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Harrison T. Finn

David S. Kennedy

Simon Green

Janet L. Taylor
*Edith Cowan University*



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# Fatigue-related feedback from calf muscles impairs knee extensor voluntary activation

Authors: Harrison T Finn [1, 2], David S Kennedy [3], Simon Green [4], Janet L Taylor [1, 2 ,5]

[1] Neuroscience Research Australia, Randwick, NSW, Australia

[2] University of New South Wales, Kensington, NSW, Australia

[3] University of Technology Sydney, NSW, Australia

[4] Western Sydney University, Campbelltown, NSW, Australia

[5] Edith Cowan University, Perth, WA, Australia

Corresponding author: Janet L Taylor

Edith Cowan University, 270 Joondalup Drive, Joondalup, WA, 6027, Australia

Telephone: +61 8 6304 3603

Email: janet.taylor@ecu.edu.au

**Running title:** Calf feedback inhibits knee extensor voluntary activation

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**ABSTRACT:**

Introduction: Fatigue-related group III/IV muscle afferent firing from agonist, antagonist or distal muscles impairs the ability to drive the elbow flexors maximally, i.e. reduces voluntary activation. In the lower limb, the effect of feedback from distal muscles on the proximal knee extensors is unknown. Here we test whether maintained group III/IV afferent feedback from the plantarflexor muscles reduces voluntary activation of the knee extensors.

Methods: On two days, voluntary activation of the knee extensors during maximal voluntary contractions (MVCs) was assessed in 12 participants before and after a 3-min fatiguing task of the plantarflexors. On one day, an inflatable cuff around the calf occluded blood flow for two mins immediately post-exercise (cuff day). The other day had no occlusion (no-cuff day). Supramaximal stimulation of the femoral nerve elicited superimposed twitches during MVCs of the knee extensors and resting twitches 2-3 s after relaxation. Pain (0-10 point scale) was reported throughout.

Results: In the 2 min after the 3-min fatiguing plantarflexor task, voluntary activation was 5.3% (SD 7) lower on the cuff day than on the no-cuff day ($P=0.045$), and MVC force was reduced by 13% (SD 16) ($P=0.021$). The resting twitch was similar on both days ($P=0.98$). Pain rated 4.9 points higher with the cuff inflated ($P=0.001$).

Conclusion: Maintained group III/IV afferent feedback from the fatigued plantarflexor muscles reduced maximal force and voluntary activation of the unfatigued knee extensors suggesting that afferents from the calf act centrally to inhibit the ability to drive the motoneurones of the knee extensors.

## Introduction:

During fatiguing exercise, mechanical and metabolic perturbations within the muscle result in increased firing of small-diameter group III/IV muscle afferents (1, 2). The firing of these afferents gives rise to sensations of muscle work and pain (3, 4) and can evoke widespread effects in the nervous system. Their firing can be evoked by fatiguing contractions, as well as by the injection of metabolites commonly present in the muscle during fatiguing contractions such as ATP, lactate, and hydrogen ions (3). Cardiorespiratory consequences of group III/IV afferent firing include reflex increases in ventilation, blood pressure, and heart rate which function to increase oxygen delivery to working muscles (5-8). During high-intensity exercise, high levels of group III/IV afferent firing are associated with suboptimal motoneuronal output and decreased voluntary activation (9-11). Group III/IV muscle afferents project to the dorsal horn of spinal cord (12-14) before projecting to higher spinal and supraspinal levels (15-19). It is suggested that these afferents can act supraspinally to affect motor cortical output (11, 20). In addition, they inhibit the excitability of motoneurones of extensor muscles, as seen for the elbow extensors (21). A high level of afferent firing can be maintained post-exercise by occlusion of blood flow to the exercised limb to block the clearance of metabolites from the muscle (22).

With fatiguing exercise, there is a progressive reduction in the maximal voluntary force that can be produced with the fatigued muscle or muscle group. Processes in the muscle and also in the nervous system contribute to the reduction. Central fatigue, the contribution of the nervous system to muscle fatigue, is defined as a progressive exercise-induced reduction in voluntary activation, i.e., the ability to drive a muscle or muscle group maximally during a voluntary contraction (23). Firing of group III/IV muscle afferents is one contributor to decreased voluntary activation of the fatigued muscle. For the knee extensors, voluntary activation decreases during fatiguing exercise and remains lower during periods of post-

exercise blood flow occlusion which maintains firing of the group III/IV muscle afferents (24). Similar effects are seen for the elbow flexor and the thumb adductor muscles (11, 20, 25).

The effects of group III/IV muscle afferents are not confined to muscles where the feedback originates, but have been shown to act on some nearby non-fatigued muscles. For the knee extensors, occlusion after a 2-min MVC of the antagonist knee flexor muscles resulted in decreased voluntary activation and maximal force (24). A similar relationship between fatigue of an antagonist muscle and reduction in voluntary activation has also been shown from the elbow extensors to elbow flexors (11). Additionally, for the upper arm, more remote effects have also been reported. Maintained firing of group III/IV muscle afferents of distal muscles (hand muscles) resulted in reductions in voluntary activation of the elbow flexors of the same arm (25). However, for the leg, it is unknown if the more distal muscles can affect the proximal muscles of the thigh. Any effects of fatigue-related feedback from the calf muscle could be of interest to exercising and patient populations. For patient populations characterised by low muscle perfusion, such as peripheral artery disease, pain due to claudication is common, especially in the calf muscles (26, 27). Thus, effects of this sensory feedback on motor performance of other muscle groups of the lower limb may be important for understanding poor movement quality and increased risk of falls.

Therefore, the aim of the current experiment was to assess maximal voluntary force and voluntary activation of the knee extensor muscles after fatiguing exercise of the plantarflexors. To determine whether fatigue-related afferent feedback from the calf impairs knee extensor performance, blood flow occlusion was applied to the lower leg after plantarflexor exercise to maintain afferent feedback. We hypothesised that, as in the arm, increased firing of muscle afferents from distal muscles of the leg would cause reductions in

voluntary activation and maximal force of proximal muscles of the same limb. That is the force and voluntary activation would be lower with the cuff inflated.

# Materials and methods:

## Participants

Twelve healthy participants (8 males, 4 females) with an average age of 27.1 (SD 1.5) years (mean and standard deviation) were recruited for the study. The participants were tested on two separate days (with or without post-exercise blood flow occlusion) with the order of conditions chosen by block randomisation. All studies were approved by Human Research Ethics Committee at the University of New South Wales. Written informed consent was obtained from each of the participants. The sample size estimation was based on data from Kennedy et al. 2014 (25). The number of 12 participants was chosen to show an effect size around 0.564 (partial eta squared) for an effect of condition on voluntary activation.

