Mechanisms for the age-related increase in fatigability of the knee extensors in old and very old adults

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Abstract

The mechanisms for the age-related increase in fatigability during high-velocity contractions in old and very old adults (≥80 yr) are unresolved. Moreover, whether the increased fatigability with advancing age and the underlying mechanisms differ between men and women is not known. The purpose of this study was to quantify the fatigability of knee extensor muscles and identify the mechanisms of fatigue in 30 young (22.6 ± 0.4 yr; 15 men), 62 old (70.5 ± 0.7 yr; 33 men), and 12 very old (86.0 ± 1.3 yr; 6 men) men and women elicited by high-velocity concentric contractions. Participants performed 80 maximal velocity contractions (1 contraction per 3 s) with a load equivalent to 20% of the maximum voluntary isometric contraction. Voluntary activation and contractile properties were quantified before and immediately following exercise (<10 s) using transcranial magnetic stimulation and electrical stimulation. Absolute mechanical power output was 97 and 217% higher in the young compared with old and very old adults, respectively. Fatigability (reductions in power) progressively increased across age groups, with a power loss of 17% in young, 31% in old, and 44% in very old adults. There were no sex differences in fatigability among any of the age groups. The age-related increase in power loss was strongly associated with changes in the involuntary twitch amplitude ($r = 0.75$, $P < 0.001$). These data suggest that the age-related increased power loss during high-velocity fatiguing exercise is unaffected by biological sex and determined primarily by mechanisms that disrupt excitation contraction coupling and/or cross-bridge function.

NEW & NOTEWORTHY

We show that aging of the neuromuscular system results in an increase in fatigability of the knee extensors during high-velocity exercise that is more pronounced in very old adults (≥80 yr) and occurs similarly in men and women. Importantly, the age-related increase in power loss was strongly associated with the changes in the electrically evoked contractile properties suggesting that the increased fatigability with aging is determined primarily by mechanisms within the muscle for both sexes.

INTRODUCTION

Human aging is accompanied by a progressive decline in neuromuscular function that can result in functional impairments and a decreased quality of life in older adults. However, the decrements in function that occur with aging can vary depending on the demands of the motor task ([22]). For example, findings on the age-related changes in fatigability are not uniform across contraction types, such as isometric and dynamic contractions ([7]), nor between old (~60–79 yr) and very old adults (>80 yr) ([26], [37]). Fatigability of limb muscle is characterized by an acute reduction in force and power that occurs in response to contractile activity ([11], [20]). Paradoxically, many studies have found that old adults (~60–79 yr) are typically less fatigable than young adults when performing isometric contractions ([5], [7], [20]). However, this fatigue resistance appears to reverse with very advanced age (>75–80 yr) ([26]) and when old adults perform dynamic contractions at moderate to high velocities ([4], [9], [10], [37], [45]).

The mechanisms for the increased fatigability with aging are not well understood but could be due to any of the age-related changes in the neuromuscular system, including cortical atrophy, reduced white matter, altered brain neurochemistry ([8], [35], [43], [44]), motor unit remodeling and instability of neuromuscular transmission ([19], [22]), or altered bioenergetics, Ca^{2+} handling and cross-bridge kinetics ([30], [31], [33], [34], [39]). Fortunately, recent advances in noninvasive stimulation procedures allow us to identify the primary mechanisms of fatigue ([52], [53]). For example, neural drive from the motor cortex can be quantified with transcranial magnetic stimulation (TMS) and is reduced and more variable with aging during and after a fatiguing isometric exercise for some muscle groups ([22]). Whether reduced voluntary drive from the motor cortex contributes to the age-related increase in fatigability with high-velocity contractions is not known. Furthermore, integrating TMS with measures of electromyography (EMG) and electrically evoked contractions of the motor nerve can localize the origin of

fatigue to changes in the excitability of the corticospinal tract ([25](#), [27](#)), neuromuscular propagation ([16](#)), and the contractile properties within the muscle ([28](#)). However, these stimulation techniques have not been integrated within a study to identify the mechanisms for the age-related increased fatigability during high-velocity contractions, particularly in old women or very old adults (≥80 yr).

Skeletal muscle atrophy and neuromuscular decline accelerates with advancing age in adults ≥80 yr ([22](#), [41](#), [47](#), [48](#)). For the ankle dorsiflexor muscles, the accelerated decline in function includes a progressive increase in fatigability ([26](#), [37](#)). For example, the power loss elicited by high-velocity contractions of the dorsiflexor muscles was greater in a group of very old (84 yr) compared with young men (26 yr) but showed no differences between the old (64 yr) and young men ([37](#)). Whether this age-related progression in fatigability is observed in a larger muscle group, such as the knee extensors, and/or differs between men and women is not known. Understanding the mechanisms of fatigue in the knee extensors of men and women is important because *1*) the knee extensors are more susceptible to age-related losses in function compared with other limb muscles ([6](#), [24](#), [32](#)), *2*) the knee extensors of old adults experience fatigue-induced power loss when performing daily activities ([15](#), [40](#)), and *3*) knee extensor power output and fatigability are associated with functional performance with aging ([2](#), [45](#)).

Women currently account for ~54% of the global population in individuals aged ≥60 yr and ~61% of the population in those aged ≥80 yr ([55](#)). Despite the large prevalence of women in the aging population, studies aimed at identifying the mechanisms for the age-related increase in fatigability during dynamic contractions have been conducted only on men and report that the increased power loss is due primarily to mechanisms within the muscle ([9](#), [10](#), [37](#)). It is unknown whether the mechanisms for the increased fatigability observed in older women ([4](#), [45](#)) are similar to those observed in older men. However, evidence from cross-sectional studies shows that age-related muscle atrophy and the decline in neuromuscular function are more pronounced in old women ([29](#), [38](#), [54](#)), suggesting that the mechanisms for the age-related increase in fatigability may differ in women compared with men.

