NEURAL AND MECHANICAL RESPONSES OF THE TRICEPS SURAE MUSCLE GROUP AFTER ONE HOUR REPEATED FAST PASSIVE STRETCHES

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Summary

Experiments were carried out to examine interaction between mechanical changes of the muscle-tendon unit and reduced reflex sensitivity after repeated and prolonged passive muscle stretching (RPS). There is some evidence that this interaction might be relevant also during active stretch-shortening cycle type of fatigue tasks. The results demonstrated a clear deterioration of voluntarily and electrically induced muscle contractions after RPS. Maximal voluntary contraction (MVC), average electromyographic activities of the gastrocnemius and soleus muscles and maximal twitch contraction decreased on average by 13.8, 10.4, 7.6, and 16.8 %, respectively. In addition, there was a 14 % lengthening in the total duration of the twitch. MVCs measured at different ankle joint angles revealed a downward and rightward shift in the torque-fascicle length curve after RPS. Interestingly, there was a crossing in the torque-fascicle length curves while measured at different activation levels but at the same joint angle before and after RPS. Even though no changes were observed in the activation level during MVCs, all the reflex parameters showed a clear reduction after RPS. This study presents evidence that repeated and prolonged passive muscle stretching can lead to some modification of material behaviour of the aponeurosis-tendon system such as stress relaxation and/or plastic deformation. In addition, altered material properties seem to affect proprioceptive feedback and, therefore, the motor unit activation in proportion to the contractile failure.
Introduction

Several experiments have demonstrated with a variety of protocols that passive dynamic or static stretching of a human skeletal muscle can impair its force generating capacity (10, 37, 1). The cause of this contractile impairment has often been contributed to the increased compliance of the muscle tendon unit induced by the stretch. This could then result in impaired force transfer from the muscle fibres via the tendon to the bone (26).

According to Taylor et al. (38) the mechanical changes of the muscle-tendon unit are time and history dependent because of the viscoelastic nature of the tissue. Two different responses in this regard have been suggested. The first one is stress relaxation (37, 27), which is purely mechanical in nature and is mainly responsible for the reduced passive tension of the muscle-tendon unit over time. The second one is called plastic deformation, which means reorientation of the supporting connective and soft tissue-supporting tissue into more parallel arrays (34). This response is suggested to be more time dependent of the maintained tissue strain.

The increased compliance of the muscle-tendon unit may also influence neural activation patterns because of neural feedback responses (10). Avela et al. (1) demonstrated a clear reduction (84.8 %) in the stretch-reflex peak-to-peak amplitude after one hour repeated fast passive muscle stretching. This reduction was related to a significant reduction in the passive stretch resisting force of the muscle. Therefore, Avela et al. (1) suggested that the origin of the reduced reflex sensitivity could be a reduction in the activity of the large diameter afferents, resulting from the reduced mechanical sensitivity of the muscle spindles to repeated stretch.

The purpose of the following study was to seek more direct evidence for interaction between mechanical changes of the muscle-tendon unit and reduced reflex sensitivity after repeated and prolonged passive muscle stretching. Since skeletal muscle consists of three components (muscle fibres, aponeurosis and free tendon) and each of them exhibits different material
behaviour, ultrasonography was used in order to differentiate the length changes of each component. It is also well known that the fast passive stretch of the muscle induces a stretch-reflex through facilitation of the α motoneuron pool. H reflexes were, therefore, recorded after several stretching ramps at the beginning and at the end of the stretching protocol to evaluate the possible modulation (state of excitability) of the motoneuronal pool induced by the stretching protocol. Consequently, the possible interaction between changes in reflex sensitivity and mechanical responses of the muscle could be taken as an evidence of the mechanical origin for the reduced reflex sensitivity. This information could be relevant also during active fatigue tasks, since simultaneous reduction in the passive stretch resisting force and reflex sensitivity have been observed after different types of active stretch-shortening cycle exercises (3, 2).
Methods

Subjects
Eight healthy male subjects, aged 20-39 years (mean 25 years) participated in the study. None of the subjects had any history of neuromuscular or vascular disease. They were fully informed of the procedures and the risks involved in this study and they gave their informed consent (code of Ethics of the World Medical Association, Declaration of Helsinki). They were also allowed to withdraw from the measurements at will. The University Research Ethics Committee approved the study.

