De Ruiter, C. J., Huijing, P. A. and De Haan, A. (2003). Low-frequency fatigue is fibre type related and most pronounced after eccentric activity in rat medial gastrocnemius muscle. *Pflügers Arch.* **447**, 239-246.

Roszek, B., Baan, G. C. and Huijing, P. A. (1994). Decreasing stimulation frequency-dependent length-force characteristics of rat muscle. *J. Appl. Physiol.* **77**, 2115-2124.

Stienen, G. J., Blange, T. and Treijtel, B. W. (1985). Tension development and calcium sensitivity in skinned muscle fibres of the frog. *Pflügers Arch.* **405**, 19-23.

Sweeney, H. L. and Stull, J. T. (1990). Alteration of cross-bridge kinetics by myosin light chain phosphorylation in rabbit skeletal muscle: implications for regulation of actin-myosin interaction. *Proc. Natl. Acad. Sci. USA* **87**, 414-418.

Sweeney, H. L., Bowman, B. F. and Stull, J. T. (1993). Myosin light chain phosphorylation in vertebrate striated muscle: regulation and function. *Am. J. Physiol.* **264**, C1085-C1095.

Thompson, L. V., Balog, E. M., Riley, D. A. and Fitts, R. H. (1992). Muscle fatigue in frog semitendinosus: alterations in contractile function. *Am. J. Physiol.* **262**, C1500-C1506.

Tubman, L. A., MacIntosh, B. R. and Maki, W. A. (1996). Myosin light chain phosphorylation and posttetanic potentiation in fatigued skeletal muscle. *Pflügers Arch.* **431**, 882-887.

Wallinga-de Jonge, W., Boom, H. B., Boon, K. L., Griep, P. A. and Lammeree, G. C. (1980). Force development of fast and slow skeletal muscle at different muscle lengths. *Am. J. Physiol.* **239**, C98-C104.

Westerblad, H., Duty, S. and Allen, D. G. (1993). Intracellular calcium concentration during low-frequency fatigue in isolated single fibers of mouse skeletal muscle. *J. Appl. Physiol.* **75**, 382-388.