Fatigue-related firing of muscle nociceptors reduces voluntary activation of ipsilateral but not contralateral lower limb muscles

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Kennedy DS, Fitzpatrick SC, Gandevia SC, Taylor JL. Fatigue-related firing of muscle nociceptors reduces voluntary activation of ipsilateral but not contralateral lower limb muscles. J Appl Physiol 118: 408–418, 2015. First published December 18, 2014; doi:10.1152/japplphysiol.00375.2014.—During fatiguing upper limb exercise, maintained firing of group III/IV muscle afferents can limit voluntary drive to muscles within the same limb. It is not known if this effect occurs in the lower limb. We investigated the effects of group III/IV muscle afferent firing from fatigued ipsilateral and contralateral extensor muscles and ipsilateral flexor muscles of the knee on voluntary activation of the knee extensors. In three experiments, we examined voluntary activation of the knee extensors by measuring changes in superimposed twitches evoked by femoral nerve stimulation. Subjects attended on 2 days for each experiment. On one day a sphygmomanometer cuff occluded blood flow of the fatigued muscles to maintain firing of group III/IV muscle afferents. After a 2-min extensor contraction (experiment 1; n = 9), mean voluntary activation was lower with than without maintained ischemia (47 ± 19% vs. 87 ± 8%, respectively; P < 0.001). After a 2-min knee flexor maximal voluntary contraction (MVC) (experiment 2; n = 8), mean voluntary activation was also lower with than without ischemia (59 ± 21% vs. 79 ± 9%; P < 0.01). After the contralateral (left) MVC (experiment 3; n = 8), voluntary activation of the right leg was similar with or without ischemia (92 ± 6% vs. 93 ± 4%; P = 0.65). After fatigue exercise, activity in group III/IV muscle afferents reduces voluntary activation of the fatigued muscle and nonfatigued antagonist muscles in the same leg. However, group III/IV muscle afferents from the fatigued left leg had no effect on the unfatigued right leg. This suggests that any “crossover” of central fatigue in the lower limbs is not mediated by group III/IV muscle afferents.

PAIN AND FATIGUE are often associated with impaired performance during high-intensity exercise and exercise associated with pathology such as peripheral vascular disease, multiple sclerosis, and syndromes with myalgia (30, 31, 39, 53). Feedback from muscle afferents, and in particular from group III and IV muscle afferents of exercising muscles, are thought to contribute to the perceptions of pain and fatigue (16, 25, 32, 44, 48). Furthermore, central fatigue, defined as an exercise-induced attenuation of the ability to voluntarily activate a muscle to produce maximal force (10), develops, in part, because of group III and IV muscle afferent feedback (5, 11, 28, 61).

Skeletal muscle is innervated by polymodal myelinated and unmyelinated sensory receptors (group III and IV muscle afferents) with slow conduction velocities and small-diameter axons. Within both groups, animal studies have identified classes of these afferents based on their physiological properties. Some of the afferents respond to mechanical stimuli and some to metabolic stimuli (21–24, 44, 45, 48). Others that respond to mechanical stimulation can also be sensitized by metabolic stimuli, which not only increases their responses to mechanical stimuli but may also result in responses to metabolic stimuli (56, 57). With respect to fatigue, two classes of group III and IV muscle afferents have been identified in studies in mice (20, 33) and more recently humans (50). These afferents respond strongly to changes in the concentration of the products of muscle work, in particular ATP, H+, and lactate. One class responds strongly to small increases in the concentrations of these products and thus would provide feedback of the sensation of fatigue related to muscle work associated with low-to-moderate intensity, nonpainful exercise, whereas the other appears to respond strongly to higher concentrations of these products and thus would provide feedback of the sensation of pain related to muscle work associated with high-intensity and/or ischemic exercise (20, 33, 50). It should be noted that few studies have directly recorded from group III and IV muscle afferents in humans (e.g., 36), and thus most of the research on group III and IV muscle afferents in humans infers the firing of these afferents from reports of muscle pain or by analogy with studies in animals.

Several methods have been employed to examine the effects of group III and IV muscle afferents during pain and fatigue in humans. First, injection of hypertonic saline into the muscle has been used to activate group III and IV muscle afferents to examine muscle pain (e.g., 16, 25). While this method has generated sensations of pain and decreased maximal voluntary force, central fatigue has not been demonstrated (16, 29). Another method to stimulate these afferents is to maintain muscle ischemia via ligation or the use of a sphygmomanometer cuff during or after exercise. This method was initially used to examine the effects of pain related to ischemia during exercise (32, 44, 46, 48) and then later to identify a reflex that arises from muscle afferents and acts to increase blood pressure during muscle contraction (1, 6, 24, 40). Further experiments using ischemia during fatigue have suggested that afferent feedback slows motoneuron firing rates of muscles in both the upper and lower limbs and that the afferents responsible were likely fatigue-sensitive group III and IV muscle afferents (5, 12, 14, 61). However, the specific effect of these afferents in the lower limb is unclear, as the influence of fatigue without ischemia was not reported (12, 14, 61). Recently we have used this method to examine the effects of group III and IV muscle afferents during postexercise ischemia on force generation and voluntary drive in the upper limb. Our studies show that after fatiguing exercise of the elbow flexors, elbow extensors, and even a remote hand muscle (adductor pollicis), maintained

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firing of group III and IV muscle afferents reduces voluntary drive not only to the fatigued muscles but also to unfatigued muscles (the elbow flexors) within the same limb (11, 27, 28).

