

Sex differences in human skeletal muscle fatigue are eliminated under ischemic conditions

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Russ, David W., and Jane A. Kent-Braun. Sex differences in human skeletal muscle fatigue are eliminated under ischemic conditions. *J Appl Physiol* 94: 2414–2422, 2003. First published January 31, 2003; 10.1152/jappphysiol.01145.2002.—Several studies have suggested that women may be more resistant to muscle fatigue than men (Fulco CS, Rock PB, Muza SA, Lammi E, Cymerman A, Butterfield G, Moore, LG, Braun B, and Lewis SF. *Acta Physiol Scand* 167: 233–239, 1999) possibly because of differences in muscle oxidative metabolism. We evaluated muscle fatigue produced by intermittent, maximal volitional isometric contractions of the dorsiflexor muscles of healthy young (21–34 yr) men ($n = 8$) and women ($n = 8$) under two conditions: free-flow (FF) circulation and ischemia. Measures of voluntary and stimulated (10- and 50-Hz) force, central activation ratio (CAR), and compound muscle action potential (CMAP) were collected in each session. The ischemic protocol induced greater fatigue than the FF protocol, in both sexes, and was associated with greater reductions in CAR, CMAP, stimulated force, and the ratio of 10- to 50-Hz force compared with the FF condition. Women fatigued less than men in FF but not during ischemia, and this difference was roughly paralleled by a difference in CAR. No sex effects on the CMAP, tetanic force, and measures of excitation-contraction coupling function were found in the FF condition, suggesting that the primary mechanism behind the difference in fatigue was a relatively greater impairment of central activation in men. The observation that ischemia eliminated the sex differences in fatigue is consistent with a number of studies (Kent-Braun JA, Ng AV, Doyle JW, and Towse TF. *J Appl Physiol* 93: 1813–1823, 2002) relating fatigue to muscle metabolism and might be the result of sex-based differences in metabolic pathway utilization during muscle contraction.

blood flow; central fatigue; muscle activation

MUSCLE FATIGUE MAY BE THE result of impairments at any of a number of sites within the neuromuscular system (1, 11), which manifests as a decline in the maximum force-generating capacity of the muscle. One of the factors contributing to the complexity of fatigue is its task-specific nature, which means that the mechanisms underlying the decline in force are likely to vary depending on the nature of the activity associated with the fatigue (9).

In addition to variations in the extent and mechanisms of fatigue with differences in task performance, recent studies have suggested that human skeletal

muscle fatigue may be influenced by sex (17). The majority of these studies have shown that women exhibit greater resistance to fatigue (endurance time) than men during submaximal, isometric contractions (12, 13, 21, 27, 35). Moreover, the results of other studies have suggested that the greater fatigue resistance of women declines as the intensity of the contraction increases (7, 27), although in at least one study women showed persistent increases in endurance times compared with men during maximal isometric contractions (18).

The observation that sex-related differences in fatigue may disappear as the force of the fatiguing contractions increases has led to the suggestion that these differences in fatigue are largely due to differences in muscle mass between men and women (17). Men typically have greater muscle mass than women and, as a result, produce greater absolute forces during muscle contraction than do women, even when submaximal contractions are performed at the same relative force (i.e., at the same percentage of maximal force). These higher absolute forces in men vs. women generate a greater metabolic demand and may produce greater mechanical compression of the vascular bed. Both the greater metabolic demand and reduced availability of oxygen would be expected to increase the reliance on anaerobic metabolic pathways in men. Because the by-products of these pathways (H^+ , P_i , $H_2PO_4^-$) have been correlated to fatigue (2, 15, 24), one would expect to see greater fatigue in men relative to women.

If differences in muscle mass and force do not explain fully the difference between sexes with regard to fatigue, differences in metabolic capacity may. It appears, from studies of whole body exercise (38) and isolated whole muscle metabolism (26), that men may rely on glycolytic pathways of metabolism to a greater extent than women, whereas women have a greater capacity for utilizing oxidative metabolism, thus reducing the reliance on glycolytic pathways.

Thus, although the mechanisms underlying gender differences in fatigue remain unclear, there is recent evidence suggesting that these differences are related to an “oxidative advantage” of women relative to men (26). The goal of the present study was to examine sex-specific differences in fatigue under conditions of

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free-flow circulation (FF) and ischemia (I). Operating under the assumption that sex-related differences in fatigue are related to a preferential utilization of oxygen by women, we hypothesized that women would exhibit less fatigue than men in the FF condition but that women and men would fatigue equally during I. The I condition would negate the oxidative advantage of female subjects, whether it was due to a higher oxygen delivery or a greater use of oxidative phosphorylation. In addition, this oxidative advantage would, in theory, decrease the reliance on glycolytic pathways in women. It has been suggested that the by-products of these pathways may inhibit central motor drive (3, 24, 32). Thus we also hypothesized that we would observe greater central activation failure in men than in women in FF but not I conditions.

METHODS

This study was designed to make comparisons between men and women under different circulatory conditions: FF circulation and I. The mechanical (force) and electrical [surface electromyogram (EMG)] responses of the ankle dorsiflexor muscle group to a series of voluntary and stimulated contractions were recorded before, each minute during, and 0, 2, 5, and 10 min after the end of the fatigue protocols. The combination of force and EMG data allowed us to make measurements of voluntary and stimulated force, contractile properties, and central and peripheral activation of the muscle (24).