Experimental protocol
All eight subjects underwent a repeated passive stretching (RPS) of the calf muscles twice and each of them lasted for one hour. A minimum of two weeks was allowed to elapse between the two RPS exercises to ensure full recovery from the first exercise. In both stretching sessions the subjects were instructed not to resist the mechanical stretching of the calf muscles. Both RPS exercises and all the measurements included in this study were performed on the ankle ergometer. The whole experimental protocol is summarised in Figure 1.

In the first RPS exercise (RPS1), pre- and post-test measurements included several measurements in the following order: 1) maximal voluntary isometric plantar flexion torque (MVC) at 90° ankle joint angle, 2) muscle contractions of 10% and 50% of the MVC at 90° ankle joint angle, 3) mechanical response of the relaxed plantar flexor muscles to double supramaximal electrical impulse (passive double twitch) and 4) maximal voluntary isometric plantar flexion torque (MVC) with superimposed double electrical impulse (28). The two latter measurements were done at ankle joint angles of 80°, 90° and 115° in order to construct a torque/fascicle length relationship. Ultrasonography was used during the isometric tests and at the beginning and at the end of the stretching protocol in order to calculate the fascicle length changes. No EMG measurements were done in the first RPS exercise.
In the second RPS exercise (RPS2) performed at least two weeks later, all the isometric measurements were done at 90° ankle joint ankle. Passive double twitch and MVC with superimposed double twitch were again measured in order to test the repeatability of the two RPS exercises. The other pre- and post-tests in the RPS2 included the measurements of the maximal M-wave and H-reflex responses. In these measurements at least 30 s were allowed to elapse between MVCs and H reflex recordings to avoid the problem of post-contraction depression of the H-reflex (39). In addition, H-reflexes and passive stretch-reflexes were measured during the RPS2 exercise for 10 stretching ramps in the beginning and at the end of the exercise.

Stretch model
Both one-hour RPS exercises were induced by repeated dynamic stretching of the calf muscles, performed by an ankle ergometer similar to that of Gollhofer and Schmidtbleicher (16) and used in our earlier studies on stretch-reflex sensitivity during stretch-shortening cycle fatigue (2, 30,32). In all the experimental conditions the subjects sat in the ergometer chair. The foot was mounted on the rotation platform so that the rotation axes of the ankle joint and the motor driven platform coincided. The torque around the rotational axis of the motor was measured by a piezoelectric crystal transducer (Kistler) and the angular movement of the ankle joint with respect to plane of the ergometer was monitored by a linear potentiometer. The initial ankle position was 90 degrees and the knee angle was fixed at 120 degrees. The stretching amplitude corresponded to 10-degree dorsiflexion of the ankle joint with an average velocity of 200 deg·s⁻¹ (figure 2). The waveform of the stretching signal was trapezoidal and the frequency of these stretches was 1.5 cycles per second.

Stretch- and H-reflex measurements
The stretch reflexes were measured with the same mechanical stimulation as used in RPS. Ten consecutive stretch reflexes were averaged and peak-to-peak amplitudes of the EMG responses were analysed together with latency times, torques and displacements of the ankle
joint (for details, see 30). Torque analyses were divided into two different time periods. An
average plantar flexion torque was analysed for the first 40 ms of the stretch, before the
torque increases due to the stretch-reflex could take place. This torque was defined as a
passive stretch resisting torque of the muscle (PsrT) (1). In addition, an average torque was
also measured for the time period of 50 to 80 ms after the onset of stretch. This torque
corresponds to the torque output of the short latency stretch reflex response and was
therefore defined as a reflex torque (RT) (for other details see 30, 32).

To evaluate the possible modulation of the α motoneuron pool induced by the RPS exercise,
Hoffmann (H) reflexes were recorded during the pre- and post-tests and also during different
phases of the second RPS exercise. After preparing the skin, stimulation electrodes
(pregelified Ag/AgCl electrodes, Niko, Denmark) were positioned for the H-reflex and M-
wave (muscle compound action potential) testing. The leg cathode (1.5 x 1.5 cm) was placed
over the tibial nerve in the popliteal fossa and the anode (5 x 8 cm) was placed superior to
the patella. Since standing position facilitates the H-reflex, the position of the stimulation
electrodes were tested first in the upright stance, and then checked in the experimental
position to ensure constant recording conditions. The electrical stimulus used for the H-
reflex and M-wave recording was rectangular and single mode signal with pulse duration of
1 ms (frequency of 0.2 Hz) and it was delivered from a signal generator of the evoked
potential measuring system (MEB-5304K, Nihon Kohden, Japan). In addition, all the
responses were amplified (bandwidth 10 Hz to 1 KHz), stored and analysed by the same
system. The intensity of the stimulus was set in every testing unit to elicit maximal H-
response (Hmax) and M-response (Mmax). The reflex excitability was calculated according
to the method used by Garland and McComas (14). Maximal H-reflex peak-to-peak
amplitudes were expressed in relation to the maximal M-wave peak-to-peak amplitudes.
Theoretically the H/M ratios, so determined, should not have been affected by any changes
in the peripheral excitability of the muscle fibres.