In contrast to methods that increase fatigue-related firing of group III and IV afferents, blocking these afferents by lumbar intrathecal injection of fentanyl has been used to examine their effects during whole body exercise (2, 4). These studies have shown that during high-intensity cycling voluntary drive and peripheral muscle fatigue increased, whereas cardiorespiratory responses decreased. This suggests that group III and IV muscle afferent feedback from the lower limbs, at least during cycling, contributes to a decrease in voluntary drive and to characteristic increases in cardiorespiratory responses. Amann and colleagues (3) have also recently used sequential bouts of fatiguing knee extension exercise to assess the effects of group III and IV muscle afferent feedback during fatigue. They concluded that feedback from group III and IV muscle afferents from the fatigued contralateral leg added together with the feedback from the subsequently fatigued ipsilateral leg to compromise endurance performance of the ipsilateral leg (3). Therefore, it is postulated that fatigue-related firing of group III and IV muscle afferents reduces the ability to activate leg muscles maximally whether the firing derives from the homonymous muscle or another muscle in the same limb (as seen in the upper limb: 27, 28), or whether afferent feedback arises from a muscle in the contralateral leg.

The current study used postexercise muscle ischemia to examine the specific effects of increased fatigue-related firing of group III and IV muscle afferents from muscles of the lower limb on voluntary drive and force production. The aims of the study were to determine whether maximal voluntary activation and maximal voluntary force of the knee extensor muscles were reduced by activity in group III and IV muscle afferents from fatigued ipsilateral knee extensor muscles, ipsilateral knee flexor muscles, or the knee extensor muscles of the contralateral leg.

MATERIALS AND METHODS

Three experiments were undertaken to determine the effects of group III and IV muscle afferents from fatigued ipsilateral and contralateral extensor muscles and ipsilateral flexor muscles of the knee on voluntary activation of the knee extensor muscles. Twelve subjects participated in experiment 1 (n = 12; mean age: 28 years; male: 6; female: 6). Three subjects from experiment 1 and eight additional subjects participated in experiment 2 (n = 11; mean age: 24 years; male: 6; female: 5). Six subjects plus two subjects from experiments 1 and 2 and two additional subjects from experiment 2 participated in experiment 3 (n = 10; mean age: 23 years; male: 8; female: 2). Some subjects from each experiment were excluded from data analysis to result in n = 9, n = 8, and n = 8 for experiments 1, 2, and 3, respectively (see Data Analysis and Statistics for details). All subjects were right leg dominant as per self-report of leg preference for kicking a ball. For each experiment, subjects attended on two separate days (“cuff” and “no-cuff” day) with at least 2 days between sessions. The Human Research Ethics Committee at the University of New South Wales approved the study, and written informed consent according to the Declaration of Helsinki.

In the first experiment, subjects performed a 2-min sustained maximal voluntary contraction (MVC) of the knee extensors followed by brief (2–3 s) knee extensor MVCs performed either during maintained ischemia of the lower limb, which prevented recovery of the muscles from fatigue, or with the muscles allowed to recover. In the second experiment, brief knee extensor MVCs were performed following a 2-min sustained MVC of the ipsilateral knee flexors, again with or without maintained ischemia of the fatigued limb. In the third experiment, brief knee extensor MVCs were performed following a 2-min sustained MVC of the contralateral knee extensors, with or without maintained ischemia of the fatigued contralateral limb. When required, rapid inflation of a standard, double-bladder adult thigh cuff using compressed air occluded circulation of the lower limb. To minimize compression to the muscles of the thigh, only the proximal bladder was inflated to a pressure of 370 mmHg. Subjects were randomly assigned to perform the cuff or no-cuff day first.

The subject was seated on a stationary chair with the leg fixed at the ankle, just above the malleoli, with a strap attached to a force transducer to measure force from the right knee extensors (linear to 1 kN; XTran, Melbourne, Australia). For study 2, the setup was similar except that an additional force transducer was attached to the anterior portion of the strap to measure force from the ipsilateral knee flexors. For experiment 3, the contralateral leg was also attached to a second force transducer to measure force from the left knee extensors similar to the setup for the right leg. For experiments 1 and 2, surface electromyogram (EMG) was acquired from the vastus lateralis and biceps femoris muscles of the right leg through Ag-AgCl electrodes (Conmed ClearTrace ECG Sensor Electrodes Utica, NY). For experiment 3, surface EMG was acquired as in experiment 1 with the addition of surface EMG from the vastus lateralis of the left leg. Electromyography was recorded at 2000 Hz and EMG data at 2000 Hz and recorded to a computer with a 16-bit A/D converter (1401, Cambridge Electronic Design) and Spike2 software (v 6.06, CED). Visual feedback of knee extensor and flexor force was provided with separate arrays of LEDs.