Subjects. Sixteen (8 men, 8 women) healthy, nonsmoking volunteers, aged 20–34 yr, participated in this study. Subjects were free from orthopedic, neurological, or vascular impairments of the lower extremity, and none were currently on any medications. All subjects were informed of the purpose and procedures of the study and gave written, informed consent to their participation, as approved by the University of Massachusetts Human Subjects Review Board. Each subject was asked to keep an activity record for the 7 days between the two protocols. No specific activity criteria were established with regard to inclusion in the study, but subjects were encouraged to maintain their customary level of activity during the period between tests. In addition, subjects were instructed to refrain from any strenuous exercise for 24 h before the experimental sessions. After finishing the second experimental session, all subjects completed the Stanford Seven-Day Physical Activity Questionnaire (SPAQ) (34), the score of which was expressed in kilocalories per kilogram per day.

Experimental sessions. Subjects participated in one habituation session before the first of two fatigue study sessions. During this session, a pair of stimulating electrodes was placed roughly longitudinally along the peroneal nerve, just posterior to and ~1 cm distal to the right fibular head, taped in place, and further stabilized with a foam pad strapped over the electrodes. An EMG recording electrode was taped to the belly of the tibialis anterior (TA) muscle, and the corresponding reference electrode was taped to the tendon of the TA on the anterior portion of the talocrural joint. To minimize the stimulus artifact, a copper ground plate was placed over the calf midway between the stimulating and recording electrodes. All electrodes were 10-mm, gold-plated disks, applied with conductive gel.

Once the electrodes were in place, subjects lay supine with the trunk supported and the right leg in a custom-built apparatus designed to measure dorsiflexion force. The knee

was kept at ~0° flexion, and the foot was positioned at a fixed angle of ~120° to the shank. The foot was held flat to the footplate of the leg apparatus with an inelastic strap pulled tightly across the foot, just below the metatarsal-phalangeal joints. The right lower limb was stabilized through the use of a knee splint and inelastic straps with Velcro closures.

Stimulation intensity was determined by monitoring the amplitude of the compound muscle action potential (CMAP) in response to single pulses (200- μ s duration) from a constant current stimulator (model DS7A, Digitimer, Hertfordshire, UK). Intensity was increased incrementally until an increase in voltage produced no increase in CMAP amplitude. The voltage was then increased by 15% to give a supramaximal intensity, which was maintained for all subsequent stimuli.

Subjects then practiced maximal volitional isometric contractions (MVICs) of the dorsiflexor muscles and were acclimated to the supramaximal electrical stimulation of this muscle group. They were trained to perform MVICs during the administration of a 50-Hz stimulation train, delivered for the purpose of assessing central activation (see *Central activation measurements*, below). Subjects were also taught to relax during other electrical stimulation trains, which were used to evaluate stimulated force and contractile properties. All stimulation trains were 500 ms in duration.

At least 48 h after the habituation session, subjects began the first of the two fatigue sessions, which were separated by at least 7 days. The fatigue sessions were conducted at approximately the same time of day (± 1 h) for each subject. Electrode placement, subject positioning and determination of stimulus intensity were conducted in exactly the same manner as during the habituation session. Once the stimulation intensity was set, three single twitches, separated by 1 min, were delivered to the subject. Next, the subject performed three MVICs, each separated by 2 min. During the final MVIC, a 50-Hz train was delivered to the muscle (see *Central activation measurements*, below). Approximately 2 s after the completion of the final MVIC, a single pulse was delivered to the muscle to produce a potentiated twitch. After a further 2 min, a 50-Hz train was delivered to the relaxed muscle, followed by 2 min of rest and a 10-Hz train.

The subject rested for ~5 min and then began one of the two fatiguing protocols. These protocols were identical, except that one was performed under conditions of open, FF circulation and the other was performed under I conditions. I was induced through the use of a pneumatic thigh cuff that was inflated to ~230 mmHg with a Hokanson E20 rapid cuff inflator (Seattle, WA), just before (≤ 10 s) the fatiguing exercise was started. In a magnetic resonance spectroscopy study using a comparable setup, no postcontractile PCr recovery was seen during the application of the same pressure, indicating that blood flow was completely occluded (33). The order in which these protocols were performed was randomly determined for each subject. Each protocol consisted of 4 min of 5-s MVICs, with a 5-s rest between contractions (24 total contractions). At the end of *minutes 1, 2, and 3* during each protocol, a single stimulus was delivered during the 5-s rest period. A 50-Hz train was delivered during the twelfth and final MVIC (see *Central activation measurements*, below). Immediately (≤ 3 s) after the final MVIC, the subject received a single pulse, a 50-Hz train, and a 10-Hz train, in that order. In the case of the I protocol, the pressure in the thigh cuff was released immediately after the 10-Hz train. At 2 and 5 min after the end of the fatigue protocol, the subject performed a MVIC, and received a single pulse, a 50-Hz train, and a 10-Hz train. At 10 min after the end of the fatigue protocol, the same sequence was administered, except that a 50-Hz

train was delivered during the MVIC. All subjects completed both protocols.