Thus the purpose of this study was to determine the fatigability of the knee extensor muscles and identify the mechanisms of fatigue in young (≤35 yr), old (60–79 yr), and very old (≥80 yr) men and women elicited by high-velocity concentric contractions. We hypothesized that the reductions in mechanical power during the fatiguing exercise would progressively increase with age (i.e., fatigability in young < old < very old) but that there would be no sex differences in any of the age cohorts. We also hypothesized that mechanisms within both the nervous system and the muscle would contribute to the power loss during the fatiguing exercise for all age groups but that the increased fatigability in the old and very old men and women would be due primarily to mechanisms originating within the muscle.

METHODS

Participants and Ethical Approval

One-hundred and four individuals participated in this study: 30 young (19–28 yr, 15 men and 15 women), 62 old (61–79 yr, 33 men and 29 women), and 12 very old adults (80–93 yr, 6 men and 6 women). Participants provided written informed consent and underwent a general health screening that included a questionnaire where older participants were excluded if they scored <26 on the mini-mental state ([14](#)). Participants were healthy, community-dwelling adults free of any known neurological, musculoskeletal, and cardiovascular diseases. All experimental procedures were approved by the Marquette University Institutional Review Board and conformed to the principles in the Declaration of Helsinki. Anthropometrics and physical activity levels for the participants are reported in [Table 1](#).

Table 1. Anthropometrics and physical activity levels for the young, old, and very old men and women

	Young		Old		Very Old	
Variable	Men (15)	Women (15)	Men (33)	Women (29)	Men (6)	Women (6)
Age,* yr	22.6 ± 2.4	22.6 ± 2.2	70.0 ± 4.6	71.0 ± 5.9	89.0 ± 3.6	83.0 ± 2.6
Height,† cm	176.8 ± 8.4	164.6 ± 5.5	176.9 ± 8.2	161.2 ± 4.3	176.0 ± 8.3	158.3 ± 6.2
Weight,†‡ kg	75.6 ± 10.6	65.1 ± 9.6	84.7 ± 12.1	66.6 ± 12.1	72.3 ± 5.0	72.9 ± 13.3
Body mass index,* kg/m2	24.1 ± 1.7	24.0 ± 3.2	27.1 ± 3.6	25.6 ± 4.7	25.5 ± 1.7	29.1 ± 4.6
Body fat,*† %	17.4 ± 3.1	29.6 ± 7.3	30.5 ± 5.5	38.8 ± 7.3	28.7 ± 6.6	41.4 ± 6.3
Physical activity,* steps/day	8,623 ± 3,973(12)	9,825 ± 2,840(14)	8,510 ± 3,998(30)	7,825 ± 3,784(26)	4,590 ± 3,606(5)	2,654 ± 1,185

Values are reported as means _ SD. Body fat percentage was measured via dual X-ray absorptiometry (Lunar iDXA; GE, Madison, WI). Physical activity was measured via triaxial accelerometery (GT3X; ActiGraph, Pensacola, FL). The sample sizes (n) for each cohort and certain variables are reported in parentheses. *P _ 0.05, significant effect of age. †P _ 0.05, significant effect of sex. ‡P _ 0.05, significant age _ sex interaction.

Experimental Setup and Protocol

Participants reported to the laboratory on three occasions, twice for familiarization and once for the experimental session to measure fatigability and the associated mechanisms elicited by high-velocity concentric contractions of the knee extensor muscles.

Experimental setup.

The experimental setup to measure knee extension torque and velocity was similar to the setup described previously ([18]). Briefly, testing was performed on the dominant leg of each participant (preferred kicking leg) except when the participant reported a previous surgical procedure, knee or leg pain, or osteoarthritis of the dominant leg (1 young woman, 2 old women, 3 very old women, 2 old men, and 1 very old man). In all sessions, participants were seated upright in the high Fowler's position with the starting knee position set at 90° flexion in a Biodex System 4 Dynamometer (Biodex Medical, Shirley, NY). The position of the dynamometer was adjusted so that the axis of rotation of the dynamometer's lever arm was aligned with the axis of rotation of the participant's knee. The length of the dynamometer's lever arm was adjusted for each participant and secured with a Velcro strap proximal to the malleoli. Extraneous movements and changes in the hip angle were minimized by securing the participant to the seat with the dynamometer's four-point restraint system ([Fig. 1]). To ensure the measured torques and velocities were generated primarily by the knee extensor muscles, participants were prohibited from grasping any part of the dynamometer with their hands.
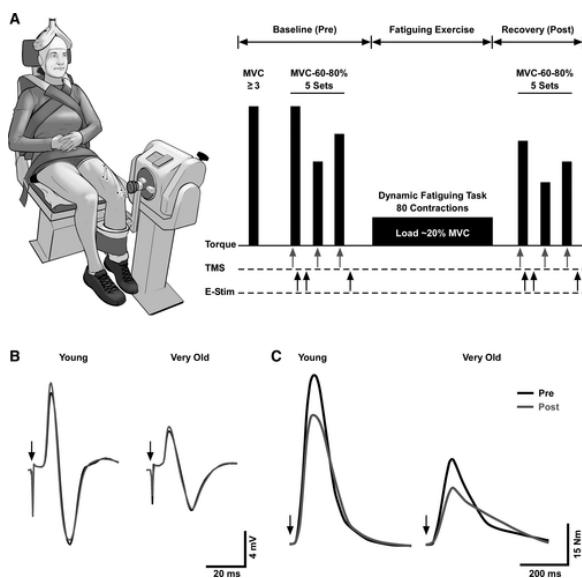