Femoral nerve stimulation. To determine the vastus lateralis maximal compound muscle action potential (Mmax), a constant current stimulator (model DS7AH, Digitimer, Welwyn Garden City, UK) delivered single stimuli (500-μs pulse width) through a custom-built steel dome-shaped cathode (2-cm diameter) and a self-adhesive anode electrode. The skin over the femoral nerve was marked with indelible ink. The cathode was placed over the femoral nerve and secured manually during each stimulation by the same experimenter for each session. The anode was placed 1–2 cm above the greater trochanter. For each session, the stimulus intensity was increased until the peak-to-peak amplitude of the M wave from vastus lateralis showed no further increase. The stimulus intensity (30–140 mA) was set to 150% of the current required to elicit Mmax. Throughout the rest of the study, paired stimuli (doublet) with an interstimulus interval of 10 ms were delivered. The doublets were delivered to the femoral nerve to evoke increments in force from the knee extensors during (superimposed twitch) and after (resting twitch) maximal efforts to provide a measure of voluntary activation.

Experimental Procedures

Experiment 1: sustained MVC of knee extensor muscles with and without subsequent ischemia. The subject was secured on the chair in 90° of knee flexion (0° is full extension), and 60°–70° of hip flexion (0° is full extension). For familiarization, subjects performed brief knee extensor MVCs with and without the cuff inflated. Subjects then performed 6, brief knee extensor MVCs, 3 with and 3 without the cuff inflated and 90–120 s rest between contractions to minimize fatigue. Doubles were delivered during each contraction and at rest, ~2 s after the contraction. Before the start of each brief cuff MVC, the cuff was inflated and remained so until after the resting doublet. Subjects then performed a 2-min sustained MVC of the knee extensors.
Doublets were delivered every 15 s, and a resting doublet was delivered 2 s after the termination of the contraction. All subjects were given verbal encouragement and visual feedback to provide maximal effort. On the cuff day, the cuff was inflated 5 s prior to the end of the 2-min contraction. The cuff remained inflated for 2 min while subjects performed 5 brief MVCs of the knee extensors, with the first at 15 s after the end of the sustained contraction and then every 25 s thereafter until the end of the 2 min. A doublet was delivered during each brief MVC which was followed by a resting doublet. The cuff was then deflated and subjects performed a further four contractions over the next 2 min (Fig. 1). On the no-cuff day, the procedure was the same but the cuff was not inflated after the 2-min fatiguing contraction. Subjects were asked after the cuff was deflated (cuff day) or after 2 min (no-cuff day) to rate their pain for the previous 2 min using the Borg scale (0–11).

**Experiment 2:** sustained MVC of knee flexor muscles with and without subsequent ischemia. Similar to experiment 1, the subject was secured on the chair in 60–70° of hip flexion. However, to minimize cramping of the hamstrings during the 2-min sustained MVC, the knee was placed in 60° of knee flexion (0° is full extension). For familiarization, subjects performed brief knee extensor MVCs with and without the cuff inflated and brief knee flexor MVCs. Procedures for the rest of experiment 2 were the same as for experiment 1, but instead of a sustained knee extensor MVC subjects performed a 2-min sustained knee flexor MVC without any stimulation. After the sustained flexor MVC, subjects performed multiple, brief MVCs of the unfatigued knee extensors as in experiment 1.

**Experiment 3:** sustained MVC of contralateral knee extensor muscles with and without subsequent ischemia. The subject was secured to the chair as in experiment 1 with the left leg placed in the same joint. **Fig. 1.** Details of experimental protocols. A: in **experiment 1**, on 2 days, subjects (n = 9) performed brief maximal voluntary contractions (MVCs) of knee extensors (3 with and 3 without occlusion) followed by a 2-min MVC of the knee extensors. After the sustained contraction, subjects performed five brief extensor MVCs with (“cuff” trial) or without (“no-cuff” trial) occlusion followed by four MVCs without inflation of the cuff. B and C: experiments 2 and 3 were similar to **experiment 1** except subjects performed 2-min MVCs of the right knee flexors (**experiment 2**: n = 8) or of the contralateral (left) knee extensors (**experiment 3**: n = 8) prior to brief right extensor MVCs. For each experiment, on 1 day a blood pressure cuff occluded blood flow of the fatigued muscles and so maintained firing of group III/IV muscle afferents (closed horizontal rectangle). Paired electrical stimuli (doublet) were delivered to the femoral nerve to evoke increments in force from the knee extensors during (superimposed twitch) and after (resting twitch) maximal efforts to provide a measure of voluntary activation. Arrows indicate time when stimulation was delivered.
Significance was set at 0.05. Data from some subjects were excluded from analysis, two from experiment 1, three from experiment 2 and two from experiment 3. For experiment 1, one subject was excluded because the superimposed twitches after the sustained MVC were not measurable. This subject showed an increase in biceps femoris EMG during the brief MVCs after the sustained contraction compared with EMG measured during control MVCs. All other subjects showed a decrease in biceps femoris EMG during the MVCs compared with control MVCs before the sustained contraction. This increase in antagonist muscle activity likely contributed to the lack of measurable twitches in this subject. This subject did not return for the second day of testing. Another subject was excluded from experiment 1 because of inconsistent performance between trial days during control testing. For experiment 2, one subject elected not to complete the second part of the experiment. Another subject was excluded because of poor voluntary activation (<70%) during brief control MVCs. A third subject was excluded because of inconsistent performance between trial days during control testing. For experiment 3, one subject elected not to complete the second part of the experiment, and another subject was excluded because there was no difference in voluntary activation during brief control MVCs compared with brief contractions at 90% of MVC (voluntary activation = 97 and 98%, respectively).