H-reflexes were also recorded during ten stretching ramps (figure 2) selected for the
beginning and for the end of the RPS2 exercise. It is essential that stimulus strength remains
invariant between recording sessions, because the size of the H-reflex depends heavily on the stimulus intensity. Therefore, when repeated H-reflex measurements are performed stimulation intensity should be related to the size of the maximal M-wave (usually 10-30 %) to ensure that the same number of motor axons are recruited in each trial (e.g. 35). In the present experiment, the target amplitude for the M wave was 25 % of the maximal M wave (8), which was measured in an earlier ramp. An average of three highest H-responses was used as a final result. Low variability in H-reflex peak-to-peak amplitude between the measurements performed at the beginning and at the end of the RPS2 exercise (8.2 % and 7.7 %, respectively) indicated comparable recording conditions.

While measuring stretch- and H-reflexes at the beginning of the RPS2 exercise, first ten stretching ramps were disregarded in order to avoid the effect of muscle thixotropy. In addition, background EMG activity was monitored throughout all the reflex measurements on an oscilloscope to ensure that it was silent. This precaution was important in the present experiment since, muscle activation is known to affect the excitability of the H-reflex (39).

**Torque measurements and activation level calculations**

In both RPS exercises and in each condition three maximal isometric plantarflexions (MVC) were performed before the RPS exercise. This was repeated only once immediately after the exercise to avoid premature recovery during the post-tests. All the MVC measurements were done with the twitch interpolation method (28). For passive double twitch one trial was performed in each condition.

Passive and superimposed double twitches were both induced by supramaximal nerve stimuli with a frequency of 100 Hz and the duration of each pulse was 1 ms. In both cases, stimulation electrode was applied identically to the one used in H-reflex testing. In the superimposed stimulation, the stimulus intensity was set approximately 25 % higher than that of the maximal M-wave to ensure maximal response in every testing condition. In addition, in twitch interpolation special care was taken to ensure that the double twitch was applied during MVC.
The following parameters were analysed from the passive double twitch recordings: maximum twitch torque (Fdt), time to peak torque (TPdt), total duration of the twitch (TDTdt) and half relaxation time (HRTdt).

The central activation level during MVC was calculated according to the following formula (36):

$$AL = \frac{MVCT}{Tst} / Tdt \cdot 100$$

In this formula: MVCT = maximal voluntary contraction torque, Tst = maximal torque during superimposed double pulse and Tdt = maximal passive twitch torque.

All the MVC values were analysed trial-by-trial. In addition, in RPS2 exercise the integrated EMG was divided by the integration time (500 ms) to obtain an average EMG (aEMG).

**EMG instrumentation**

The recording electrodes for the H-reflexes, M-waves, stretch reflexes and the EMGs associated with MVC were bipolar surface electrodes (Beckman miniature skin electrodes 650437, Illinois, USA) fixed at a constant center-to-center interelectrode distance of 20 mm. The electrodes were placed approximately 6 cm above the superior aspect of the calcaneus on the soleus (SOL) muscle and between the center of the innervation zone and distal end of the lateral head of the gastrocnemius (GA) muscle. EMG signals were transferred telemetrically, amplified by a FM-microvolt amplifier (Glonner Electronic GmbH, Munich, Germany) and finally transferred through an AD converter (AD sampling frequency was 1 – 3 kHz, depending on the signal), to a microcomputer or in a case of H-reflex testing to Nihon Kohden measuring system.
Fascicle and tendon length measurements with ultrasonography