## Experimental setup

Participants were seated in a custom-built chair with hips at 80 degrees (0 is extended neutral position) and left knee at 85 degrees (knee fully extended is 0 degrees) (Figure 1A). For knee extension contractions, the left leg was secured between restraints which fastened around the lower leg just above the malleolus and pulled taut both backwards and forwards. The ankle was able to hang freely, in a comfortable position for the participant. In addition, an adjustable strap was placed over the upper thigh and was tightened to secure the participant during the contractions. Knee extension force was measured with a linear strain gauge (linear to 2 kN; XTran, Melbourne, Australia) attached in line with the restraint behind the ankle.

For plantarflexor contractions, the left foot was placed on a foot-plate and an adjustable strap was placed over the distal thigh, 4-6 cm proximal to the superior border of the patella (Figure 1B). The foot-plate and foot were positioned so that the knee joint was directly over

the ankle joint with both joints at 90 degrees. Plantarflexion force was measured with a linear strain gauge (linear to 2 kN; XTran, Melbourne, Australia) between the foot-plate and the ground. To measure muscle activity, electromyograms (EMG) of the three superficial quadriceps muscles, vastus medialis (VM), vastus lateralis (VL), and rectus femoris (RF), as well as the soleus were recorded. For each muscle two Ag-AgCl electrodes (20 mm diameter Conmed ClearTrace ECG Sensor Electrodes Utica, NY) were arranged in a bipolar fashion along the muscle. The VM electrodes were placed at two centimetres and seven centimetres proximal to the superior medial border of the patella. The VL electrodes were placed at 75% and 70% of the distance from the anterior superior iliac spine to the lateral border of the patella. The RF electrodes were placed at 60% and 55% of the distance from the anterior superior iliac spine to the superior border of the patella. Soleus electrodes were placed 60% and 55% of the distance from the popliteal fossa to the medial malleolus. Placement of electrodes was confirmed with palpation during brief knee extension or plantar flexion contractions. A 70 mm by 40 mm (3M Universal Electrosurgical Pad, AUS) reference electrode was placed across the patella. Force and EMG signals were recorded using a 16-bit A/D converter (CED 1401; Cambridge Electronic Design Ltd, Cambridge, UK) and Spike2 software (v. 7.12 Cambridge Electronic Design). Force and EMG signals were sampled at 1000 and 2000 Hz, respectively. EMG signals were bandpass filtered (16 - 1000 Hz) and amplified (x100) prior to sampling using CED 1902 amplifiers (Cambridge Electronic Design). During the experiment, participants were given visual feedback of knee extension force or soleus root mean square EMG (rmsEMG) activity, which was computed using Spike2 and displayed on an external monitor. For feedback, soleus EMG signal was root mean square processed and median filtered in real-time using a 40 ms time constant.

*Femoral nerve stimulation.*

Single electrical stimuli (500 µs pulse width) were delivered to the femoral nerve via a constant current stimulator (DS7AH, Digitmer, Welwyn Garden City, UK) to elicit force twitches from the knee extensor muscles for measuring voluntary activation. A custom-made cathode (20 mm diameter circular probe) was placed over the femoral nerve at the level of the inguinal ligament over the femoral triangle, and secured with an adjustable strap. The anode, 70 mm by 40 mm electrode (3M Universal Electrosurgical Pad, Australia), was placed along the iliac crest on the left gluteus minimus. Stimulation intensity was set at 150% of the current required to get a maximal compound muscle action potential (M-wave), as measured by peak-to-peak amplitude, in all three measured superficial quadriceps muscles (range 65-240 mA, median 110 mA). Stimulation was delivered during maximal voluntary contractions of the knee extensors to evoke superimposed twitches and with the muscles at rest (resting twitch).

*Blood flow occlusion.*

A standard double bladder adult arm cuff (length 45 cm, width 15 cm) was placed around the lower left leg, just below the knee joint, and when required the top bladder (width 6 cm) was inflated with compressed air to a pressure of 300 mmHg to occlude circulation to the leg. The cuff was briefly inflated during two of the four baseline MVCs, and then for 2 min after the fatiguing protocol on the cuff day (Figure 1C).

## Experimental procedures

The procedures for the two days of the experiment were identical apart from the inflation of a cuff around the lower leg during the 2 min immediately following the fatiguing protocol of the plantarflexors (cuff day) (Figure 1C). Once stimulus intensity was set, participants performed several knee extensor contractions of increasing force, then two to three maximal voluntary contractions (MVC) as a warm up and familiarisation. Participants then performed

four MVCs of the knee extensors separated by 2-min rest periods. Twitches were evoked from the quadriceps muscles via femoral nerve stimulation during each MVC and with the muscles at rest, ~2 s after the MVC. For two of the MVCs, the cuff was inflated around the top of the calf prior to the MVC and deflated after the resting twitch. After the completion of baseline MVCs, the lower leg was placed in the plantarflexor set up and participants then performed two brief plantarflexor MVCs, followed by a 4 s contraction at 70% of the highest MVC recorded. The average level of rmsEMG activity recorded from the soleus during the 70% MVC contraction was then used as the target for the fatiguing task of plantarflexors and was visually displayed to the participant in real-time. Soleus was chosen to represent plantarflexor activity although other muscles, like gastrocnemius and the toe flexors, would also contribute to force output. The 3-min fatiguing task consisted of forty-five plantarflexor contractions held for 3-s with 1-s rest between contractions. Audio cues were provided to maintain cadence. At the end of the task, the plantarflexor set up was removed before knee extensor MVCs were performed. The knee extensor MVCs were performed at 40 s, 65 s, 90 s and 115 s. As with baseline MVCs, twitches were evoked during and shortly after each MVC. On the cuff day, the cuff was inflated 6 s prior to the end of the fatigue protocol and remained inflated for 2 min (Figure 1C). On the no-cuff day, the cuff remained deflated throughout. During the fatigue protocol at 20 s, 90 s and 150 s, participants were asked to rate the perceived effort (RPE) required to maintain task performance as well as pain in the plantarflexor muscles using scales from 0 - 10. Following the fatigue protocol, pain in the plantarflexors was reported after each knee extensor MVC.

**Data processing**

During off-line analysis both Spike2 (v. 7.12) and Signal software (v. 4.06) were used to determine all measures. For the knee extensors, MVC force was the highest force recorded before stimulation and rmsEMG activity for each contraction was calculated over a 200 ms

period prior to stimulation. Force was normalised to the highest MVC during baseline contractions. Voluntary activation was calculated using the following equation:

$$\text{voluntary activation (\%)} = [1 - (\text{superimposed twitch/resting twitch})] \times 100.$$

The amplitudes of superimposed twitches and resting twitches were measured as the difference in force prior to stimulation and the peak force after stimulation. Amplitudes of the M-waves for the three quadriceps muscles were measured between cursors at the initial deflection from baseline to the second crossing of zero.