Fig. 1. Experimental protocol. *A*: schematic of the experimental setup and protocol to measure the fatigability and the mechanisms elicited by high-velocity concentric contractions of the knee extensor muscles. Participants performed a minimum of 3 knee extensor maximum voluntary contractions (MVCs) with no stimulations followed by five sets of isometric contractions that included a MVC followed by contractions at both 60 and 80% MVC (MVC-60–80%). Transcranial magnetic stimulation (TMS) to the motor cortex and electrical stimulation to the femoral nerve during the MVC-60–80% contractions are represented by the gray and black arrows, respectively. Following the baseline isometric measurements, participants completed the dynamic fatiguing exercise which consisted of a maximal velocity knee extension performed once every 3 s against a load of ~20% MVC for a total of 4 min (80 total contractions). Two sets of MVC-60–80% isometric contractions were performed in succession as rapidly as possible immediately after the exercise (Post 1 and Post 2), with additional sets completed at 2.5, 5, and 10 min into recovery. The *x*-axis for the experimental protocol is not to scale, and the timing of the stimuli and contractions are described in detail in methods. *B* and *C*: representative compound

muscle action potentials (*B*) and potentiated resting twitches (*C*) from before (Pre) and immediately after (Post 1) the fatiguing exercise are displayed for both a young (22 yr) and very old man (89 yr).

Familiarization sessions.

During the familiarization sessions, each participant was habituated to electrical stimulation of the femoral nerve and TMS to the motor cortex. Additionally, participants practiced performing brief 2- to 3-s maximal and submaximal voluntary isometric contractions and maximal voluntary concentric contractions with the knee extensors. The familiarization session also included an assessment of body composition with dual X-ray absorptiometry (Lunar iDXA; GE, Madison, WI).

Experimental session.

The experimental session began with electrical stimulation of the femoral nerve to identify the electrode placement that elicited the maximum peak-to-peak compound muscle action potential [maximum M wave (M_{max})] of the vastus lateralis (VL), rectus femoris (RF), and vastus medialis (VM). Following the electrical stimulations, participants performed a minimum of three brief (2–3 s) knee extension maximum voluntary contractions (MVCs) without stimulation interspersed with two knee flexor MVCs. Participants were provided strong verbal encouragement and visual feedback on their performance with a 56-cm monitor mounted 1–1.5 m directly in front of their line of vision. Each MVC was interspersed with at least 60-s rest, and MVC attempts were continued until the two highest values were within 5% of each other. The highest torque output from the MVCs was used to calculate *1*) the target forces for the submaximal isometric contractions needed for optimizing the TMS parameters (i.e., coil placement and stimulator intensity), and *2*) the visual feedback gain in the subsequent MVC trials used to assess voluntary activation.

Once the optimal TMS position and intensity were identified, participants performed five sets of brief isometric contractions (2–3 s per contraction) with the knee extensor muscles to obtain the baseline measures used in identifying the mechanisms of fatigue (Fig. 1 and Table 2). Each set of contractions included a MVC followed by contractions at 60 and 80% MVC (MVC-60–80%) with TMS delivered at each contraction to estimate the resting twitch amplitude for the calculation of voluntary activation (21, 53). Single-pulse femoral nerve stimulation was delivered during the MVC and at rest immediately following (<5 s) both the MVC and 80% MVC contractions. Sets of MVC-60–80% contractions were interspersed with at least a 2.5-min rest to help ensure repeatable maximal efforts were performed while minimizing residual fatigue from each set. The highest torque output from all MVC attempts was used to calculate the 20% MVC load for the dynamic fatiguing exercise, whereas the median value from the baseline sets of MVC-60–80% contractions was used to identify the mechanisms of fatigue. This approach ensured that each participant's best effort was used to calculate the load for the fatiguing exercise.

Table 2. Baseline neuromuscular performance measures from the young, old, and very old men and women