Force measurements: Force was recorded by using an Interface SSM-AJ-250 force transducer (Interface, Scottsdale, AZ), which was built into the leg apparatus. The raw voltage signal was amplified (SGA strain-gauge transducer amplifier, Interface) and converted to a digital signal (PCI MIO 16E-4 analog-to-digital board, National Instruments, Austin, TX). This signal was recorded by using customized LabView (National Instruments) software. All MVIC force was sampled at 500 Hz. During the fatiguing protocol, force was monitored continuously at 40 Hz. All of the stimulated contractions (twitch, 10 Hz, and 50 Hz) were sampled at 2,500 Hz.

The highest force value recorded during the three pre-fatigue MVICs was reported as the baseline value. The subsequent MVIC peak forces were expressed as a percentage of the baseline value, and we defined fatigue as postexercise MVIC/baseline MVIC. Similarly, the 10- and 50-Hz peak forces were expressed relative to their respective baseline values, and the twitch peak force was expressed relative to the potentiated twitch force.

Ratios of the 10- to 50-Hz (10-Hz/50-Hz force) and twitch-to-50-Hz peak forces (twitch/50-Hz force) were calculated to evaluate low-frequency fatigue (LFF), which is thought to indicate an impairment of excitation-contraction coupling (ECC) (23). The ratio of 10-Hz/50-Hz force was chosen rather than the 20- to 50-Hz force ratio previously described in the quadriceps (8, 23), because the dorsiflexors exhibit slower contractile properties (4, 6), and thus a lower frequency should more accurately reflect LFF in this muscle group.

Contractile properties. Measurements of the maximal rates of force development (+df/dt) and relaxation (-df/dt) were calculated from the force responses to the 50-Hz trains. The +df/dt and -df/dt were expressed as percentages of the peak force produced during the contraction (%peak force/ms) to account for the effect of peak force on the rate of force production (29). The calculations were performed in Excel (Microsoft, Redmond, WA), as previously reported (24).

Central activation measurements: Central activation was determined by using the CAR, as previously described (25). Briefly, a supramaximal 50-Hz stimulation train was delivered during the subject's MVIC. The CAR equaled the voluntary force produced before the delivery of this train divided by the force produced during the train, if any increase in force was observed. In the absence of any increase in force, CAR equaled 1, and any value <1 indicated a failure of central activation (25, 37). A CAR was acquired at baseline, 2 min into the fatigue protocol, at the end of the fatigue protocol, and 10 min after the end of the fatigue protocol.

Peripheral activation measurements. Changes in peripheral activation were assessed via changes in the CMAP, which reflects the excitability of the neuromuscular junction and sarcolemma. The CMAP was recorded during the twitch responses described above. The EMG response was amplified (model P55 AC amplifier, Astro-Med, Warwick, RI), converted to a digital signal, recorded, and stored by using customized software. The peak-to-peak amplitude (mV) and integrated area of the negative peak (mV·ms) were calculated from these data.

Statistical analysis. All data are presented as means \pm SE, unless otherwise noted. All statistical analyses were performed by using SAS software, and statistical significance was established at $P \leq 0.05$. Comparisons of the subject characteristics (Table 1) were made by using unpaired *t*-tests. Tests for differences in the baseline measurements

Table 1. *Subject characteristics*

	Men	Women
Age yr	27 \pm 4	24 \pm 4
Height, cm	179 \pm 5*	162 \pm 5
Weight, kg	74 \pm 5*	56 \pm 5
Physical activity, kcal·kg ⁻¹ ·day ⁻¹	37.3 \pm 4.9	36.5 \pm 4.7

Values are means \pm SD. *Significantly different from women, $P < 0.05$.

were made by using a mixed-model, two-way repeated-measures [sex \times condition (FF vs. I)] ANOVA.

To test for changes in the dependent variables during exercise and recovery, mixed-model three-way (sex \times condition \times time) ANOVAs, where condition and time were repeated factors, were performed for each variable. In the event of a significant main effect of time, Dunnett's test was used within each group (i.e., men-FF, women-I, and so forth) to determine when significant changes from baseline in a given variable were present. In the event of significant effects of sex or condition, subsequent two-way ANOVAs (sex \times condition) were performed at each time point to determine where these effects existed. Unpaired *t*-tests were used post hoc in the event of any significant sex \times condition interactions.

The CAR measurement was tested nonparametrically due to its ceiling effect. We hypothesized a priori that men would demonstrate greater declines in CAR than women in the FF condition but not during I. We therefore used separate, two-sample Wilcoxon rank-sum tests to determine the effects of sex and condition.

Finally, we performed correlational analyses on the relationships between initial MVIC force and fatigue, and between fatigue and postexercise (fatigued) CAR, in the FF and I conditions.

RESULTS

All of the subjects reported being right-leg dominant. No differences were found between men and women in daily physical activity as indicated by the SPAQ (Table 1). In addition, the range of activity values was similar for men (32.5–46.8 kcal·kg⁻¹·day⁻¹) and women (32.3–46.2 kcal·kg⁻¹·day⁻¹). All subjects reported that their activity over the period covered by the SPAQ was the same (5 men, 6 women) or less (3 men, 2 women) than their activity over the previous 3 mo. The men were heavier and taller than the women (Table 1) and produced significantly greater MVIC and 50-Hz forces (Table 2). No other sex differences were found. There was an effect of condition on MVIC but no gender \times condition interaction (Table 2). It is possible that the thigh cuff used in the I experiments afforded subjects a slight mechanical advantage during voluntary effort. However, this difference was consistent across both sexes, making comparisons between the two valid.