RESULTS

For all three experiments, knee extensor force, voluntary activation, size of the resting twitch, and EMG RMS values from vastus lateralis measured during brief control MVCs did not differ on the 2 days (Table 1), nor did they differ when the cuff was inflated. During 2-min sustained MVCs, force and EMG RMS of the exercising muscle declined a similar amount on the 2 days (Table 2). Typical superimposed twitches and resting twitches are displayed in Fig. 2.

**Experiment 1:** sustained MVC of knee extensor muscles with and without subsequent ischemia. Subjects (n = 9; mean age: 28 years; male: 5; female: 4) performed brief MVCs of the knee extensors with and without maintained ischemia (cuff/no-cuff trials) after a fatiguing extensor contraction. During the 2-min sustained MVC, voluntary activation at the end of the 2-min MVC did not differ on the 2 days (60.8 ± 17.9% and 52.1 ± 13.0%; P = 0.87).

After the 2-min extensor MVC, the next five contractions were performed during ischemic (cuff trial) or nonischemic (no-cuff trial) conditions. Mean knee extensor force during the five brief MVCs after the sustained 2-min contraction was lower in the cuff trial (14.3 ± 11.9% peak MVC) than in the no-cuff trial (66.9 ± 11.1% peak MVC), and both were reduced compared with the mean force during control MVCs (92.7 ± 3.9% peak MVC; Fig. 3A). Two-way repeated measures ANOVA examining MVC force showed significant main effects of trial (F(1.8) = 199.3, P < 0.001) and time (F(5, 40) = 133.5, P < 0.001) and a significant interaction (F(5, 40) = 49.1, P < 0.001).

<table>
<thead>
<tr>
<th>Trial Day</th>
<th>No cuff</th>
<th>Cuff</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
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<tr>
<td>Knee extensor force</td>
<td>74.6 ± 13.7%</td>
<td>69.2 ± 12.0%</td>
<td>0.19</td>
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<tr>
<td>Vastus lateralis EMG RMS</td>
<td>51.1 ± 39.6%</td>
<td>71.9 ± 11.2%</td>
<td>0.08</td>
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<tr>
<td><strong>Experiment 2</strong></td>
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<td></td>
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<tr>
<td>Knee flexor force</td>
<td>66.4 ± 17.8%</td>
<td>59.9 ± 14.7%</td>
<td>0.31</td>
</tr>
<tr>
<td>Biceps femoris EMG RMS</td>
<td>25.6 ± 37.1%</td>
<td>32.2 ± 26.0%</td>
<td>0.68</td>
</tr>
<tr>
<td><strong>Experiment 3</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Left knee extensor force</td>
<td>77.4 ± 10.6%</td>
<td>76.2 ± 10.7%</td>
<td>0.58</td>
</tr>
<tr>
<td>Left vastus lateralis EMG RMS</td>
<td>53.2 ± 32.6%</td>
<td>68.9 ± 18.4%</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Values are means ± SD.

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**Table 1. Force, voluntary activation, resting twitch, and EMG RMS values for knee extenders during control maximal contractions of the right knee extensors for experiments 1, 2, and 3**

<table>
<thead>
<tr>
<th>Trial Day</th>
<th>No cuff</th>
<th>Cuff</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Force, % peak MVC</td>
<td>92.4 ± 1.9</td>
<td>92.9 ± 2.0</td>
<td>0.64</td>
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<tr>
<td>Voluntary activation, %</td>
<td>93.7 ± 5.6</td>
<td>90.5 ± 7.5</td>
<td>0.14</td>
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<tr>
<td>Resting twitch, % peak MVC</td>
<td>55.0 ± 18.1</td>
<td>57.0 ± 9.0</td>
<td>0.62</td>
</tr>
<tr>
<td>EMG RMS, % Mmax</td>
<td>5.2 ± 1.3</td>
<td>4.7 ± 0.8</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Force, % peak MVC</td>
<td>91.9 ± 3.2</td>
<td>93.3 ± 2.7</td>
<td>0.41</td>
</tr>
<tr>
<td>Voluntary activation, %</td>
<td>82.8 ± 6.6</td>
<td>84.1 ± 7.2</td>
<td>0.28</td>
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<tr>
<td>Resting twitch, % peak MVC</td>
<td>50.0 ± 11.4</td>
<td>49.6 ± 20.2</td>
<td>0.94</td>
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<tr>
<td>EMG RMS, % Mmax</td>
<td>4.6 ± 1.1</td>
<td>4.4 ± 1.9</td>
<td>0.65</td>
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<tr>
<td><strong>Experiment 3</strong></td>
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<tr>
<td>Force, % peak MVC</td>
<td>96.2 ± 3.2</td>
<td>94.1 ± 3.7</td>
<td>0.17</td>
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<tr>
<td>Voluntary activation, %</td>
<td>93.12 ± 4.6</td>
<td>92.7 ± 5.2</td>
<td>0.79</td>
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<tr>
<td>Resting twitch, % peak MVC</td>
<td>41.2 ± 9.7</td>
<td>44.1 ± 13.0</td>
<td>0.29</td>
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<tr>
<td>EMG RMS, % Mmax</td>
<td>5.8 ± 1.7</td>
<td>5.9 ± 1.9</td>
<td>0.8</td>
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</table>