	Young (_35 yr)		Old (60–79 yr)		Very Old (_80 yr)	
Variable	Men (15)	Women (15)	Men (33)	Women (29)	Men (6)	Women (6)
Electrical stimulation						
Potentiated twitch torque-Qtw,*‡ N·m	58.8 ± 17.0	37.2 ± 9.6	46.7 ± 12.1	29.6 ± 6.4	35.6 ± 6.8	22.8 ± 4.6
Rate of torque development,*† Nm/s	1,270 ± 373	800 ± 216	945 ± 260	584 ± 132	675 ± 73	440 ± 107
Normalized rate of torque development,* s-1	21.6 ± 2.1	21.7 ± 3.4	20.2 ± 1.9	19.8 ± 1.6	19.3 ± 2.6	19.2 ± 1.9
1/2Relaxation time,*†‡ ms	69 ± 14	77 ± 14	70 ± 12	100 ± 31 (27)	78 ± 22	76 ± 20
VL Mmax amplitude,*† mV	18.1 ± 2.1	11.9 ± 2.5	10.5 ± 3.6	7.1 ± 3.1	7.8 ± 2.3	3.8 ± 1.8
VL M-wave area,*† mV·ms	98.1 ± 10.9	70.3 ± 11.3	67.8 ± 17.6	47.8 ± 15.6	53.5 ± 12.3	31.1 ± 14.8
VM Mmax amplitude, *† mV	18.9 ± 4.3	15.8 ± 3.2	14.2 ± 3.5	9.6 ± 3.1	10.0 ± 2.8	7.3 ± 1.2
VM M-wave area,*† mV·ms	119.1 ± 33.6	100.7 ± 22.7	92.1 ± 20.6	64.6 ± 22.8	76.1 ± 20.2	59.3 ± 7.2
RF Mmax amplitude,*† mV	9.9 ± 2.3	7.0 ± 1.6	6.4 ± 1.9	4.1 ± 1.7	4.5 ± 2.3	2.1 ± 1.4
RF M-wave area,*† mV·ms	54.6 ± 10.1	38.1 ± 8.2	37.5 ± 9.8	24.1 ± 8.0	28.5 ± 13.9	13.6 ± 9.3
Transcranial magnetic Stimulation						
Voluntary activation (Eq. 1), %	97.9 ± 1.9	97.4 ± 2.4	96.3 ± 3.7	97.2 ± 2.9	97.7 ± 0.5	90.8 ± 8.7 (5)
Voluntary activation (Eq. 2), %	0.3 ± 0.4	0.4 ± 0.4	0.9 ± 1.2	0.6 ± 0.7	0.5 ± 0.2	3.3 ± 3.6
Estimated resting twitch,*† N·m	37.6 ± 13.9	25.9 ± 10.6	32.3 ± 12.8	18.9 ± 7.0	20.5 ± 4.0	17.7 ± 9.8 (5)
Peak relaxation rate,*† N·m·s-1	-2,840 ± 784	-1,534 ± 388	-1,330 ± 471	-628 ± 181	-1,029 ± 309	-546 ± 179
Normalized peak relaxation rate,*† s-1	-10.5 ± 1.3	-9.0 ± 1.4	-8.1 ± 1.7	-6.5 ± 1.6	-9.1 ± 1.5	-7.7 ± 2.5
VL MEPmax,* %Mmax	37.0 ± 9.6	38.0 ± 9.8	27.7 ± 8.3	26.8 ± 8.9	29.0 ± 8.0	39.9 ± 18.7
VM MEPmax,* %Mmax	44.8 ± 13.6	39.1 ± 11.8	32.0 ± 11.7	30.3 ± 11.1	35.1 ± 12.6	43.6 ± 18.5
RF MEPmax,* %Mmax	51.7 ± 10.7	51.9 ± 7.7	40.9 ± 10.7	37.2 ± 10.5	39.9 ± 10.4	51.2 ± 16.8
VL silent period, ms	222 ± 47	237 ± 56	266 ± 55 (31)	237 ± 59 (28)	219 ± 31	248 ± 74 (3)

Values are reported as means ± SD. Variables from electrical stimulation to the femoral nerve are the median values from the stimuli delivered at rest following the maximal voluntary contraction (MVC) and 80% MVC contractions (see Fig. 1). Maximum M wave (M_{max}) for the vastus lateralis (VL), vastus medialis (VM), and rectus femoris (RF) is the peak-to-peak maximal compound muscle action potential amplitude. Variables from transcranial magnetic stimulation to the motor cortex are the median values from the 5 sets of MVC-60–80% contractions performed before the dynamic exercise. The peak-to-peak motor-evoked potential amplitudes (MEP_{max}) from the TMS during the MVC are expressed relative to the M_{max} (%M_{max}) obtained from the electrical stimulation delivered during the MVC. The sample sizes (n) for each cohort and certain variables are reported in parentheses. *$P < 0.05$, significant effect of age. †$P < 0.05$, significant effect of sex. ‡$P < 0.05$, significant age × sex interaction.

Dynamic fatiguing exercise.

Following the baseline MVC measurements, participants were habituated to performing maximal velocity knee extensions against a 20% MVC load applied by the dynamometer. With this setup, the dynamometer's motor provides a quasiconstant force while allowing velocity to vary. This approach was employed because *1*) it more closely mimics common daily activities that require moving an object with constant mass but at different velocities, and *2*) it allowed participants to generate high mechanical power outputs while still maintaining a full range of motion (ROM) ([10]). To minimize the effect of the additional braking force applied by the dynamometer at the end of the ROM, the maximum total displacement was set to 95° with the starting position set at 90° knee flexion. A compliant foam pad was placed at ~0° knee flexion, and participants were instructed to kick as fast as possible through the pad to achieve the maximal volitional shortening velocity for every contraction. Although the 95° maximum total displacement allowed for slight hyperextension of the knee, no participants reported discomfort or pain during or following the dynamic exercise.

For the dynamic fatiguing exercise, participants were verbally cued to kick once every 3 s for a total of 4 min (80 contractions). The low frequency of contractions was selected to maximize muscle perfusion during the exercise by inducing a low duty cycle, i.e., the ratio of the duration of muscle force application to the entire duration between contractions ([3], [49]). The average duty cycles were, respectively, 13 ± 1, 16 ± 1, and 16 ± 3% for the young, old, and very old adults. The 3% higher duty cycle in the old and very old compared with young adults ($P < 0.001$; $\eta_p^2 = 0.44$) was due to the slower contractile velocities with age ($P < 0.001$; $\eta_p^2 = 0.43$) and not due to differences in the duration between the start of each contraction ($P = 0.116$; $\eta_p^2 = 0.04$). Participants were provided strong verbal encouragement to generate their maximal effort and to complete the full ROM for every contraction. Upon completion of each contraction, the participant was instructed to relax, and the limb was passively returned to the starting position by the dynamometer. To identify the mechanisms of fatigue induced by the dynamic exercise, two sets of MVC-60–80% isometric contractions were performed in succession as rapidly as possible following the fatiguing exercise with additional sets performed at 2.5, 5, and 10 min following exercise cessation ([Fig. 1]).