Voluntary force (MVIC). Significant effects of sex ($P = 0.003$), condition ($P < 0.001$), and time ($P < 0.001$), as well as a condition \times time interaction ($P < 0.001$), were detected with the overall ANOVA. The I protocol produced greater fatigue than the FF protocol after 2 min of exercise (Fig. 1A). Men tended to exhibit a greater decline in MVIC than women, in both condi-

Table 2. *Baseline values*

	Free Flow		Ischemia	
	Men	Women	Men	Women
MVIC,*† N	235.0 ± 14.1	172.1 ± 10.1	249.8 ± 16.6	179.4 ± 10.6
CAR	1.00 ± 0.01	0.99 ± 0.03	1.00 ± 0.00	0.99 ± 0.04
Twitch force, N	22.1 ± 2.7	18.8 ± 2.3	26.2 ± 3.3	17.7 ± 2.5
Potentiated twitch force, N	34.1 ± 4.3	27.7 ± 4.0	35.6 ± 4.8	25.4 ± 3.0
10-Hz force, N	83.7 ± 10.7	66.8 ± 8.5	85.0 ± 12.4	64.6 ± 6.4
50-Hz force,* N	195.2 ± 16.3	138.5 ± 12.3	187.4 ± 16.3	137.9 ± 13.4
Twitch/50-Hz force	0.17 ± 0.01	0.20 ± 0.02	0.19 ± 0.01	0.19 ± 0.01
10-Hz/50-Hz force	0.43 ± 0.03	0.49 ± 0.05	0.45 ± 0.03	0.48 ± 0.04
CMAP amplitude, mV	9.7 ± 0.9	10.1 ± 0.5	9.5 ± 0.7	9.3 ± 0.7
CMAP area, mV·ms	56.5 ± 7.7	52.9 ± 2.9	56.5 ± 6.0	50.0 ± 3.5
+dF/dt, %/ms	0.55 ± 0.04	0.66 ± 0.04	0.57 ± 0.02	0.60 ± 0.03
-dF/dt, %/ms	1.00 ± 0.04	0.93 ± 0.04	0.99 ± 0.04	0.98 ± 0.03

Values are means ± SE. MVIC, maximal voluntary isometric contraction; CAR, control activation rate; twitch/50-Hz force, ratio of twitch to 50-Hz force; 10-Hz/50-Hz force, ratio of 10-Hz to 50-Hz force; CMAP, compound muscle action potential; +dF/dt, maximum rate of force development; -dF/dt, maximum rate of force relaxation. *Significant effect of sex, $P < 0.05$. †Significant effect of condition, $P < 0.05$.

tions, after only 1 min of the fatiguing exercise. However, the difference in decline between men and women during the I condition decreased by 3 min of exercise and was absent by the end of the protocol. In contrast, the sex difference persisted in FF, such that a significant sex × condition ($P = 0.049$) interaction was present at the end of exercise (Fig. 1A). Regression analyses found no significant relationships between initial MVIC force and fatigue in either the FF ($r^2 = 0.18$, $P = 0.11$) or I ($r^2 = 0.03$, $P = 0.50$) conditions.

After the I exercise, MVIC recovered rapidly, such that there was no effect of condition beyond 5 min of recovery. Women recovered their MVICs more completely than men, as indicated by the effect of sex observed after 5 and 10 min of recovery.

Stimulated force. Similar to the MVIC data, the I protocol produced greater declines than the FF protocol in stimulated force at all three test frequencies (1, 10, and 50 Hz; Fig. 2, A–C). In contrast to the MVIC data, the twitch force declined more in women than men (Fig. 2A), a difference that was significant by *minute 4* ($P < 0.001$). Twitch force did not return to baseline levels after 10 min of recovery from either protocol, and the effect of condition persisted through 5 min of recovery. There was a significant sex × condition interaction at the end of recovery, where women showed less recovery than men after FF fatigue ($P = 0.028$) but not after I fatigue ($P = 0.975$).

Similar to the twitch force data, the 10-Hz force fell lower in I than in FF and in women than in men (Fig. 2B). During recovery, there was a more rapid restoration of 10-Hz force, as indicated by the 2-min recovery responses, in men than women after the I ($P = 0.014$) but not the FF study ($P = 0.991$). As with twitch force, 10-Hz force did not return to baseline levels in men or women during the 10-min recovery period after either condition.

The I protocol produced a greater reduction in 50-Hz force than the FF protocol, but no significant effects of sex were detected and no effect of condition was present after 2 min of recovery (Fig. 2C). In both the men and women, 50-Hz force remained lower than

baseline throughout recovery from the FF protocol, but it was not different from baseline after 5 min of recovery from the I protocols. Overall, 50-Hz force was reduced less than the 10-Hz force in both FF (77 vs. 70% of initial force) and I (37 vs. 13% of initial force) conditions. Recovery of 50-Hz force was greater than that observed for the twitch or 10-Hz force after the FF (87 vs. 67 and 61% of initial force, respectively) and I conditions (88 vs. 59 and 54% of initial force, respectively).