Statistical Analysis

*Knee extensor MVCs.* During baseline MVCs, two of the four baseline MVCs were performed with the cuff inflated. A two-way ANOVA was performed with factors of day and cuff to examine whether cuff inflation had an effect and if baseline MVCs were different between days.

Two-way repeated measures ANOVAs with time (baseline, 40 s, 65 s, 90 s, 115 s) and day (no-cuff or cuff) as factors were used to examine the effect of maintained fatigue-related feedback during the 2 min that followed plantarflexor fatigue on knee extensor MVC force, voluntary activation, superimposed twitches, and resting twitches. Sphericity was verified by Mauchly's test. When sphericity was violated Greenhouse-Geisser correction was applied. To compare individual time points between days and from baseline, pairwise t-tests with Bonferroni corrected P values were used. A further two-way repeated measures ANOVA with time (40 s, 65 s, 90 s, 115 s) and day (no-cuff or cuff) examined pain during the first two min post plantarflexor fatigue task. rmsEMG and M-wave data for the three quadriceps muscles were analysed with three-way repeated measures ANOVAs with day (cuff or no-cuff), muscle (VL, VM, RF) and time (baseline, 40 s, 65 s, 90 s, 115 s) as the factors. Data from 2 min onwards (2:15, 3, 4 min) were not included in the analysis, as these data do not

test our hypothesis that maintained afferent feedback from the PF would lead to reduced VA in the knee extensors.

*Plantarflexor task.* For analysis of the plantarflexor task, the 3-min task was broken into three epochs: Start (4-32 s), Middle (80-108 s) and End (144-172 s). The value of each measure for each epoch was calculated as the average of the 7 contractions that occurred in that epoch. Two-way repeated measures ANOVAs with time (Start, Middle, End) and day (no-cuff or cuff) as factors were used to compare plantarflexor force, soleus rmsEMG, RPE, pain, VL rmsEMG and RF rmsEMG. VM rmsEMG was not able to be recorded during the plantarflexor task due to the positioning of the restraint across the knee. All data in text and figures are reported as mean (SD). The significance level was set to $P < 0.05$.

# Results:

## Fatiguing task

On each day participants performed 3 min of intermittent (3 s on, 1 s off) plantarflexor contractions to the level of soleus rmsEMG recorded during a 70% MVC force contraction. The soleus rmsEMG was well matched between days ($F_{1, 11} < 0.1$, $P = 0.87$) during the 3 min. At the beginning of the task, the level of soleus rmsEMG was 56.1% (SD 10.5) (Table 1) of maximal soleus rmsEMG and participants were able to maintain this level throughout the 3 min ($F_{2, 22} = 1.7$, $P = 0.2$). The plantarflexor force during the 3-min task decreased by 10.7% MVC (SD 9.3) during the fatigue protocol (Table 1) showing an effect of time ($F_{2, 22} = 15$, $P < 0.001$), and changed similarly on the two days as there was no effect of day ($F_{1, 11} = 1.9$, $P = 0.18$). Effort (RPE) to perform the plantarflexion task increased progressively over the 3-min task from 2.9 (SD 1.7) to 6.0 (SD 1.7) (Table 1). Two-way ANOVA showed an effect of time ($F_{2, 22} = 43.2$, $P < 0.001$) and no difference between days

($F_{1, 11}$ = 0.1, P = 0.7) or interaction ($F_{2, 22}$ = 0.8, P = 0.45) for RPE rating. Muscle activity from the quadriceps muscles was minimal during the plantarflexor task (Table 1)

**Maximal knee extensions before and after fatiguing plantarflexion**

*Knee extensor MVC*

Knee extensor MVC was 521.1 N (SD 171) at baseline with no difference between days ($F_{1, 11}$ = 0.5, P = 0.5) and no effect of cuff ($F_{1, 11}$ < 0.1, P = 0.9). Post the 3-min plantarflexor fatiguing task, the MVCs during the first 2 min were lower on the cuff day (75.9% (SD 9) of maximal MVC) than the no-cuff day (88.8% (SD 11.3); Figure. 2A). There was a main effect of day ($F_{1, 11}$ = 7.2, P = 0.021), time ($F_{4, 44}$ = 7.8, P < 0.001) and interaction ($F_{4, 44}$ = 5.1, P = 0.002). Post hoc comparisons showed that on the cuff day the MVC was lower than without the cuff at each time point (P < 0.015) during the period of cuff inflation. Compared to baseline, the MVC was significantly lower on the cuff day (P < 0.001 for all points during the period of cuff inflation) but not different on the no-cuff day (P > 0.14).

*Voluntary activation*

The voluntary activation at baseline was 77.4% (SD 8.6), with no difference between days ($F_{1, 11}$ = 0.2, P = 0.67), nor was there a difference with the cuff inflated ($F_{1, 11}$ = 0.02, P = 0.9). Analysis of the baseline values and those during the first 2 min post fatiguing task showed an effect of day ($F_{1, 11}$ = 5.1, P = 0.045), but no effect of time ($F_{4, 44}$ = 1.0, P = 0.39), or an interaction ($F_{4, 44}$ = 1.5, P = 0.2) (Figure 2B). Voluntary activation was lower on the cuff day than the no-cuff day by an average of 5.3% (SD 7). Post hoc analysis showed that on the cuff day, activation for the second and third post-fatigue MVCs were significantly lower when compared to the no-cuff day (P < 0.014).

*Superimposed twitch*

The average superimposed twitch at baseline was 38.3 N (SD 18.5) and was not different between days ($F_{1, 11} = 2.9$, $P = 0.114$) or with and without cuff inflation ($F_{1,11} = 0.3$, $P = 0.6$). Analysis of the baseline values and those during the first 2 min of recovery showed main effects of time ($F_{4, 44} = 3.7$, $P = 0.01$) and day ($F_{1, 11} = 6.7$, $P = 0.025$) but no interaction ($F_{4, 44} = 1.1$, $P = 0.37$; Figures 2C and 3). During the 2 min after the fatiguing task, the superimposed twitches on the cuff day were larger than on the no-cuff day for the last three MVCs ($P < 0.021$). The superimposed twitches were larger than baseline on the cuff day during the 4th MVC ($P < 0.003$).