Measurements and Data Analysis

Torque and mechanical power output.

Torque, position, and angular velocity from the dynamometer's transducers were digitized at 500 Hz with a Power 1401 A/D converter and stored online using Spike 2 software (Cambridge Electronics Design, Cambridge, UK). The torque during each MVC was quantified as the average value over a 0.5-s interval centered on the peak torque of the contraction. The baseline MVC value for each participant was the median torque output recorded during the MVC from the five sets of MVC-60–80% contractions performed before the dynamic fatiguing exercise. To compare the changes in MVC among the young, old, and very old men and women following the dynamic exercise, MVC values are expressed as a percentage of the individual-specific baseline MVC value.

For the dynamic fatiguing exercise, contraction-by-contraction mechanical power outputs (W) were calculated as the product of the measured torque (N·m) and angular velocity (rad/s) and averaged over the entire shortening phase of the knee extension. Because power output increased over the first few contractions in some participants, the recorded baseline power output for each participant was the highest average obtained from 5 sequential contractions within the first 10 contractions. To quantify the relative reductions in power for each participant, the average power output from the last five contractions is expressed as a percentage of the individual-specific baseline power output value.

Electromyography.

Surface Ag/AgCl EMG electrodes (Grass Products; Natus Neurology, Warwich, RI) were adhered to the skin in a bipolar arrangement overlying the muscle bellies of the VL, VM, RF, and biceps femoris with an interelectrode

distance of 2.5 cm. The skin was shaved and cleaned with 70% ethanol before electrode placement, and the reference electrodes were placed on the patella. Analog EMG signals were amplified (×100), filtered (13-1,000-Hz band pass; Coulbourn Instruments, Allentown, PA), and digitized at 2,000 Hz with a Power 1401 A/D converter and stored online using Spike 2 software (Cambridge Electronics Design).

Electrical stimulation.

The femoral nerve was stimulated with a constant-current, variable high-voltage stimulator (DS7AH; Digitimer, Welwyn Garden City, Hertforshire, UK) to obtain M_{max} of the VL, VM, and RF. The cathode was placed over the nerve high in the femoral triangle, and the anode was placed over the greater trochanter. Single 200-μs square-wave pulses were delivered with a stimulus intensity beginning at 50 mA and increased incrementally by 50–100 mA until both the unpotentiated resting twitch torque amplitude and M_{max} for all three quadriceps muscles no longer increased. The intensity was then increased by an additional 20% to ensure the stimuli were supramaximal (range: 120–720 mA).

Contractile properties of the knee extensor muscles were quantified with the potentiated resting twitches from the single-pulse femoral nerve stimulations delivered after the MVC and 80% MVC contractions (Fig. 1). Stimuli were delivered after both the MVC and 80% MVC contractions to ensure that at least one of the stimuli was delivered while the participant was fully relaxed. The baseline values for each participant are the median obtained from the five sets of MVC-60–80% performed before the dynamic fatiguing exercise and are reported for the amplitude of the potentiated resting twitch torque (Q_{tw}: N·m), the half relaxation time (ms), and the absolute (Nm/s) and normalized (s^{-1}) peak rates of torque development. The peak rate of torque development was quantified with the derivative of the torque channel as the highest rate of torque increase over a 10-ms interval. To provide an indication of neuromuscular propagation and the ability of the action potential to propagate across the sarcolemma, the peak-to-peak amplitude (M_{max}) and area of the M wave are reported for all three quadriceps muscles (16).

TMS and voluntary activation.

The motor cortex was stimulated by delivering a 1-ms duration magnetic pulse with a concave double-cone coil (110-mm diameter: maximum output 1.4 T) connected to a monophasic magnetic stimulator (Magstim 200^2; Magstim, Whitland, UK). The coil was initially positioned with the center of the coil ~1 cm lateral to the vertex of the motor cortex contralateral to the limb under investigation. The orientation of the coil induced a posterior-to-anterior current flow in the underlying cortical tissue. Identification of the optimal stimulator position was guided by moving along a 1-cm grid drawn on an electroencephalography cap and was determined as the location that elicited the greatest motor-evoked potential (MEP) in the VL while the subject contracted at 20% MVC. This position was marked to ensure repeatable placement of the coil for the remainder of the experiment.

Once the optimal stimulator position was determined, the stimulator intensity for the voluntary activation measurements was identified during brief (2–4 s) isometric contractions at 40% MVC. Single-pulse TMS was delivered during each contraction with an intensity starting at 50% stimulator output and increased incrementally by 10% until the peak-to-peak MEP amplitude of the VL failed to increase further or began to decrease. If the latter occurred, then the stimulator output was reduced in 5% decrements until the largest peak-to-peak MEP amplitude was achieved in the VL. The intensity eliciting the largest MEP was compared with the intensity eliciting the largest twitch torque at the 40% MVC to verify that the stimulator intensities were approximately similar. This additional step ensured that the stimulus intensity did not elicit large activation of the antagonist muscles. This method was used instead of quantifying the biceps femoris MEP amplitude (%M_{max}) because of the difficulty of maximally stimulating the sciatic nerve with surface electrical stimulation. It should also be noted that the knee flexor MVC was on average only 38 ± 8% of the knee extensor MVC at the 90° knee

flexed position. Thus the effect of any inadvertent activation of the antagonist muscle group on measurements of voluntary activation would be diminished due to the positioning of the participant.