10-Hz/50-Hz force and twitch/50-Hz force. The 10-Hz/50-Hz force declined with fatigue, indicating a greater attenuation of the 10-Hz vs. the 50-Hz force in both men and women (Fig. 2D). As with voluntary and stimulated force, 10-Hz/50-Hz force was reduced more after the I than the FF protocol (Fig. 2D). After fatigue, some recovery was seen, but values did not return to baseline for men or women after either protocol. No main effect of sex was observed at any time. A significant sex × condition interaction ($P = 0.006$) at 2 min of recovery was observed, because men showed greater recovery than women after the I fatigue, but women recovered more than men after the FF fatigue. However, post hoc testing showed no significant difference between men and women after either protocol.

The twitch/50-Hz force (data not shown) exhibited the same overall pattern of change as the 10-Hz/50-Hz force. The only difference from 10-Hz/50-Hz force was that a significant effect of condition persisted until after 5 min of recovery.

Central activation measurements. CAR fell more in response to the I protocol than the FF protocol, in both sexes (Fig. 1B). There was a trend toward a greater reduction in CAR for men than women at the second minute of both the FF ($P = 0.081$) and I ($P = 0.079$) protocols. As hypothesized, men showed a greater decline in CAR than women at fatigue in the FF condition ($P = 0.036$) but not during I ($P = 0.490$). The CAR was fully recovered in men and women in both conditions after 10 min of recovery (Fig. 1B).

The CAR recorded at the end of the exercise protocols was significantly correlated to fatigue in both the FF

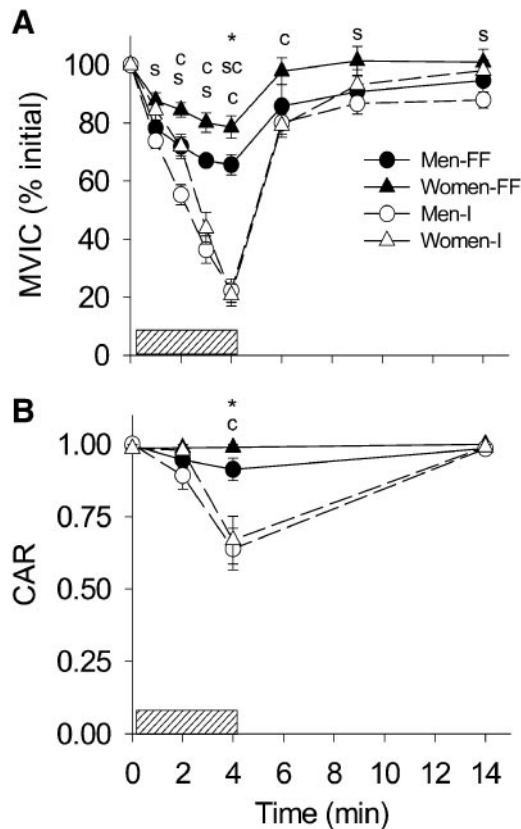


Fig. 1. Voluntary fatigue and central activation for men and women in free-flow (FF) and ischemic (I) conditions. Values are means \pm SE. Hatched bar, exercise. **A:** percent changes in maximal volitional isometric contraction (MVIC) during exercise and recovery. For all 4 experimental groups, force was significantly lower than baseline from the first minute of fatigue until the end of fatigue ($P < 0.001$ for all time points in all protocols). After 2 min of recovery, MVIC force was still significantly reduced for all groups ($P \leq 0.016$), except for the women in the FF condition. After 5 min of recovery, no differences from baseline were observed in any of the groups. **B:** central activation ratio (CAR) values in fatigue and recovery. At end of the I fatigue protocols, CAR values were significantly reduced relative to the initial values in both men and women ($P \leq 0.001$). During the FF fatigue protocols, only the men exhibited a significant reduction in CAR. In all groups, CAR was fully recovered after 10 min. For a given time point, ^seffect of sex, ^ceffect of condition, ^{sc}sex \times condition interaction. *Difference between men and women in the FF but not in the I condition.

($r^2 = 0.48$, $P = 0.003$) and I conditions ($r^2 = 0.51$, $P = 0.002$), although the ceiling effect of the CAR measurement should be taken into account when interpreting these results. In the FF condition, six of eight women but just three of eight men had a CAR of 1.0, whereas in the I condition, only one of eight men and one of eight women had a CAR of 1.0.

Peripheral activation measurements. No differences between men and women in CMAP amplitude or area were present at baseline (Table 2). During the fatigue studies, CMAP amplitude was reduced below baseline during the I but not the FF protocol, but no sex differences were present (Fig. 3). The effect of I was apparent at the third and fourth minutes of exercise, and it persisted through 5 min of recovery. The CMAP area exhibited similar patterns of change to the CMAP

amplitude, with an effect of condition that was present after the first minute of exercise and persisted until the fifth minute of recovery (data not shown).

Contractile properties. There were no differences in contractile properties between men and women at baseline (Table 2). Generally, $+dF/dt$ did not change during the fatigue protocols, whereas $-dF/dt$ exhibited a slight decline. The $-dF/dt$ quickly recovered to baseline, more rapidly after the FF than the I protocols (Fig. 4). There were a few exceptions to this overall pattern, however. Most surprising was the observation of an increase in both $+dF/dt$ and $-dF/dt$ for women at the end of the I fatigue protocol. To investigate this unexpected finding, we calculated the maximal absolute rates of force development ($+N/ms$) and relaxation ($-N/ms$) for both protocols at this time point, and we found that these absolute rates were indeed reduced after fatigue. We believe that declines in the relative rates were a function of the extremely low forces produced by the women after the I session. After 2 min of recovery, both $+dF/dt$ and $-dF/dt$ were still significantly reduced after the I vs. the FF protocols in both men and women, but they had recovered by 5 min.