*Resting Twitch*

The resting twitches, which were elicited after each MVC, were on average 171 N (SD 38.4) at baseline with a difference between days ($F_{1, 11} = 5.6$, $P = 0.037$) but no difference with the cuff ($F_{1, 11} = 0.1$, $P = 0.7$) (Figure 2D). Two-way ANOVA, including the baseline values and those in the 2 min after the fatiguing task, showed an effect of time ($F_{4, 44} = 6$, $P < 0.001$) but no effect of day ($F_{1, 11} < 0.01$, $P = 0.95$). There was a significant interaction ($F_{4, 44} = 6.6$, $P < 0.001$). At baseline, the amplitude of the resting twitch on the cuff day was higher than on the no-cuff day ($P = 0.004$). On the cuff day, the twitch amplitude was significantly lower than baseline for the first two measures after the fatiguing task ($P < 0.043$). On the no-cuff day the twitch amplitude was significantly higher than baseline on the last two measures of the first two mins ($P < 0.048$).

*Pain*

Pain rated in the calf increased during the 3-min fatiguing plantarflexion exercise from 0.6 (SD 1), *extremely weak*, to 3.6 (SD 2.6), *moderate/strong* (Figure 4). Two-way ANOVA showed an effect of time ($F_{2, 22} = 27.8$, $P < 0.001$), with no effect of day ($F_{1, 11} = 0.9$, $P = 0.3$). During the first 2 min of recovery, the average pain rating was 6.1 (SD 3), *strong to*

*very strong*, on the cuff day but 1.5 (SD 1.6), *very weak to weak*, on the no-cuff day. There was a significant effect of day ($F_{1, 11}$ = 56.2, P < 0.001) but no effect of time ($F_{3, 33}$ = 0.6, P = 0.6) nor interaction ($F_{3, 33}$ = 1.7, P = 0.17). When the cuff was deflated, pain rating decreased to 2.5 (SD 2.4) after 15 s.

*EMG*

A 3-way ANOVA with day, muscle (VL, VM, RF) and time was performed to compare rmsEMG during brief knee extensor MVCs during the 2 min with or without the cuff. There were significant effects of time ($F_{4, 44}$ = 6.8, P < 0.001) and day ($F_{1,11}$ = 22.6, P < 0.001) but not muscle ($F_{2, 22}$ = 1.4, P = 0.25; Figure 5). There was only one significant interaction, which was for day by time ($F_{4, 66}$ = 2.6, P = 0.038). The day effect showed that quadriceps EMG was lower on the cuff day when compared to the no-cuff day but post hoc tests were only significant during the 1st MVC with the cuff inflated (P = 0.03).

*M-waves EMG*

A 3-way ANOVA with day, muscle (VL, VM, RF) and time was performed to compare M-wave amplitude during the resting twitches at baseline and the 2 min with or without the cuff. There was no muscle by day by time interaction ($F_{8, 321}$ = 0.03, P = 1). There was a significant effect of muscle ($F_{2, 22}$ = 69.7, P < 0.001) indicating differences between muscles. The average RF M-wave was 10.5 mV (SD 6.7), VM was 18.3 mV (SD 3.5), and VL was 13 mV (SD 4.7). There was no effect of time ($F_{4, 44}$ = 0.11, P = 0.978) or day ($F_{1, 11}$ = 0.91, P = 0.342).

## Discussion:

The main findings of this study are that when fatigue-related feedback from group III/IV afferents of the calf muscles is maintained after exercise, there are reductions in knee

extensor maximal force, voluntary activation, and EMG activity despite the knee extensors performing no exercise.

With no blood flow to the fatigued plantarflexors after exercise, we expect that the firing of group III/IV muscle afferents was maintained or increased during the two mins of occlusion (2). While afferent firing was not measured directly, activation of muscle afferents through high concentrations of metabolites results in pain (3). On the cuff day participants rated pain as *strong*, to *very strong* (mean 6.1 on a 0-10 point scale), whereas without cuff inflation, pain ratings were much lower, averaging 1.5 out of 10. Thus, the higher levels of pain provide indirect evidence for higher group III/IV afferent feedback from the plantarflexor muscles during the two minutes of post-exercise occlusion.

With occlusion of blood flow to the calf after fatiguing exercise of the plantarflexors, there was a reduction in the maximal force produced with the knee extensors. Knee extensor force was ~14% lower than without occlusion when it was unchanged from baseline. This observed decrease in knee extensor force was greater than the ~6% seen for elbow flexor force with distal hand muscle afferent firing (25), but less than the ~25% decrease of knee extensor force, with knee flexor afferent firing (24). A 14% reduction in maximal force with plantarflexor afferent firing suggests a moderate impairment of the knee extensors' strength. The knee extensors were minimally activated throughout the plantarflexor task (Table 1). However, there were minor changes in resting twitch force (Figure 2D) which could suggest peripheral fatigue. At baseline the resting twitches differed by ~6% across days and on the cuff day, the resting twitch was initially decreased by ~16% after the plantarflexor task, but this decrease disappeared over time despite the continued ischaemia. This pattern is not consistent with fatigue but rather a variable degree of potentiation of the muscle by preceding activity, and depotentiation over the plantarflexor task when knee extensor activity was low. Although all resting twitches were elicited after brief MVCs, a single 2-3 s MVC is not

always sufficient to potentiate the muscle fully (28). Post-activation potentiation reflects changes in calcium sensitivity of the contractile apparatus. Its effect is greatest on twitch responses, whereas for voluntary contractions, it does not increase maximal force (29). Moreover, in the current study, the MVC remained low when the resting twitch regained its baseline amplitude over the latter part of the period of occlusion. This suggests that the impairment in maximal force of the knee extensors did not occur within the muscle but in the central nervous system.

To explore central influences on force production, we measured voluntary activation and EMG of the knee extensors. With post-exercise occlusion of the calf, quadriceps voluntary activation and EMG activity were reduced, whereas without occlusion, there was no change in voluntary activation from baseline. Thus, the preceding fatiguing plantarflexor task by itself was not sufficient to cause central impairment limiting drive to the knee extensors when measured after 40 s of recovery with freely perfused muscle. The reduction in voluntary activation of 5.3% on the cuff day compared to the no-cuff day was similar to the 5.3% change in elbow flexor voluntary activation seen with maintained firing of afferents in the muscles of the hand (25) but was less than the reduction in knee extensor voluntary activation seen with maintained high levels of feedback from knee flexor fatigue (~25%; Kennedy *et al.*, 2014 (24)). This could indicate stronger inhibition between proximal muscles (agonist-antagonist pair) than from a distal to a proximal muscle, but could also be a function of the number of afferents firing and/or their level of activity. It should be noted that in the current study minimal recovery of MVC and voluntary activation occurred with deflation of the cuff despite a quick reduction in pain ratings. As similar previous studies have all shown obvious recovery on cuff deflation (11, 24, 25), this lack of recovery was unexpected and needs confirmation before any conclusion, such as a muscle specific interaction, could be made.