Values are means ± SD. EMG RMS, root mean square electromyogram; MVC, maximal voluntary contraction; Mmax, maximal compound muscle action potential.
Mean voluntary activation of the knee extensors during the 5 brief MVCs after the sustained 2-min contraction was also lower in the cuff trial (47.1 ± 18.8%) than in the no-cuff trial (86.8 ± 7.7%; Fig. 3B). Two-way repeated measures ANOVA examining voluntary activation showed significant main effects of trial (F(1,8) = 97.7, P < 0.001) and time (F(5,40) = 17.7, P < 0.001) and a significant interaction (F(5,40) = 10.92, P < 0.01). In the cuff trial, mean voluntary activation during the five brief MVCs after 2-min contraction was lower than the mean voluntary activation during control MVCs (P > 0.001). Post hoc analysis using Dunnett’s test was used to compare voluntary activation for each of the brief contractions with the mean voluntary activation during control MVCs. This analysis showed that voluntary activation during each of the brief MVCs was lower than voluntary activation during control MVCs (P < 0.01). In the no-cuff trial, mean voluntary activation during the brief MVCs after the 2-min contraction was also lower than the mean voluntary activation during control MVCs, but was of borderline significance (P = 0.05). Post hoc analysis showed that voluntary activation during each of the brief MVCs was not different to that during control MVCs (P = 0.06 to 0.83). As expected, mean EMG RMS for vastus lateralis for the five brief MVCs after 2-min contraction was lower in the cuff trial (1.39 ± 0.79% Mmax) than in the no-cuff trial (3.67 ± 1.05% Mmax). Two-way repeated measures ANOVA examining EMG RMS for vastus lateralis showed significant main effects of trial (F(1,8) = 60.4, P < 0.01) and time (F(5,40) = 22.5, P < 0.001) and a significant interaction (F(5,40) = 7.0, P < 0.01).

The mean size of the resting twitch of the knee extensors following each of the 5 brief MVCs after the sustained 2-min contraction was smaller in the cuff trial (36.0 ± 9.7% vs. 42.2 ± 16.1%, expressed as a percent of peak MVC force during control contractions; Fig. 3C). Two-way repeated measures ANOVA showed no significant differences between the size of the resting twitch in the two trials (F(1,8) = 3.1, P = 0.12). However, there was a significant effect over time (F(5,40) = 34.4, P < 0.01), and a significant interaction (F(5,40) = 10.8, P < 0.01). Pairwise contrasts between the cuff and no-cuff trials of twitches after the sustained MVC at each time point showed significant decreases (P < 0.05) for the third, fourth, and fifth twitches after the 2-min knee flexor MVC.

Pain perception on the Borg scale (0–11) during the cuff trial ranged from 7.5 (very strong) to 11 (extremely strong) with a mean rating of 9.3 (very strong to extremely strong). Pain was localized to the right thigh. Perceived pain during the no-cuff trial was rated from 0 (nothing at all) to 5 (strong) with a mean rating of 2.2 (weak).

Experiment 2: sustained MVC of knee flexor muscles with and without subsequent ischemia. Subjects (n = 8; mean age: 24 years; male: 4; female: 4) performed brief MVCs of the knee extensors with and without maintained ischemia (cuff/no-cuff trials) after a fatiguing ipsilateral knee flexor MVC. During the 2-min sustained knee flexor MVC, on the cuff and no-cuff trial days, EMG RMS of vastus lateralis decreased, respectively, from 4.4 ± 1.9% and 4.6 ± 11% Mmax during control MVCs of the knee extensors to 0.4 ± 0.3% and 0.4 ± 0.2% Mmax at the start of the 2-min flexor contraction and remained low throughout the rest of the contraction.

After the 2-min flexor MVC, the next five contractions were performed during ischemic (cuff trial) or nonischemic (no-cuff trial) conditions. Mean knee extensor force during the 5 brief MVCs after the sustained 2-min contraction was lower in the
cuff trial (67.6 ± 22.8% peak MVC) than in the no-cuff trial (95.7 ± 9.8% peak MVC) with the cuff trial force reduced compared with the mean force during control MVCs (92.6 ± 3.0% peak MVC; Fig. 4A). Force showed significant main effects of trial ($F_{1,7} = 10.3, P < 0.05$) and time ($F_{5,35} = 5.4, P < 0.05$) and a significant interaction ($F_{5,35} = 11.9, P < 0.01$). Pairwise contrasts between the cuff and no-cuff trials were significant ($P < 0.01$) except for the first brief MVC immediately after the 2-min knee flexor MVC ($P = 0.12$).