DISCUSSION

In this study, we evaluated muscle fatigue produced by intermittent, maximal isometric contractions of the dorsiflexor muscles of men and women under two conditions: FF circulation and I. As expected, the I protocol induced greater fatigue than the FF protocol, in both men and women. The observation of greater fatigue in the I condition was associated with greater reductions in CAR, CMAP amplitude and area, stimulated tetanic force, and 10-Hz/50-Hz force vs. the FF condition. Thus the greater fatigue in I was associated with central and peripheral activation failure and marked intramuscular dysfunction, including an impairment of ECC.

The principal new findings of this study related to differences in the degree and mechanisms of fatigue between sexes. As hypothesized, men exhibited greater fatigue than women at the completion of FF but not I exercise. The CAR exhibited a similar sex \times condition interaction. No sex differences were found in stimulated tetanic force, low-frequency fatigue (10-Hz/50-Hz force), or peripheral activation, in either condition. Together, these results suggest that 1) the sex difference in fatigue is blood flow dependent, and 2) a greater impairment of central activation in men is a primary contributor to the greater fatigue in men relative to women. Moreover, the effect of I is most likely due to the absence of oxygen, and not the restriction of blood flow per se, on the basis of the findings of Hogan et al. (19). In an in situ study of mammalian muscle, these investigators found similar fatigue during intermittent stimulation in ischemic (no blood flow) and hypoxic (no O_2 , but normal blood flow) conditions.

In the present study, the restriction of oxygen delivery in the I protocol was associated with an increased onset and amount of fatigue. Similarly, it is possible that our results with regard to the sex difference in

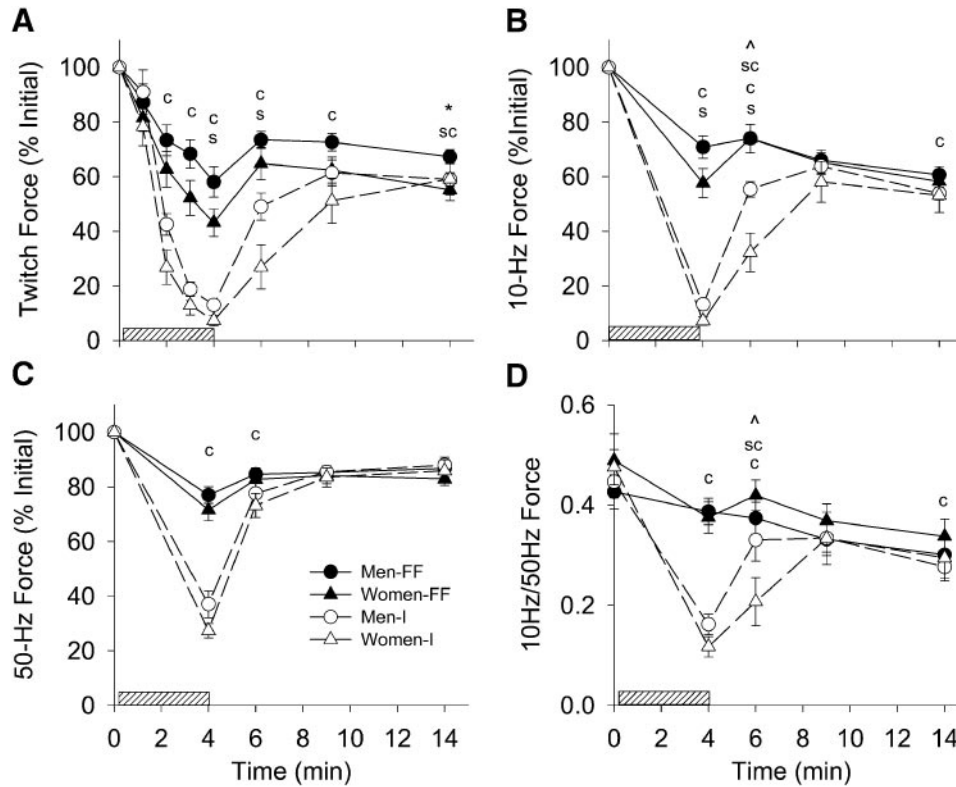


Fig. 2. Fatigue of stimulated force measures. Values are means \pm SE. Hatched bar, exercise. A: changes in twitch force during fatigue and recovery, scaled to potentiated twitch force. Regardless of condition, the twitch forces were reduced in women by the end of the first minute of the fatigue protocols but not in men. By the second minute, twitch forces were reduced in all groups, and this difference persisted throughout recovery ($P \leq 0.03$). B: percent changes in 10-Hz peak force. All groups showed a reduction in 10-Hz force at the end of exercise, and force remained lower than baseline throughout recovery ($P \leq 0.006$). C: percent changes in 50-Hz peak force, scaled to initial value. Force was reduced in all groups at the end of exercise and after 2 min of recovery ($P \leq 0.03$). D: ratio of 10-Hz to 50-Hz peak force (10-Hz/50-Hz force) during fatigue and recovery. All values were reduced relative to baseline at the end of exercise, and remained so throughout recovery. The only exceptions occurred 2 min after the FF protocol, when both men and women exhibited only a trend for a decline ($P = 0.06$ and $P = 0.08$, respectively). For a given time point, *effect of sex, ^ceffect of condition, ^{sc}sex \times condition interaction. *Difference between men and women in the FF but not in the I condition and [^]difference between men and women in the I but not in the FF condition.