Length of the medial gastrocnemius (MG) fascicle was calculated on the basis of ultrasonographic measurements before, during and after the first RPS exercise. With the subjects seated on the ankle ergometer, an ultrasound probe (40 mm, 7.5 MHz, B-mode, Aloka SSD-2000 with scanning frequency of 42 Hz) was firmly attached to the muscle belly of the MG muscle. The real-time images were captured on videotape at 50 Hz. Fascicle interfaces appear as light stripes in the ultrasound image. One of these stripes was chosen for analysis and its length was tracked throughout the movement. A parallelogram model (figure 3a) was used when images were digitized and analysed with Motus software (Peak Performance Technologies, USA). For three subjects the entire fascicle was not fully visible within the image area during passive muscle conditions. In those cases the total fascicle length ($l_{fasc}$) was calculated according to Finni et al (9). When the aponeuroses were not in parallel, the angle between them was subtracted from $\alpha$ to make the calculation possible (figure 3a). Length changes in the tendinous tissue ($l_{\text{length}} =$ tendon and aponeurosis) were calculated according to Fukunaga et al. (12) (figure 3b).

Model of Hawkins and Hull (18) was used to estimate the length changes in the muscle-tendon unit ($MTU_{\text{length}}$). This model requires information about joint angles. For the ankle joint angle this was obtained by using a potentiometer of the ankle ergometer. The knee angle was kept constant (120°). Information of the MTU length changes was needed for the tendon length calculations.

To compensate for the low scanning frequency of the ultrasonography, ten consecutive stretch cycles were averaged. For this purpose manually triggered electrical signal was used to synchronize the video and analogue data. In MVC, the fascicle length changes for the zero torque and maximal torque were analysed as an average of 50 data points.

Ultrasonography has been widely used in studying muscle function and tendinous tissue behaviour in isometric and isokinetic movements (e.g. 11, 19). Many researchers have reported good reliability and repeatability of the method (e.g. 24, 29). In addition, the error
due to the linear extrapolation of fascicle length has been investigated in our laboratory. Finni et al. (9) and Ishikawa et al. (23) respectively reported it to be 2-7% and 4.5-5.9% for vastus lateralis muscle. For the medial gastrocnemius muscle, however, the error is considerably smaller than in vastus lateralis muscle because greater proportion of the total fascicle length could be visualized. Due to these reasons the reliability of the fascicle length calculations was not tested in this experiment nor discussed in this paper.

**Statistical analysis**

Mean and standard deviation values were calculated for the various parameters in all the different tests. Pearson’s correlation coefficients were calculated in order to reveal significant relationships between selected parameters. As the whole data showed normality in every case, Student’s t-test for paired samples was used to determine differences between two parameters. The statistical significances for MVC and AL between different RPSs were determined according to multivariate analysis of variance (MANOVA). When a significant F-ratio occurred for the main effects, profile analysis was carried out by MANOVA to locate the source of the difference. Level of significance in all tests was set to p<0.05, p<0.01 and p<0.001.
RESULTS

Isometric measurements

One hour of repeated passive stretching of the triceps surae muscle resulted in reduction of maximal voluntary plantarflexion torque by 13.8 ±14.1% (p<0.05) after the first session (RPS1) and by 13.2 ±12.7% (p<0.05) after the second session (RPS2) (figure 4). Maximal EMGs of the Ga and SOL muscles, measured only during RPS2 showed also a reduction in the mean values, but the statistical analysis revealed that the decreases of 10.4 ±12.9 % and 7.6 ±7.0 %, respectively, were not significant. Similarly, the EMG/torque ratio decreased slightly by 4.7 ±13.2% (n.s.) and 4.2 ±16.1% (n.s.) for the SOL and Ga muscles, respectively.

The cause of the impairment in MVC could have been due to reduction in activation levels (AL), but no exercise session induced reduction was observed in this parameter. AL values at 90° ankle joint angle were the same before and after one hour RPS1: 97.9 ±2.0 % and 97.7 ±2.5 %, respectively. Similar results were obtained for the other two MVC measurement angles (range of change was from 97.2 to 98.5 %).

Because MVC was measured at three different ankle angles of 80°, 90° and 115°, this gave the possibility to use the simultaneously measured MG fascicle lengths to construct the torque/fascicle length relationship. These relationships have been drawn in figure 5, which shows that the overall shift was downward and to the right during RPS1 session. The fascicle length increased during one hour stretching more at shorter fascicle lengths (90° and 115° ankle joint angles): from 2.54 ± 0.62 cm to 2.85 ± 0.90 cm (p<0.05) and from 2.34 ± 0.84 cm to 2.49 ± 0.97 cm (p<0.05), respectively.