Mean voluntary activation of the knee extensors during the 5 brief MVCs after the 2-min flexor MVC was also lower in the cuff trial (95.7 ± 9.8% peak MVC) with the cuff trial force reduced compared with the mean force during control MVCs (92.6 ± 3.0% peak MVC; Fig. 4A). Force showed significant main effects of trial ($F_{1,7} = 10.3, P < 0.05$) and time ($F_{5,35} = 5.4, P < 0.05$) and a significant interaction ($F_{5,35} = 11.9, P < 0.01$). Pairwise contrasts between the cuff and no-cuff trials were significant ($P < 0.01$) except for the first brief MVC immediately after the 2-min knee flexor MVC ($P = 0.12$).

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cuff trial (59.0 ± 20.6%) than in the no-cuff trial (79.1 ± 8.9%; Fig. 4B). Voluntary activation showed significant main effects of trial ($F_{1,7} = 15.2, P < 0.01$) and time ($F_{5,35} = 8.5, P < 0.01$) and a significant interaction ($F_{5,35} = 11.1, P < 0.01$). Pairwise contrasts between the cuff and no-cuff trials were significant ($P < 0.01$) except for the first brief MVC immediately after the 2-min knee flexor MVC ($P = 0.11$). In contrast to experiment 1, EMGRMS for vastus lateralis showed no effect of trial ($F_{1,7} = 2.9, P = 0.13$) but a significant effect of time ($F_{5,35} = 8.0, P < 0.01$), but no interaction ($F_{5,35} = 2.5, P < 0.12$). Post hoc analysis showed that during the cuff trial, EMGRMS during the fourth and fifth brief MVC after the 2-min contraction was significantly lower than that during control MVCs ($P < 0.05$). For the no-cuff trial, EMGRMS during each of the five contractions was not different from that during control contractions ($P > 0.29$).

The mean size of the resting twitch of the knee extensors following each of the five brief MVCs after the 2-min flexor contraction showed no significant differences between the two trials ($F_{1,7} = 4.6, P = 0.07$; Fig. 4C). However, there was a significant effect over time ($F_{5,35} = 10.7, P < 0.001$), but not a significant interaction ($F_{5,35} = 2.18, P < 0.15$). Post hoc analysis showed that twitches after each of the five contractions were not different to those after the control contractions for both the cuff trial ($P > 0.58$) and the no-cuff trial ($P > 0.75$).

Ratings of pain during the cuff trial ranged from 3.5 (moderate) to 10 (extremely strong) with a mean rating of 6.3 (strong to very strong). Pain was localized to the right thigh. Perceived pain during the no-cuff trial was rated from 0 (nothing at all) to 3 (moderate) with a mean rating of 1.1 (very weak).

**Experiment 3: sustained MVC of contralateral knee extensor muscles with and without subsequent ischemia.** Subjects ($n = 8$; mean age: 23 years; male: 6; female: 2) performed brief MVCs of the knee extensors with and without maintained ischemia (cuff/no-cuff trials) of the contralateral (left) leg after a fatiguing extensor contraction of that leg. During the 2-min sustained contralateral (left) MVC, on both the cuff and no-cuff trial days, EMGRMS of the right (nonexercising) vastus lateralis decreased respectively from 5.8 ± 1.9% and 5.9 ± 1.7% Mmax during control MVCs of the right knee extensors to 0.8 ± 0.7% and 0.6 ± 0.5% Mmax at the start of the 2-min contralateral (left) extensor MVC and remained low throughout the rest of the contraction.

After the 2-min contralateral (left) MVC, the next five contractions were performed with the nonexercised right leg during ischemic (cuff trial) or nonischemic (no-cuff trial) conditions of the contralateral (left) leg. There were no effects of trial, time, or an interaction for mean knee extensor force of the nonexercised right leg after the sustained contralateral MVC ($F_{1,7} = 0.5, P = 0.48, F_{5,35} = 2.3, P = 0.13, F_{5,35} = 1.4, P = 0.27$, respectively; Fig. 5A), nor for mean voluntary activation ($F_{1,7} = 0.2, P = 0.65, F_{5,35} = 2.1, P = 0.15, F_{5,35} = 1.1, P = 0.36$, respectively), nor mean vastus lateralis EMGRMS ($F_{1,7} = 1.4, P = 0.28, F_{5,35} = 2.5, P = 0.13, F_{5,35} = 0.6, P = 0.59$, respectively; Fig. 5A). The mean size of the resting twitches of the knee extensors after the 2-min contralateral MVC showed no significant differences between the two trials ($F_{1,7} = 3.7, P = 0.10$; Fig. 5C). However, there was a significant effect over time ($F_{5,35} = 12.6, P < 0.01$) but not a significant interaction ($F_{5,35} = 4.9, P = 0.6$). Post hoc analysis showed that twitches after each of the brief contractions were not different to those after control contractions for either the cuff trial ($P > 0.95$) or the no-cuff trial ($P > 0.44$).