Peripheral nerve stimulation was used to assess voluntary activation and this technique gives no insight into the specific site at which feedback from group III/IV muscle afferents of the plantarflexors might act centrally to reduce knee extensor voluntary drive. Group III/IV muscle afferents terminate in the dorsal horn of the spinal cord where their projections are diffuse. In animal models that investigate referred pain, there is evidence that the neurones in the spinal cord that respond to nociceptive feedback have large receptive fields that may include more than one muscle (30, 31). Thus afferents from one muscle may act on dorsal root neurones that primarily receive input from another muscle (31, 32). Excitation of these neurones may result in the activation of inhibitory circuits that change motor output in the same way regardless of whether input was received from the primary muscle or others. Changes in motor output could occur at a spinal level through effects on motoneurone excitability, or through actions in higher central nervous system locations such as motor cortical areas.

The evidence of inhibition of knee extensor motoneurones by group III/IV muscle afferents is mixed. When measured during voluntary contraction, no change in motoneurone excitability is reported with blockade of group III/IV feedback (33), or with post-exercise occlusion (34). However, we have observed a reduction in knee extensor motoneurone excitability during post-exercise ischaemia after knee extensor or knee flexor exercise (unpublished data) when motoneurone excitability was tested without ongoing descending drive at time of assessment (35). This result is consistent with the similar inhibition of elbow extensor motoneurones (21). Thus, it is likely that the knee extensor motoneurones are sensitive to inhibition by group III/IV feedback from the knee extensors and knee flexors, but we can only speculate that afferents from the plantarflexor muscles also cause reductions in knee extensor motoneurone excitability.

Supraspinal changes may also contribute to the reduction in knee extensor force and voluntary activation with firing of group III/IV afferents of distal muscles. For both the elbow flexors and the knee extensors, motor cortical excitability, as assessed by TMS-evoked motor evoked potentials (MEPs) are apparently unaffected by post-exercise ischaemia (34, 36). However, with maintained fatigue-related feedback from the hand muscles, elbow flexor voluntary activation as assessed by TMS was decreased (25). This finding indicates reduced drive from the motor cortex to the motoneurones as the motoneurones of the elbow flexors are excited by group III/IV muscle afferent feedback (21, 25). Corresponding data are not available for the knee extensor muscles. Nevertheless, taking together all the evidence, we would expect changes both at the motoneurones and at supraspinal centres.

Knee extension voluntary activation has been shown to be impaired by feedback from group III/IV muscle afferents from the homonymous muscle, the antagonist knee flexor muscles (24) and now the distal plantarflexor muscles of the same limb. Therefore, during full body or locomotion exercise/tasks these three muscle groups could all act to impair knee extensor function. However, the combined effect of these inputs is as yet unknown. They may summate to induce greater impairment or there may be a ceiling to the impairment. It is unknown whether they use the same interneuronal or synaptic networks. One note is that sensory feedback from the contralateral knee extensors during post-exercise ischaemia does not cause reductions in voluntary activation (24) despite reports of cross-over inhibition resulting in reductions in central motor drive (37).

In conclusion, post-exercise occlusion of the fatigued plantarflexor muscles resulted in a reduction of voluntary activation, maximal force, and muscle activity of the knee extensor muscles, but these were unchanged if normal blood flow allowed recovery of the plantarflexor muscles. This suggests that high levels of fatigue-related group III/IV muscle afferent feedback from the calf impairs knee extensor function.

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## Conflict of interest

We report no conflict of interests

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**Captions**

**Table 1.** rmsEMG, force and effort during the 3-min plantarflexor (PF) task. Mean (SD). * indicates significant difference to Start ($P < 0.05$)

**Figure 1. Setup and Protocol. Setup for knee extensor MVCs (A). Setup for plantarflexor contractions (B). Protocol outline (C).** On each of the two days, at baseline, four brief knee extensor MVCs were performed with twitches evoked via femoral nerve stimulation during the MVC and at rest (arrows). Two of these MVCs were performed while the cuff was inflated around the lower leg. The fatiguing task consisted of intermittent plantarflexor contractions (3s on, 1s off) performed to the level of soleus rmsEMG required to generate a plantarflexor force of 70% maximum. After the fatiguing task, more brief knee extensor MVCs with twitches were performed. On one of the days a cuff was inflated around the lower leg at the end of the fatiguing task for 2 mins.

**Figure 2**. **Panels display group means (mean and SD, n = 12) at baseline (bl) and then post plantarflexor task for knee extensor MVC (A), voluntary activation (B), superimposed twitch (C) and resting twitch (D)**. Grey bar represents the performance of the 3-min plantarflexor task. Black bar indicates the period when the cuff was inflated on one day and not on the other. Open circles denote the no-cuff day and closed circles represent the cuff day. # indicates significant ($P < 0.05$) difference between days and * denotes difference ($P < 0.05$) from baseline.

**Figure 3. Force traces of superimposed twitches for a single participant on the no-cuff and cuff days at baseline and during the immediate 2 min after the fatiguing task**. Dotted horizontal lines represent the average amplitudes of the superimposed twitches during the baseline MVCs. Arrow represents femoral nerve stimulation. Note the greater increase in the size of the superimposed twitch on the cuff day.

**Figure 4. Group data (mean and SD, n = 12) for pain rated in the plantarflexors during the 3-min plantarflexor task and during recovery with and without the inflation of the cuff.** Grey box represents the plantarflexor task. Black bar indicates the period of cuff inflation on one day. Open circles denote the no-cuff day and closed circles represent the cuff day. # indicates significant difference between days ($P < 0.05$).

**Figure 5. Panels display group means (mean and SD, n = 12) of the EMG activity during knee extensor MVCs at baseline (bl) and after the plantarflexor task for vastus lateralis (VL) (A), vastus medialis (VM) (B) and rectus femoris (RF) (C)**. Grey bar represents the performance of the 3-min plantarflexor task. Black bar indicates the period of cuff inflation on the cuff day. Open circles denote the no-cuff day and closed circles represent the cuff day.