In RPS1, the fascicle lengths were also calculated for four different activation levels at 90° ankle joint angle (figure 6). During zero activation, the fascicles became shorter by 0.30 ± 0.13 cm (p<0.05) after RPS. At maximal activation, the length changes were to the opposite direction by 0.24 ± 0.09 cm (p<0.05). Figure 6 shows also the respective changes for the...
tendon length calculations. Since the ankle joint displacements were constant and the model of Fukunaga et al. (12) merely subtracts the fascicle length changes from that of the tendomuscular unit, the tendon length changes were the same but to the opposite direction from those of the fascicle length changes.

Double supramaximal electrical stimulus applied to the passive muscle before and after RPS at 90° ankle joint angle revealed impaired responses. This was shown by a 16.8 ± 16.8% (p<0.01) reduction in the maximum twitch torque and by a 14.0 ± 8.7% increase in the total duration of the twitch. In addition, time to peak torque and half relaxation time were lengthened by 15.1 ± 7.7% (p<0.001) and 19.7 ± 11.9 (p<0.01), respectively.

The maximal H-reflex amplitude decreased by 29.1 ±22.1% (p<0.01) immediately after RPS2. This reduction was not associated with any changes in the maximal M-wave, indicating that there was no failure in neuromuscular transmission. Therefore, the changes in the maximal H-reflex resulted in a reduced maximal H/M ratio (mean decline 29.2 ±20.4 %, p<0.01), suggesting impaired excitation of the α-motoneuron pool.

**Measurements during passive stretching**

EMG responses of the stretch reflexes showed a dramatic peak-to-peak reduction at a very end of RPS (figure 7). These reductions were 41.0 ± 37.3% (p<0.05) and 27.5 ± 25.2% (p<0.05) for the GA and SOL muscles, respectively. The passive stretch resisting torque (PsT) showed very similar changes during the RPS stimulation. The immediate after-RPS reduction was 11.2 ± 8.2% (p<0.05). In addition, the changes in the PsT were significantly related to changes in MVC, to fascicle length changes during MVC and to the stretch reflex amplitude changes (figure 8). There was also a very clear drop in the reflex induced torque (RT) immediately after RPS (31.7 ± 17.9%, p<0.05). Thus, a moderate relationship existed between RT and PsT (R=0.63, n.s.).

Figure 9a shows average recordings of the fascicle length changes for the whole subject group. This figure represents one passive stretch cycle (average of 10 cycles) at the
beginning and at the end of RPS1. In addition, figure 9b shows clearly that the fascicles became shorter throughout the passive stretch cycle (p<0.05) at the end of RPS1. This result is very similar to the one in isometric condition with relaxed muscle at 90° ankle joint angle (figure 5). During the stretch cycle the measured H-reflex was reduced from 3.6 ± 0.3mV (beginning of the stretching session) to 1.9 ± 0.1 mV (p<0.01) (end of session). Figure 7 presents the H-reflex signals of one representative subject measured at the beginning and at the end of the first RPS exercise.
DISCUSSION

This study supports the earlier finding (1) that repeated and prolonged passive stretching of a human skeletal muscle can cause considerable impairment of its torque output. The mean reduction was 13.8 and 13.2 % in RPS1 and RPS2 exercises, respectively. This was clearly less than the 23.2 % reduction reported earlier by Avela et al. (1) with a similar protocol. It should be noted that in that experiment the post-RPS measurements were performed under ischemia and even though the effect of ischemia was tested it could have led to a systematic force deficit. In addition, the activation level during MVC was not tested in that study. Lieber et al. (26) found a very similar (13 %) reduction in maximal tension of the rabbit tibialis anterior muscle induced by electrical stimulation (100 Hz) of the isolated peroneal nerve after 30 minutes of cyclic passive stretching. In their experiment, alterations in muscle length were avoided by readjusting the muscle always to the length at which twitch tension was maximal. Therefore, they proposed that the origin of the force deficit could be damage related impairment in force transfer from the muscle fibres to the tendon. Damage could have taken place in the portions of the myotendinous junction, a location that has been shown to be susceptible to acute injury because of stress concentration at the ends of the tapered muscle fibres (15). However, Lieber et al. (26) did not observe any abnormalities within the muscle fibres. On the other hand, they also suggested the possibility of mechanical breakage of stable cross-bridges, which are responsible for passive tension of the muscle. In the present experiment the changes in the twitch properties support these findings at least partly. However, since the muscle length was not readjusted in the present study, reduced twitch amplitude could also be explained by a greater time to take up the slack in compliant in-series elements (7) induced by the RPS exercise. This greater slack is likely to explain the reduction in the twitch torque; however, it cannot explain the reduced MVC since all the slack should be taken up during maximal contraction.