Ratings of pain during the cuff trial ranged from 7 (moderate) to 9 (very strong to extremely strong) with a mean rating...
of 6.3 (strong to very strong) for the left leg. Pain was localized to the left thigh. Perceived pain for the right leg during the 2 min of ischemia of the left leg ranged from 0 (nothing at all) to 1.5 (very weak to weak) with a mean rating of 0.4 (extremely weak). Perceived pain for the left leg during the no-cuff trial was rated from 0 (nothing at all) to 2 (weak) with a mean rating of 0.9 (very weak). Perceived pain for the right leg during the no-cuff trial ranged from 0 (nothing at all) to 2 (weak) with a mean rating of 0.4 (extremely weak).

**DISCUSSION**

The main findings of this study were that voluntary activation and maximal voluntary force of the knee extensors were reduced >45% when activity of group III and IV muscle afferents from fatigued knee extensor muscles was maintained and >25% when group III and IV muscle afferent activity was maintained from fatigued knee flexor muscles. However, we found no crossover of fatigue when the contralateral knee extensor muscles were fatigued prior to brief knee extensor MVCs. Furthermore, maintained firing of group III and IV muscle afferents from the contralateral homologous muscle did not alter voluntary activation of the unfatigued knee extensor muscles or their maximal voluntary force.

In these studies, muscle afferent activity was not measured directly. However, the critical comparison in these studies is between voluntary activation during MVCs that were performed after the fatiguing 2-min contraction either with or without maintained ischemia. Reports of high levels of muscle pain strongly suggest that muscle nociceptive afferents fired more with circulatory occlusion of the fatigued muscles than when normal circulation was allowed. Furthermore, during maintained ischemia after exercise, metabolites from the muscle are restricted to the occluded limb and thus are not available to contribute to any nonafferent-mediated feedback to alter voluntary activation. In addition, previous studies have documented that blood pressure remains high during postexercise occlusion, and this is attributed to reflex cardiovascular actions of group III and IV muscle afferents (47, 59). Taken together, the use of circulatory occlusion to maintain high metabolite concentrations after maximal fatiguing contractions, the high pain responses from our subjects, and previous work on cardiovascular responses, it is highly likely that enhanced group III and IV muscle afferent feedback from the fatigued muscle is the primary mechanism underlying the decrease in voluntary activation during postexercise occlusion of blood flow.

**Effects on fatigued homonymous muscles.** The hallmark of central fatigue is a progressive decline in voluntary drive to the muscle, and it is accompanied by reduced motoneuron firing rates or in some instances the cessation of firing of motoneurons (10, 49). While many factors can contribute to central fatigue, there is strong evidence that feedback, originally described as a reflex, from fatigue-sensitive afferents contributes to modulation of motoneuron output (5, 12, 13, 17, 61). Our data indicate that significant central fatigue occurred when firing of group III and IV muscle afferents was maintained via ongoing ischemia after a fatiguing contraction of the knee extensors. Voluntary activation was reduced by as much as ~48% and maximal voluntary force by ~79% compared with when the muscles recovered with normal postexercise perfusion. These findings are consistent with a similar development of central fatigue for both the elbow flexor muscles and adductor pollicis when firing of homonymous group III and IV muscle afferents was maintained by postexercise ischemia (11, 27, 28). Because we used peripheral nerve stimulation to determine voluntary activation in the current study, we cannot determine at what level in the motor pathway group III and IV muscle afferent effects occurred. However, it is likely that these afferents act at a supraspinal level to affect the amount of voluntary drive to the muscle, though not necessarily at the site of the motor cortical output neurons. We have shown previously that group III and IV muscle afferents act at a supraspinal level to reduce voluntary activation of the elbow flexors (11, 27, 28), in spite of their direct facilitation of the elbow flexor motoneurons (38). For leg muscles, evidence that group III and IV muscle afferent input does not reduce motoneuronal output through actions at the spinal level comes from Sidhu and colleagues (58). They have recently reported that the responsiveness of the motoneuron pool of vastus lateralis was unchanged during cycling to exhaustion and at task failure. Similarly, we have briefly reported that responses elicited in vastus lateralis by descending tract (corticospinal) stimulation are unchanged by a 2-min maximal knee extension contraction and subsequent postexercise ischemia in a similar protocol to that used in the current study (26). Although group III and IV muscle afferents may not affect the excitability of the motoneuron pool directly, they may do so indirectly by presynaptic inhibition of Ia afferents (8, 54, 55), and thus reduction of reflex facilitation of the motoneurons (34, 35). In turn, this would mean more descending drive would be needed to drive the motoneurons at the same rate. However, the importance of this effect is unclear given the profound fatigue-related reduction of motoneuron excitability due to activity-dependent changes to the intrinsic properties of the motoneurons of the exercising limb (41). Indeed, in the brief absence of voluntary drive caused by the silent period after a transcranial magnetic stimulus, cervicomedullary motor evoked potentials are abolished within 30 s of fatiguing contraction (42). On the whole, it would appear that group III and IV muscle afferent feedback at the spinal level does little to alter the responsiveness of the motoneuron pool. Hence, we propose that like group III and IV muscle afferents of the upper limb, group III and IV muscle afferents from fatigued knee extensor muscles likely act at a supraspinal level to reduce voluntary drive to the motoneurons to such an extent as to also reduce force of the fatigued muscle.