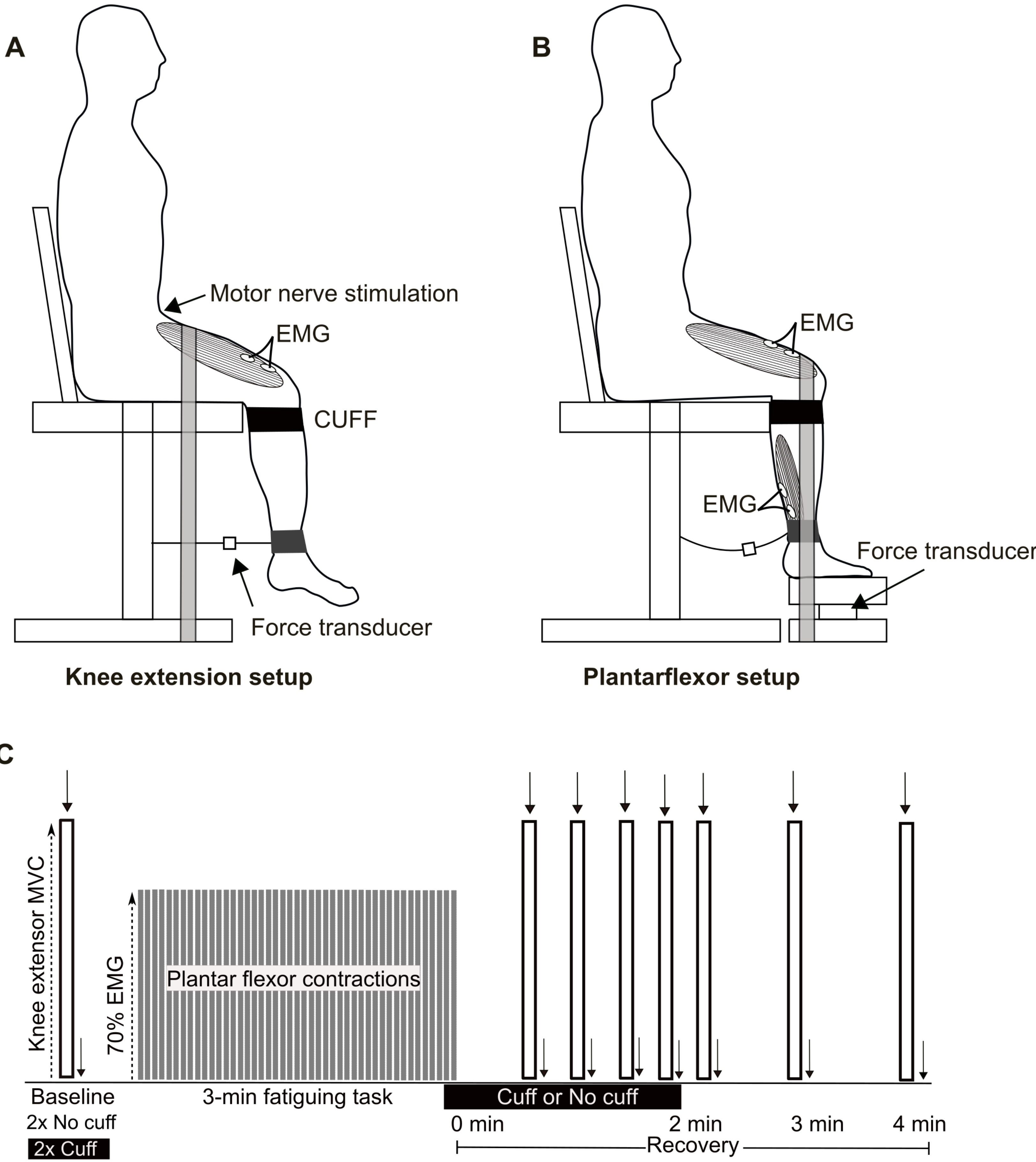
Table 1.

| | | 3-min contraction | | |
|---|---|---|---|---|
| | | **Start** | **Middle** | **End** |
| **Soleus rmsEMG** | *No-cuff* | 55.8% (SD 12) | 55.2% (SD 12) | 53.9% (SD 11) |
| % maximal | *Cuff* | 56.4% (SD 9) | 54.3% (SD 8) | 53.9% (SD 8) |
| **PF force** | *No-cuff* | 61.5% (SD 12) | 58.2% (SD 12) | 51.8% (SD 9.8)* |
| % MVC | *Cuff* | 59.3% (SD 12.5) | 52% (SD 12)* | 47.3% (SD 9.5)* |
| **Effort** | *No-cuff* | 2.8 (SD 1.5) | 4.3 (SD 1.9)* | 6.2 (SD 2)* |
| 0-10 scale | *Cuff* | 3.1 (SD 1.9) | 4.2 (SD 1.7)* | 5.8 (SD 2.2)* |
| **VL rmsEMG** | *No-cuff* | 2.9% (SD 2.2) | 3.7% (SD 3.9) | 4.2% (SD 3.7) |
| % maximal | *Cuff* | 3.7% (SD 3.3) | 5.1% (SD 4.4) | 4.8% (SD 4.9) |
| **RF rmsEMG** | *No-cuff* | 2.6% (SD 2.2) | 3% (SD 3.8) | 3.6% (SD 2.6) |
| % maximal | *Cuff* | 1.7% (SD 0.9) | 2.6% (SD 2.5) | 2.3% (SD 1.6) |