Voluntary activation was quantified from each set of MVC-60–80% contractions based on the technique originally developed for the elbow flexors ([53]) and later for the knee extensors ([46]). Briefly, single pulse TMS was delivered during the MVC and 60 and 80% MVC contractions, and the amplitude of the superimposed twitch torque was measured for each contraction. A linear regression was performed between the superimposed twitch torque and the voluntary torque to obtain an estimated resting twitch by extrapolating the regression to the *y*-intercept ([Fig. 2]).The resting twitch evoked by TMS was estimated rather than measured directly, because the excitability of the corticospinal tract increases markedly from rest to maximal activation ([12]). Any three-point regression with an $R^2 < 0.8$ ([21]) was excluded from the voluntary activation calculations using *Eq. 1* but was still included in the calculations using *Eq. 2*. This occurred in 9.7% of the baseline measurement MVC-60–80% sets, 16.0% of the two sets performed immediately after the fatiguing exercise, and 10.1% of the three sets performed in recovery. In addition, we were unable to obtain estimated resting twitches during either the baseline or following the fatiguing exercise from one old woman. As a result, to ensure all participants were included in the analysis and to provide comparison of the data to all other studies that have used TMS for voluntary activation, we quantified voluntary activation for each set of MVC-60–80% contractions in two ways:

$$\text{Voluntary Activation (\%)} = (1 - \frac{\text{SIT}}{\text{eRT}}) \times 100 \text{ (1)}$$

$$\text{Voluntary Activation (\%)} = (\frac{\text{SIT}}{\text{SIT+MVC}}) \times 100 \text{ (2)}$$
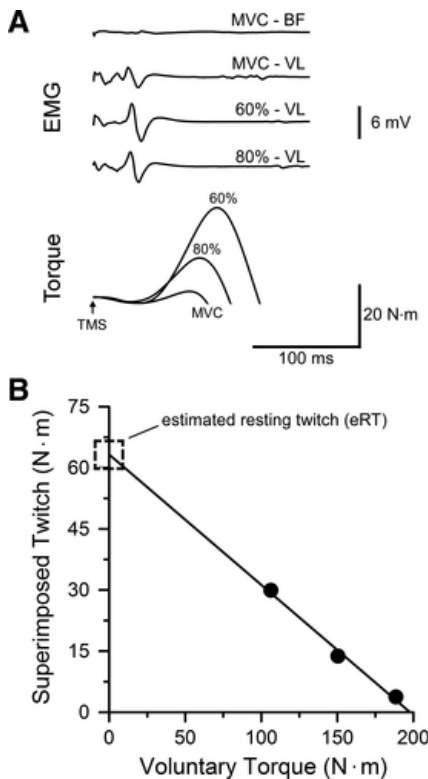

Fig. 2. Representative data of the method used to assess voluntary activation with transcranial magnetic stimulation (TMS). *A*: raw torque and EMG responses evoked by TMS delivered to the motor cortex from a 71-yr-old man during the maximum voluntary contraction (MVC) and 60 and 80% MVC contractions. The EMG response is depicted for the vastus lateralis (VL) from all 3 stimuli and for the biceps femoris (BF) from the MVC only. The superimposed twitch torques are offset and overlaid to depict the amplitude of the twitches from the

MVC and 60 and 80% MVC contractions. *B*: linear regressions were performed between the superimposed twitch torque and the voluntary torque to obtain an estimated resting twitch by extrapolating the regression to the *y*-intercept. For comparison of our data set to all other studies that have used TMS to assess voluntary activation, we calculated voluntary activation both with (*Eq. 1*) and without the estimated resting twitch (*Eq. 2*).

where SIT is the amplitude of the superimposed twitch torque elicited by TMS during the MVC and eRT is the calculated estimated resting twitch torque. The reported baseline voluntary activation for each participant was the median from the five MVC-60–80% sets performed before the dynamic exercise. To compare the changes in voluntary activation among the young, old, and very old men and women, voluntary activation levels immediately following the dynamic exercise were compared with the individual-specific baseline values.

Absolute (Nm/s) and normalized (s^{-1}) peak rates of torque relaxation were also determined from the TMS delivered during the MVC contractions ([51]). When TMS is delivered to the motor cortex during a MVC, there is a brief transient withdrawal of the descending neural drive following the stimulus that causes the muscle to involuntarily relax. The peak rate of torque relaxation was quantified with the derivative of the torque channel as the greatest rate of torque decrease over a 10-ms interval and was compared before and immediately following the fatiguing dynamic exercise.

Physical activity assessment.
Physical activity was quantified for each participant with a triaxial accelerometer (GT3X; ActiGraph, Pensacola, FL) worn around the waist for at least 4 days (2 weekdays and 2 weekend days) as reported previously ([18]). Data are reported for each participant as long as the accelerometer was worn for a minimum of 3 days ([17]).

Statistical Analyses
Individual univariate ANOVAs were performed between the subject characteristics and baseline values and age (young, old, or very old) and sex (men or women) as the grouping variables. Repeated-measure ANOVAs were performed on the measures of fatigability (power and MVC torque) and the associated mechanistic measurements (voluntary activation, M waves, MEPs, and contractile properties) with age (young, old, and very old) and sex (men and women) as the between subject factors. The relative changes in mechanical power, MVC, and the mechanistic measurements from the beginning to the end of the fatiguing exercise were also compared with an individual univariate ANOVA with age and sex as the grouping variables. When a significant main effect for age or an interaction was found, pair-wise post hoc comparisons were performed using Tukey's test. Simple linear regression analyses were performed between the reductions in mechanical power and the mechanistic measurements to identify the primary mechanisms of fatigue.