fatigue in the FF condition were due to an effect of oxygen delivery, namely a difference in perfusion between the sexes. Although a similar degree of capillarization in men and women has been demonstrated in the TA (30), a recent study found that endurance time in men and women was similar when initial MVIC was used as a covariate during fatigue (21). This result supports the concept that the fatigue resistance of women is a result of their producing less vascular occlusion during muscle contraction, as a result of lower absolute forces, thus allowing relatively greater perfusion. However, this previous study evaluated a sustained contraction. By contrast, Fulco and colleagues (13) reported less fatigue in women compared with strength-matched men during an intermittent contraction protocol, similar to the one used in the present study. Furthermore, linear regression analyses found no significant relationships between initial MVIC force and fatigue in the FF or I conditions ($r^2 = 0.18$, $P = 0.11$ and $r^2 = 0.03$, $P = 0.50$, respectively). Thus we believe that initial MVIC force is unlikely to

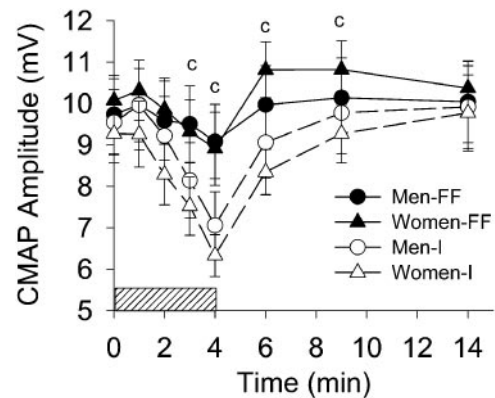


Fig. 3. Peak-to-peak compound muscle action potential (CMAP). Hatched bar, exercise. There were no differences from baseline values in men and women during the FF protocols. During the I protocols, men demonstrated significantly reduced CMAP amplitudes and areas at the third and fourth minutes of exercise. In women, CMAP amplitude was reduced relative to baseline by the second minute of exercise. Amplitude recovered rapidly in both men and women and was not different from baseline after 2 min of recovery. ^cEffect of condition.

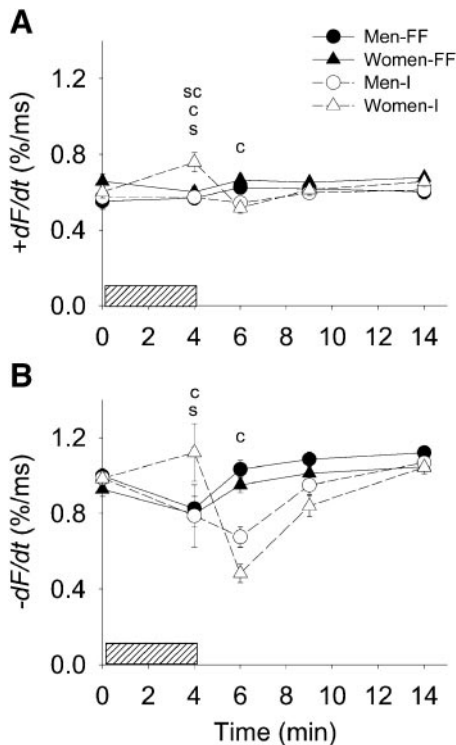


Fig. 4. Contractile properties. Values are means \pm SE. Hatched bar, exercise. **A:** maximal rate of force development ($+dF/dt$). **B:** maximal rates of force relaxation ($-dF/dt$). Increases seen at the end of I exercise in women (in **A** and **B**) accounted for the significant main effects observed at the end of exercise. These changes were likely an artifact of the extremely low forces produced at the end of the exercise, because the absolute maximum rates of force development and relaxation were reduced after fatigue at these time points (see text). *Effect of sex, ^ceffect of condition, ^{sc}sex \times condition interaction. Note that an effect of condition was present in both force development and relaxation after 2 min of recovery but not at any other time.

have played a major role in the sex differences we observed in the present study.

Our measures of stimulated tetanic force (Fig. 2C), LFF (Fig. 2D), and peripheral activation (Fig. 3) suggest that the differences in fatigue between men and women during FF exercise were not due to sex differences in cross-bridge function, ECC coupling, or neuromuscular junction and/or membrane excitability. It appears that the difference in CAR accounts for most of the difference in fatigue between men and women, particularly in light of the observation that twice as many women (6 of 8) as men (3 of 8) had a postfatigue CAR of 1.0 in the FF condition. This difference was not observed after the I protocol, where only one woman and one man maintained a CAR of 1.0. Overall, CAR was significantly correlated to fatigue in both the FF and I conditions, although the ceiling effect of the CAR and the nonlinear relationship between voluntary effort and CAR (37) make interpretation of these analyses somewhat problematic.