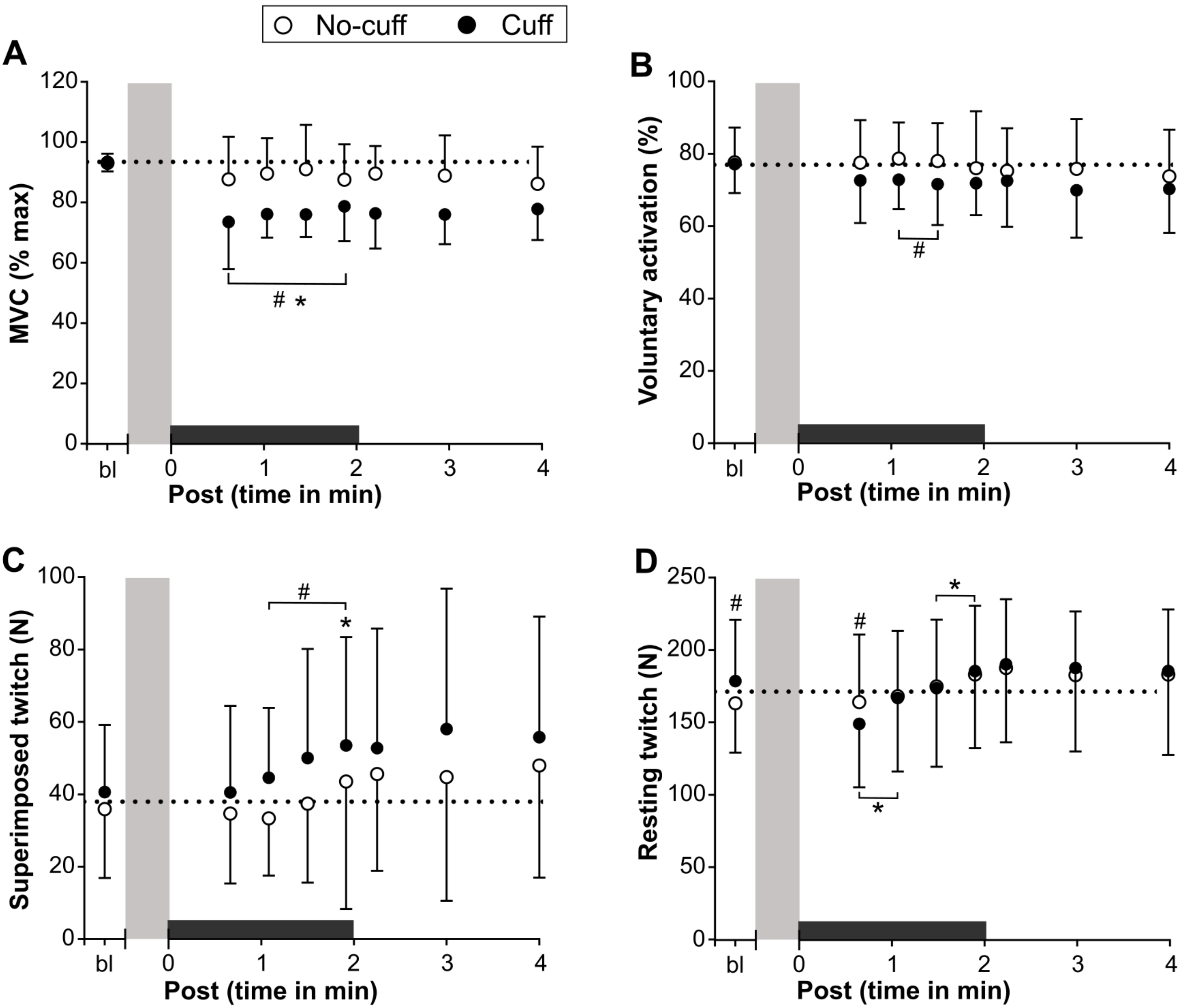
1

**Table 1.** rmsEMG, force and effort during the 3-min plantarflexor task. Mean (SD). * indicates significant difference to Start (P < 0.05)

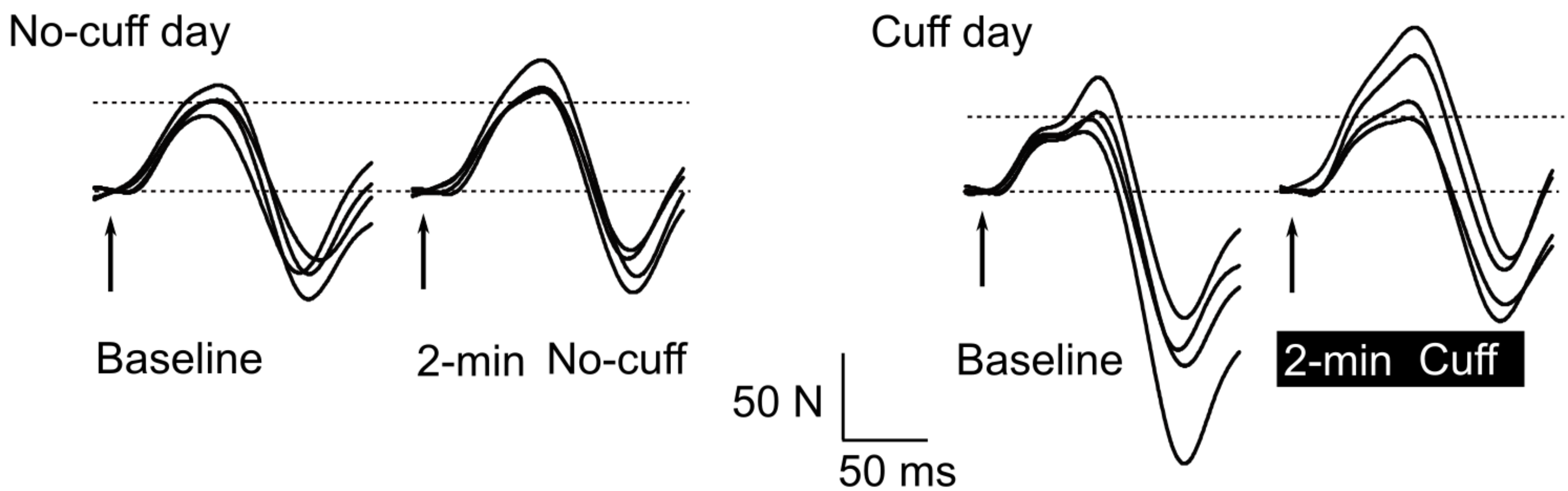
Figure


A
B
Motor nerve stimulation
EMG
CUFF
Force transducer
EMG
EMG
Force transducer
Knee extension setup
Plantarflexor setup
C
Knee extensor MVC
70% EMG
Plantar flexor contractions
Baseline
2x No cuff
2x Cuff
3-min fatiguing task
Cuff or No cuff
0 min
2 min
3 min
4 min
Recovery