Normal distributions and homogeneity of variance of the data were performed before any statistical comparisons and were assessed using the Kolmogorov-Smirnov test and Levene's statistic, respectively. If the assumptions of a normal distribution and/or homogeneity of variance were violated, then the nonparametric Kruskal-Wallis test was performed instead of the univariate ANOVA, with age and/or sex as the grouping variables (e.g., voluntary activation). If the assumptions were violated for the repeated-measure ANOVAs, then the nonparametric Friedman's test was performed (e.g., voluntary activation). All significance levels were set at *P* < 0.05, and all statistics were performed using SPSS (version 24; IBM, Chicago, IL). Data are presented as the means ± SD in the text and tables and means ± SE in the figures.

RESULTS

Mechanical Power Output

The power output at the beginning of the dynamic exercise showed a main effect of age ($P < 0.001$; $\eta_p^2 = 0.51$) and was 97 and 217% higher in the young (245.3 ± 75.7 W) compared with old (124.8 ± 51.7 W; $P < 0.001$) and very old adults (77.3 ± 17.2 W; $P < 0.001$), respectively, and 61% higher in the old compared with the very old ($P = 0.001$) (Fig. 3). In addition, the initial power outputs were 64% higher in all men (189.4 ± 86.2 W) compared with all women (115.8 ± 59.2 W; $P < 0.001$; $\eta_p^2 = 0.22$) irrespective of age.
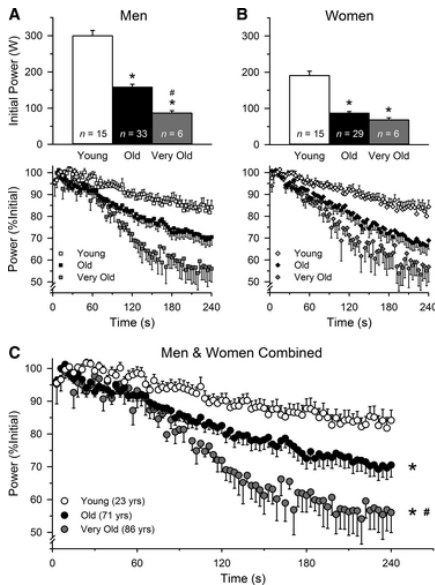
Fig. 3. Power output during the high-velocity fatiguing exercise. *A* and *B*: mean absolute mechanical power outputs and contraction-by-contraction relative power outputs (%Initial) measured from the dynamic fatiguing exercise for the young, old, and very old men (*A*) and women (*B*). *C*: because the relative reductions in power did not differ between men and women in any of the 3 age cohorts ($P > 0.05$), the men and women were combined for the young, old, and very old. Fatigability progressively increased with age, so that the least amount of relative power loss occurred in the young adults and the most occurred in the very old adults. Values are means ± SE. *$P < 0.05$, significantly different from young; #$P < 0.05$, significantly different from old.

Fatigability (reductions in power).

The relative reductions in power from the beginning to the end of the dynamic exercise showed a main effect of age ($P < 0.001$; $\eta_p^2 = 0.19$) and were greater in the very old (44 ± 15%; $P < 0.05$) and old (31 ± 20%; $P < 0.05$) compared with the young (17 ± 12%) and greater in the very old compared with the old ($P < 0.05$) (Fig. 3). However, there were no sex differences in the relative reductions in power ($P = 0.801$; $\eta_p^2 = 0.00$) for the young (men = 17 ± 12% and women = 17 ± 12%), old (men = 30 ± 20% and women = 32 ± 21%), or very old adults (men = 44 ± 16% and women = 44 ± 16%) (Fig. 3).

Range of Motion

The total ROM at the beginning of the fatiguing exercise showed a main effect of age ($P < 0.001$; $\eta_p^2 = 0.12$) and was greater in both the young (91.3 ± 2.5°; $P < 0.001$) and the old (89.5 ± 5.3°; $P = 0.007$) compared with the very old adults (86.9 ± 3.4°) but did not differ between the young and old adults ($P = 0.211$). There were also no sex differences in the ROM at the beginning of the dynamic exercise between the men (89.9 ± 4.2°) and women (89.6 ± 5.0°; $P = 0.755$; $\eta_p^2 = 0.00$).

However, the ROM at the end of the dynamic exercise was lower than at the start of the exercise by $1.8 \pm 2.1°$ in the young ($P < 0.001$; $\eta_p^2 = 0.60$), $7.7 \pm 8.6°$ in the old ($P < 0.001$; $\eta_p^2 = 0.51$), and $17.7 \pm 12.3°$ in the very old adults ($P = 0.001$; $\eta_p^2 2 = 1.00$). The reductions in the ROM over the course of the fatiguing exercise showed a main effect of age ($P < 0.001$; $\eta_p^2 = 0.25$) and were greater in the old and very old compared with the young adults ($P < 0.001$) and in the old compared with the very old ($P < 0.001$) but did not differ between the men ($7.1 \pm 9.8°$) and women ($7.3 \pm 8.3°$; $P = 0.675$; $\eta_p^2 = 0.00$) (Table 3).