**Effects on unfatigued agonist muscles from fatigued antagonist muscles.** In previous work, we have demonstrated that maintained firing of group III and IV muscle afferents from fatigued muscles (elbow extensors and adductor pollicis) can affect voluntary activation and maximal force of unfatigued elbow flexors of the same limb (27, 28). After a fatiguing contraction of the antagonist elbow extensors and subsequent ongoing ischemia to maintain firing of group III and IV muscle afferents, voluntary activation of the unfatigued agonist elbow flexors was reduced ~14%. We show a similar, though greater reduction in voluntary activation of the knee extensors (~25%) when firing of group III and IV muscle afferents was maintained from the fatigued antagonist flexors of the knee. In addition, the reduction of voluntary activation and force of the knee extensors during maintained group III and IV muscle afferent firing after the flexor MVC showed a similar time course to that observed in the upper limb. For the first MVC
after the fatiguing contraction, voluntary activation and force were no different during ongoing ischemia compared with when the muscle was allowed to recover. However, by the second MVC (~40 s after the termination of the 2-min MVC) voluntary activation and force were lower with than without ongoing ischemia, and remained low until blood flow was restored. Given the use of postexercise ischemia to investigate fatiguing, painful exercise (1, 15, 25, 32), and the high pain ratings reported by participants, it is likely that group III and IV nociceptors were responsible for the changes in voluntary drive. Nociceptive group III and IV muscle afferents respond to high concentrations of muscle metabolites produced by high-intensity, fatiguing contractions, which in the current experiment would not have been cleared during postexercise occlusion and resulted in the “strong to very strong” sensations of pain. The delayed effects of muscle nociceptors may be explained by animal studies investigating the mechanisms underlying referred pain. Muscle nociceptors may excite centrally projecting neurons in the dorsal horn by spatial and temporal summation. These neurons could then convey nociceptive signals to higher centers in the absence of noxious input from their primary muscle (18, 19, 43). This “referred” input could then lead to the slightly delayed decrease in voluntary activation. Indeed, we have observed a similar time course for changes in voluntary activation and force for the elbow flexors after a fatiguing antagonist contraction (28). Thus for both the upper and lower limbs, group III and IV muscle nociceptors from fatigued antagonist muscles act to reduce voluntary drive and force of unfatigued agonist muscles of the same limb.

Effects on unfatigued homologous muscles from fatigued contralateral muscles. Although we have shown that fatigue-related firing of group III and IV muscle afferents from one muscle can affect other muscles of the same limb (27, 28), there is little definitive data on the spread of central fatigue from an exercised limb to an unexercised limb. In the upper limb, a slight decrease in voluntary activation occurs after fatigue of the contralateral homologous muscle although this does not result in a concomitant decrease in force production (51, 60, 62). Thus in the upper limb, there appears to be a negligible crossover effect of fatigue. However, for the lower limb there are more mixed reports. With a single bout of fatiguing exercise, a slight but significant crossover effect was found, albeit more so in males than in females (9 vs. 3% decrease, respectively) (37, 52). Given this sex difference, further investigations using male subjects have shown decreased voluntary activation and force with contralateral fatiguing contractions only after a second bout of fatiguing isometric exercise (7), and no crossover effect on power output of the rested leg was demonstrated after a 10-min maximal unilateral cycling time trial of the other leg (9). More recently, Amann and colleagues (3) have shown reduced central motor drive, as measured by integrated EMG responses and force production during fatiguing knee extension exercise after fatigue of the contralateral leg. They proposed that changes in central motor drive and force arise from inhibitory feedback from the ensemble firing of group III and IV muscle afferents generated during and after the fatiguing contractions of both legs. Thus although crossover effects of fatigue may depend on gender, muscle, and task, sustained firing of group III and IV afferents from contralateral muscles has been postulated to reduce force production and voluntary activation. In the current study, our data clearly show that voluntary drive and force are not reduced after a 2-min fatiguing contraction of the contralateral leg. Even if the two female subjects in the current study are removed from analysis, no reduction in voluntary activation or force generation are seen. It is possible that longer or successive bouts of exercise may be needed to reveal a crossover of fatigue, although this is not clear since single bouts of slightly shorter duration exercise (100 s) than that in the present study (120 s) have previously resulted in some crossover of central fatigue (37, 52). Importantly, in the present study when group III and IV muscle afferent firing was maintained during postexercise ischemia, voluntary drive and force were unchanged. Hence, any crossover of central fatigue that may occur is very unlikely to be due to group III and IV muscle afferent input.

In conclusion, after a fatiguing lower limb contraction, activity of group III and IV muscle afferents can act to reduce voluntary activation and, hence, force production of the fatigued muscle and of a nonfatigued antagonist muscle in the same limb. However, continued firing of group III and IV muscle afferents from the contralateral leg had no effect on the unfatigued leg. This suggests that group III and IV muscle afferents do not mediate a crossover effect on central fatigue in the lower limbs.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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