Figure

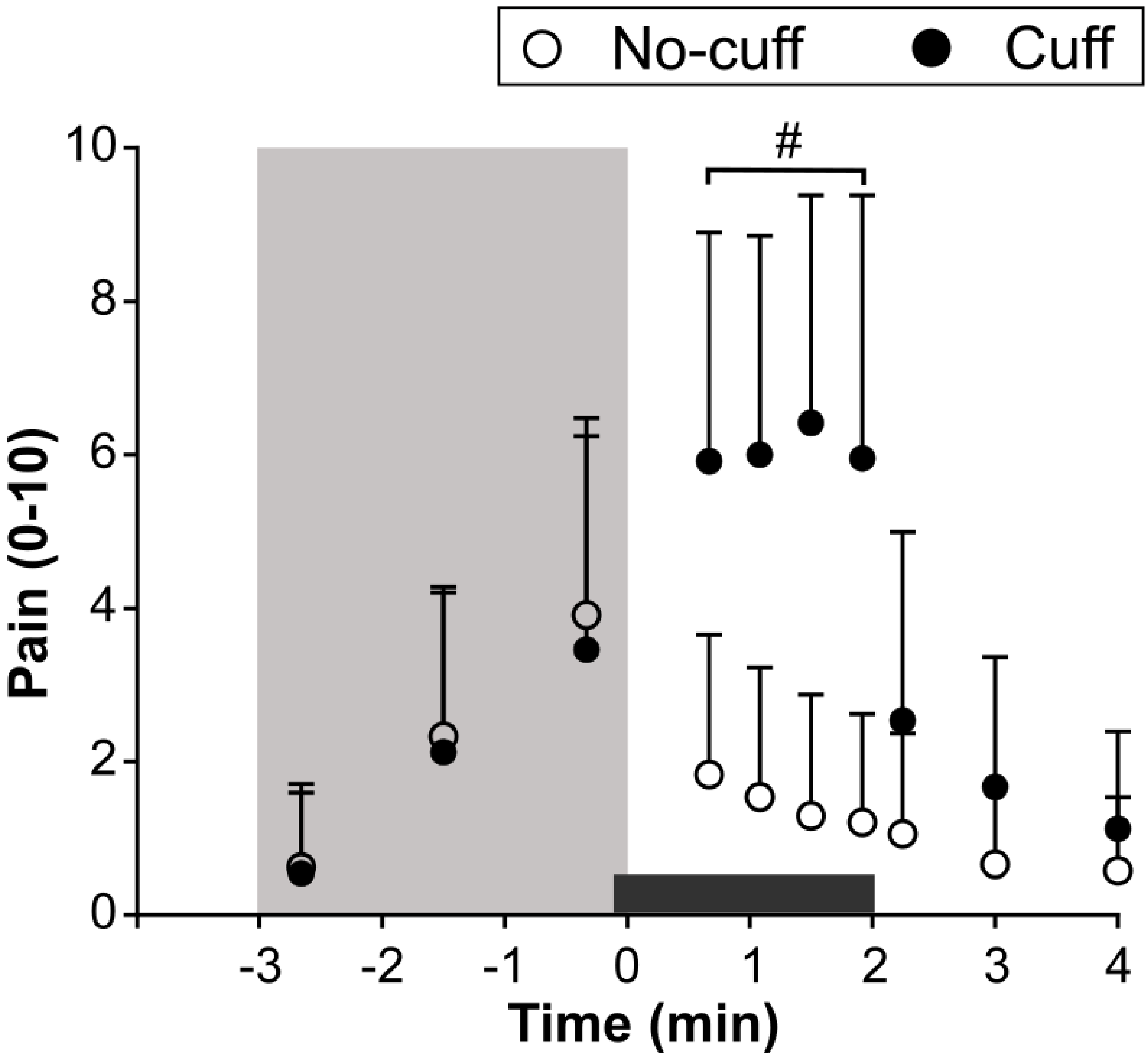

No-cuff
Cuff
A
MVC (% max)
120
100
80
60
40
20
0
bl
0
1
2
3
4
Post (time in min)
# *
B
Voluntary activation (%)
100
80
60
40
20
0
#
C
Superimposed twitch (N)
#
*
D
Resting twitch (N)
250
200
150
100
50
#
*

Figure

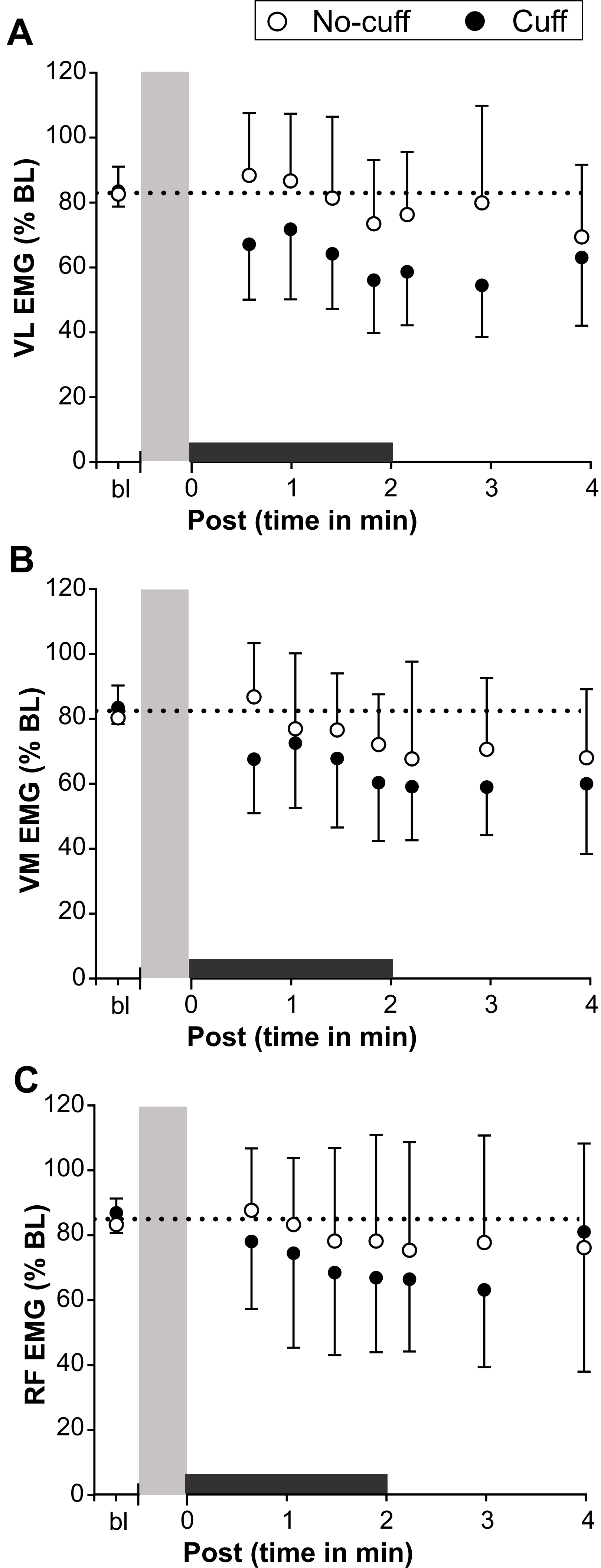


No-cuff day
Baseline
2-min No-cuff
Cuff day
Baseline
2-min Cuff
50 N
50 ms

Figure



Figure



No-cuff
Cuff
A
VL EMG (% BL)
120
100
80
60
40
20
0
bl
0
1
2
3
4
Post (time in min)
B
VM EMG (% BL)
120
100
80
60
40
20
0
bl
0
1
2
3
4
Post (time in min)
C
RF EMG (% BL)
120
100
80
60
40
20
0
bl
0
1
2
3
4
Post (time in min)