Additional explanation could be sought from possible alterations in the force-length relationship of the muscle-tendon unit induced by modifying contribution of the length changes of its different components. In a passive muscle-tendon system this elongation can
be facilitated by stress relaxation and/or plastic deformation. Stress relaxation is viscoelastic in nature and according to Taylor et al. (38) it occurs primarily in the connective tissue. Stress relaxation is indicated by a descending passive torque over time for a given stretched muscle length (37). Contribution of the stress relaxation is difficult to estimate in a cyclic RPS protocol. In addition, in the present experiment the time course of the behaviour of the PsrT was not followed during the RPS. However, our previous experiment (1) with identical protocol showed that 50 % of the total reduction in the PsrT was achieved already 15 minutes after the beginning of the RPS. This implies that stress relaxation may indeed play a role also in a cyclic passive stretching protocol especially during the early part of it.

Usually, plastic deformation (creep) is related to maintained tissue strain, which causes a reorientation of the supporting connective tissue to more-ordered arrays (34). In our earlier experiment (1), one third of the reduction in the PsrT took place during the latter part of the one-hour RPS. In addition, a clear drop in the passive torque was seen during the plateau phase of the cyclic RPS exercise at the end of the present experiment. Therefore, it could be suggested that plastic deformation seems to depend on the total tissue strain regardless of the mode of the stretching.

In the present experiment, torque/fascicle length and torque/tendon length relationships were calculated for different activation levels. After RPS, the fascicle length was shorter during low torques and longer during high torques as compared to the ones measured before the RPS exercise. Because of the tendon model and the isometric nature of the contraction, the tendon length changes were exactly the opposite. These results indicate altered tendomuscular material properties. As discussed above, stress relaxation and plastic deformation occur mainly on connective tissue (38, 34). Therefore, it could be suggested that the major changes in the tendomuscular material properties that could affect the torque/fascicle length relationship take place somewhere in the aponeurosis-tendon system. This is supported also by the finding of Herbert et al. (20) that for the relaxed tibialis anterior and gastrocnemius muscles more than half of the total change in muscle-tendon length was taken up by stretch of tendon. It should be noted that their tendon model was not
able to separate the length changes of the aponeurosis from that of the outer tendon. In addition, Lieber et al. (25) found in a frog semitendinosus muscle that aponeurosis strain during contraction was significantly below that measured during passive loading. Therefore, they suggested that aponeurosis actually changes its intrinsic properties during muscle contraction. Huijing and Ettema (21) reported also similar differences in aponeurosis properties between passively and actively loaded muscle. Unfortunately, in the present experiment the tendon model was unable to differentiate the behaviour of the aponeurosis and the tendon. However, crossing of the present torque/tendon length and torque/fascicle length curves with different activation levels before and after RPS could be a result of material modification of the aponeurosis due to passive stretching.

If the previous suggestion holds true and RPS induces a slack in aponeurosis, this could also lead to impaired torque production. Garfin et al. (13) reported a relationship between muscle stiffness and contractile performance. They found that surgical small release of fascia resulted in 15% reduction in force production due to lower compartment pressure during contraction. Therefore, as suggested by Fowles et al. (10) plastic deformation could decrease fascia stiffness to a point that could result in reduced force production. The fact that in the present study PsrT had a rather high relationship with MVC and fascicle length changes measured during MVC could also support this suggestion.

In addition, the reported length changes could place muscle fascicles to a less optimal portion of the torque/fascicle length curve and result in reduced MVC. In the present experiment, the torque/fascicle length curve was also measured during MVC at three different ankle joint angles (figure 5). Pre and post measurements gave indication of the rightward shift in this curve. Fowles et al. (10) reported a similar finding in a twitch torque-joint angle relation after passive stretch of the human plantarflexors. If this rightward shift means that the fascicles are replaced at a length corresponding to a descending limb of the torque-length relationship, this would obviously result in a reduced maximal torque production.
Altered torque/fascicle length characteristics may also influence the neural activation patterns. For example Fowles et al. (10) found a significant reduction in motor unit activation and electromyogram after 13 passive maximal stretches (30 min of time under stretch) of the human plantarflexors. In the present study, activation level was calculated during all MVCs. In contrast to the experiment of Fowles et al (10), our results showed that motor unit activation was not a limiting factor for the maximal torque production after the RPS exercise. These contradictory results can be explained by the differences in the passive stretching protocols. In our experiment RPS was cyclic and the stretch amplitude corresponded only 10° dorsiflexion of the ankle joint, which was much less than to one used by Fowles et al. (10). Therefore, it seems that the level of neural modification induced by passive stretching depends on the total strain of the muscle-tendon complex induced by the protocol.