It was somewhat surprising to find a significantly greater decline in twitch and 10-Hz force in women than men (Fig. 2, A and B). The two most obvious explanations for these results would be that women

exhibited a greater impairment of peripheral excitation or ECC. However, we did not observe any sex differences in the M-wave or 10-Hz/50-Hz force changes, suggesting that these are unlikely mechanisms for the observed differences in force. Furthermore, the similarity in the resting $+dF/dt$ and $-dF/dt$ values (Table 2) suggests that there was no difference in fiber type distribution between sexes (16). Perhaps sex-specific changes in the force-frequency curve that we could not detect with only three stimulation frequencies occurred. In any case, this differential response of men and women with regard to volitional and stimulated contractions warrants further investigation.

Two likely remaining candidates for the mechanisms underlying the sex effect on fatigue and CAR are differences in metabolic fuel utilization or motor unit discharge rates. Although there is little evidence to suggest fiber type differences between men and women, several studies using muscle biopsies of the vastus lateralis have shown that men exhibit higher activities of several glycolytic enzymes than women (15, 22, 28, 36), whereas women exhibit greater activities of enzymes associated with oxidation of carbohydrate (28) and lipid (15, 28). In line with these findings, Esbjornsson-Liljedahl et al. (9) found that glycogen depletion during sprint exercise was reduced 50% more in type I fibers of women vs. those of men. A greater degree or rate of glycolysis in men would be associated with a larger reduction in intramuscular pH, which has been implicated in central fatigue (24, 32), possibly by acting on the metaboreceptors of small-diameter afferent fibers to produce reflex inhibition (14, 32). Although we did not collect any metabolic data in the present study, our finding of greater fatigue associated with greater central activation failure in men is consistent with such a metabolic scenario.

It should be noted that, similar to the FF condition, men appeared to show a greater decline in MVIC over the first 2 min of the I protocol but that this difference had disappeared by the end of the protocol. We believe that this finding may be a function of oxygen availability. Only a few seconds elapsed between cuff inflation and the start of exercise, which was not sufficient time to deplete the oxygen in the blood trapped in the lower limb. We believe that the women were better able to utilize this residual oxygen to support muscle contraction than men during the first 2 min of exercise, thus accounting for the observed sex difference. As exercise progressed and the stored oxygen was used up, the sex difference disappeared.

Fulco et al. (12) reported that endurance time for men was decreased in hypobaric hypoxic conditions (4,300-m altitude) relative to normoxia with a 50% duty cycle, similar to that used in the present study, but that the endurance time for women was not different in the two conditions. They suggested these findings indicated a greater oxidative capacity in women, which allowed them to utilize the available oxygen in a more efficient manner than men. Despite the many similarities between the present study and that of

Fulco et al., their finding that the fatigue resistance of women was accentuated during hypoxia appears at odds with our results showing that the difference in fatigue between men and women disappeared during I conditions. Differences in the protocols may account for this discrepancy. Under hypoxic conditions some oxygen is available, whereas none is available during ischemia. It is reasonable to suppose that if women are better able to utilize oxidative metabolic pathways than men, they would be able to make better use of any available oxygen and thus be less affected by hypoxia than men. In contrast, during ischemia this oxidative advantage would be lost, and women, forced to depend on glycolytic mechanisms, would fatigue similarly to men, as observed in the present study.

The suggestion that the sex differences in fatigue and CAR observed in the present study are due to differences in metabolism between men and women is consistent with our laboratory's recent work using magnetic resonance spectroscopy (26). It was observed that men exhibited greater declines in pH and increases in the ratio of P_i to PCr during a sustained, submaximal isometric protocol that increased in intensity over 16 min, suggesting that women may utilize oxidative metabolism to a greater degree than men. It is important to note that in this submaximal fatiguing protocol, there were no differences in fatigue or CAR between men and women, despite the differences in metabolism. It may be that the differences in metabolites did not reach a level sufficient to produce a difference in fatigue.

Another potential mechanism for a portion of the sex difference in fatigue observed in the present study relates to neural activation of the muscle. Our laboratory has previously suggested that the differences in fatigue between men and women could occur if there were reduced motor unit discharge rates in women (17). If women are able to achieve MVIC with relatively lower discharge rates, they may be less vulnerable to changes in central drive and thus more resistant to central fatigue. Moreover, if this difference in motor unit activation proves true, it might result in a lower metabolic cost of contraction in women due to differences in the contributions of the contractile and non-contractile ATPases (20). This lower contractile cost could allow women to meet the demands of muscle contraction with less of a contribution from anaerobic metabolic pathways.

Conclusions. We found that women fatigued less than men in FF conditions, but not during I, and that the sex differences in fatigue were roughly paralleled by differences in central activation. With the lack of sex effects on the CMAP, tetanic force, and measures of ECC function, these results suggest that the primary mechanism behind the sex difference in fatigue is a relatively greater impairment of central activation in men. The intermittent nature of the exercise protocol, coupled with the lack of a significant relationship between initial MVIC force and fatigue, suggests that differences in vascular occlusion due to strength differences between men and women are unlikely to have

played a major role in our findings. The observation that ischemia eliminated the differences in fatigue is consistent with a number of studies relating fatigue to muscle metabolism and might be the result of sex-based differences in metabolic pathway utilization during muscle contraction.

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