Despite the fact that no changes in the activation level were observed in the present experiment, a clear reduction in maximal H/M –ratio was observed after RPS. This result indicates that some neural modification has indeed taken place. In addition, since there was a 14 % increase in the total twitch duration, slowing of the excitation-contraction coupling could be suggested. Therefore, it would be tempting to use the concept of muscle wisdom (4) to explain these parallel changes in neural input and muscle mechanics.

In any case, it is puzzling why the neural modifications were so clear after passive stretching in the present experiment. To explore these possible mechanisms stretch and H –reflexes were measured during the RPS exercise. However, in the interpretation of these reflexes, the mechanisms of post-activation depression will not be discussed, since in the present beginning / end -comparison this effect should be systematic in both and, therefore, in regard to the reflex modulation of the RPS exercise, insignificant.

The underlying mechanisms for the reflex modification pose a challenging question because several aspects should be considered. It is known that the supraspinal influences on the H-reflex are very strong (33). This was tested in our previous experiment with RPS (1) by
using contra lateral leg as a control leg. The assumption was that in a case of supraspinal modulation, similar changes in H-reflex should be observed in both legs. However, this was not the case. The effect of Ib-afferents can also be excluded since, first of all they are known to be very sensitive during active contraction but much less sensitive to passive stretches (6), and secondly Guissard et al. (17) showed that in a case of small amplitude stretching (10° of dorsiflexion) the premotoneuronal mechanisms are mainly involved. In a case of mechano-receptors of the skin and joint capsule, it has been shown that ischaemia with induced blockage of these afferents did not change the soleus stretch and H-reflexes (22). In addition, presynaptic inhibition induced by group III and IV muscle afferents (5) is not a very likely candidate, since these afferents are known to be operative in a presence of metabolic fatigue and/or muscle damage. However, to the best of our knowledge no data has conclusively shown that such a small amplitude passive stretching of the muscle could induce either of these.

Since the above-mentioned mechanisms did not seem to be ideal for the reflex modification during the RPS, proprioceptive feedback should be taken into consideration. In our previous RPS experiment (1) we suggested that the most likely explanation for the depressed H-reflex is a reduction in the excitatory drive from the Ia afferents onto the α-motoneurones. In addition, we proposed that the origin for this reduction could be decreased resting discharge of the muscle spindles because of the increased compliance of the muscle. This assumption was made on the basis of the reduction in PsrT. Our present experiment verifies this result. However, since there was shortening in the passive fascicle length after the RPS exercise, this increased compliance seems to take place somewhere in the aponeurosis-tendon system as discussed earlier. In addition, the above-mentioned hypothesis seems to be valid for two reasons. Firstly there was a high correlation between PsrF and the stretch reflex response of the soleus muscle (figure 8). Secondly the H-reflex, measured at the end of the stretching ramp, showed almost a fifty percent reduction at the end of the RPS exercise. These results could be interpreted so that the increased compliance of the muscle results in reduced mechanical response of the muscle spindle leading to disfacilitation of the α-motoneuron pool.
In summary, repeated and prolonged passive muscle stretching impairs both electrically and voluntarily induced muscle contractions. This impairment was mainly related to modification of the torque/fascicle length relationship due to altered material behaviour of the aponeurosis-tendon system. In addition, altered material properties seem to affect proprioceptive feedback and, therefore, the motor unit activation in proportion to the contractile failure. Similar changes in muscle mechanics and parameters related to neural behaviour have also been observed during SSC type of muscle fatigue (2, 31). Therefore, it would be interesting to examine the corresponding mechanisms also during active conditions by applying the present methodology.
References


Figure legends:

Figure 1. Experimental protocol illustrating repeated passive stretching protocols (RPS1 and RPS2). MVC = maximal voluntary contraction, DT = double twitch, US = ultrasound measurement, $M_{\text{max}}$ = maximum M-wave, $H_{\text{max}}$ = Maximum H-reflex and H-reflex = H-reflex measured at intensity of 25% of the maximal M-wave during the RPS exercise.

Figure 2. An example of one cycle of the repeated dorsiflexions of the ankle joint (stretching of the calf muscles). Passive mechanical stretch-reflex was also measured with this model. 10 deg and 3.5 deg·s$^{-1}$ represents the amplitude and the speed of the stretch, respectively. Stim. 1 represents the onset of the H-reflex stimulation.

Figure 3. Two schematic models that show how the fascicle length (A.) and tensile structures (B.) of the medial gastrocnemius muscle were identified and their changes measured during RPS exercise. $L_{\text{mtu}}$ = length of the muscle-tendon unit, $L_{\text{dt}}$ = length of the distal tendon and $L_{\text{pt}}$ = length of the proximal tendon.

Figure 4. Mean values ($\pm$SD) for all eight subjects in maximal plantarflexion torque measured before and after RPS1 and RPS2. In addition, corresponding average EMG levels of the SOL and GA muscles resulting from RPS2. *P<0.05 refers to the statistical significance between the values compared to the before-condition.

Figure 5. Torque-fascicle length relationship (mean $\pm$SD) at ankle joint angles of 80° (▲), 90° (●) and 115° (■) before and after the RPS1. Torque at each joint angle represents MVC. *P<0.05 refers to the statistical significance between the values compared to the before-condition.
Figure 6. Torque-fascicle length and torque-tendon length relationship (mean ±SD) at different activity levels (0, 10, 50 and 100 % of the MVC) before and after the RPS1, *P<0.05 refers to the statistical significance between the values compared to the before-condition.

Figure 7. Stretch- and H-reflex signals recorded from one subject at the beginning and at the end of the second RPS exercise. The filled dot shows the timing of the H-reflex stimulation.

Figure 8. Relationship between changes in the passive stretch-resisting torque and changes in fascicle length during MVC (above), stretch-reflex response of the soleus muscle (middle) and MVC (below).

Figure 9. Mean data from all the subjects illustrating the behaviour of the fascicle length changes (p<0.05 – 0.01) during one passive stretch cycle (average of ten cycles) in the beginning and at the end of the RPS1 both in absolute (A.) and relative (B.) scale. Please note that during the repeated passive stretching of one hour the fascicle displacement remained the same, but its length decreased.
PROTOCOL 1.
Subject pool
n = 8

PROTOCOL 2.
Subject pool
n = 8

PRE-TEST
Passive DT
(at 80°, 90° and 115° ankle joint ankle)

MVC + superimposed DT + US
(at 80°, 90° and 115° ankle joint ankle)

10% and 50% MVC + US
(at 90° ankle joint ankle)

RPS1
Beginning
Cycles (10-20)
- Ultrasound
End
Cycles (2380-2390)
- Ultrasound

POST-TEST
Passive DT
(at 80°, 90° and 115° ankle joint ankle)

MVC + superimposed DT + US
(at 80°, 90° and 115° ankle joint ankle)

10% and 50% MVC + US
(at 90° ankle joint ankle)

RPS2
Beginning
Cycles (10-50)
- H-reflex
- Stretch-reflex
End
Cycles (2350-2390)
- H-reflex
- Stretch-reflex

POST-TEST
Passive DT
(at 90° ankle joint ankle)

MVC + superimposed DT
(at 90° ankle joint ankle)

H_max / M_max

PRE-TEST
Passive DT
(at 90° ankle joint ankle)

MVC + superimposed DT
(at 90° ankle joint ankle)

H_max / M_max

> 2 weeks

1 hour
Mechanical Stimulation

100 ms

10 deg and 200 deg s⁻¹

200 ms

Stim. 1
Estimated fascicle length

$\text{Measured fascicle length } l_1$

Total fascicle length \( (L_f) \) = $l_1 + \frac{h}{\sin \alpha}$

Total tendon length = $L_{dt} + L_{pt} = L_{mtu} - L_f \cos \alpha$
Torque vs. fascicle length

Before

After
Stretch-reflex

H-reflex

Beginning

End

\[ 40 \text{ ms} \]

\[ 10^\circ \text{ angle} \]

\[ 1 \text{ mV Ga} \]

\[ 1 \text{ mV SOL} \]

\[ 2 \text{ mV SOL} \]
Passive stretch cycle

A. Time (ms)

B. Δ fascicle length

Ankle displacement

Deg.

cm

ms

%