Table 3. Mechanical outputs and range of motion at the beginning and end of the fatiguing dynamic exercise

Variable	Young (_35 yr)			Old (60–79 yr)		Very Old (_80 yr)	
	Men (15)	Women (15)		Men (33)	Women (29)	Men (6)	Women (6)
Power							
Beginning,*† W	300.0 ± 57.6	190.8 ± 47.0		158.0 ± 45.8	86.9 ± 25.1	86.5 ± 15.5	68.2 ± 14.4
End,*† W	247.7 ± 56.3	156.4 ± 38.0		108.9 ± 41.3	57.0 ± 19.6	50.7 ± 20.3	37.0 ± 10.4
Velocity							
Beginning, *† °/s	254.6 ± 11.6	235.9 ± 19.8		211.9 ± 23.9	192.0 ± 25.5	185.2 ± 13.3	185.2 ± 20.3
End,* °/s	232.5 ± 21.3	216.1 ± 27.4		165.7 ± 35.9	146.2 ± 34.2	117.8 ± 38.3	124.0 ± 28.4
Torque							
Beginning, *† N·m	68.9 ± 10.5	48.5 ± 8.1		44.1 ± 9.3	27.9 ± 4.6	30.7 ± 4.4	23.7 ± 3.4
End,*† N·m	63.0 ± 10.3	44.0 ± 7.1		38.6 ± 8.9	23.5 ± 4.0	26.1 ± 5.1	18.4 ± 2.3
Range of Motion							
Beginning,* °	91.3 ± 2.3	91.3 ± 2.8		90.3 ± 4.4	88.6 ± 6.1	84.3 ± 2.4	89.6 ± 1.7
End,* °	89.8 2.2	89.2 ± 3.2		83.1 ± 8.6	80.3 ± 10.2	64.1 ± 15.7	74.3 ± 8.0

Values are reported as means ± SD. Power, torque and velocity are the average values calculated over the entire shortening phase of the knee extension. Because the power outputs increased over the first few contractions in some participants, the recorded baseline power, torque, and velocity for each participant are the highest average obtained from 5 sequential contractions within the first 10 contractions at the beginning of the dynamic exercise. The reported power, torque, velocity, and range of motion at the end of the fatiguing dynamic exercise are the average values from the last 5 contractions. *P < 0.05, significant effect of age. †P < 0.05, significant effect of sex.

MVC Torque Output

Baseline isometric MVC torque outputs obtained from the five sets of MVC-60–80% contractions showed a main effect of age ($P < 0.001$; $\eta_p^2 = 0.41$) and were 67 and 145% higher in the young (223.8 ± 74.8 N·m) compared with the old (134.2 ± 48.1 N·m; $P < 0.001$) and very old adults (91.2 ± 28.8 N·m; $P < 0.001$), respectively, and 47% higher in the old compared with the very old ($P = 0.003$) (Fig. 4). Additionally, the MVC torque outputs were 61% higher in all men (189.9 ± 73.3 N·m) compared with all women (117.6 ± 47.4 N·m; $P < 0.001$; $\eta_p^2 = 0.32$) irrespective of age.
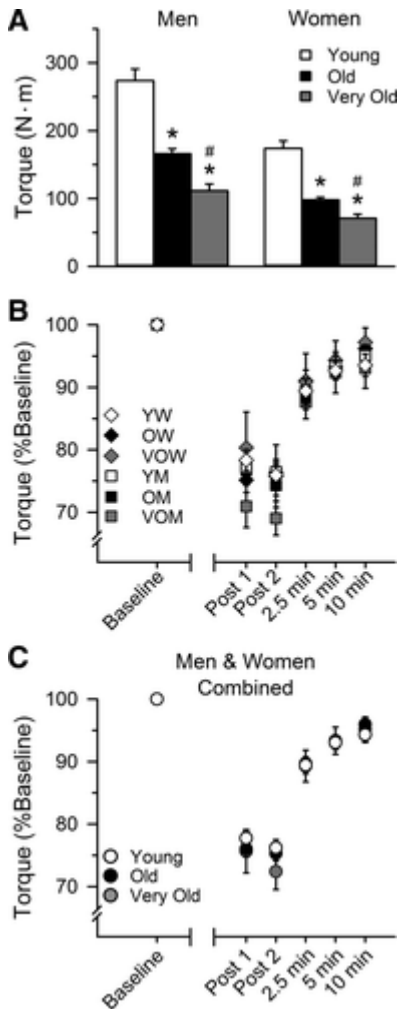
Fig. 4. Maximum voluntary contraction (MVC) isometric torque output before and after the high-velocity fatiguing exercise. *A*: absolute isometric torque outputs before the dynamic fatiguing exercise progressively decreased with age and were greater in men compared with women. *B*: the relative loss in isometric torque immediately following the fatiguing exercise (Post 1 and Post 2) and into recovery (2.5, 5, and 10 min) did not differ between the sexes for the young men (YM) and women (YW), old men (OM) and women (OW), or the very old men (VOM) and women (VOW). *C*: similarly, when the men and women were combined in the three age cohorts, the relative loss in isometric torque did not differ between the age groups at any time point. Values are means ± SE. *$P < 0.05$, significantly different from young; #$P < 0.05$, significantly different from old.

Fatigability (reductions in MVC torque).

All cohorts (young, old, and very old men and women) had a significant reduction in their MVC torque following the dynamic fatiguing exercise ($P < 0.001$; $\eta_p^2 = 0.86$) (Fig. 4). However, the relative reductions in MVC

immediately following the exercise (Post 1) were not different between the age groups (young = 22 ± 6%, old = 24 ± 10%, and very old = 24 ± 12%; P = 0.665; η_p^2 = 0.01) or between men (24 ± 9%) and women (23 ± 10%; P = 0.680; η_p^2 = 0.00). There were also no differences in MVC torque (%Baseline) during the recovery measurements based on age (P = 0.192; η_p^2 = 0.03) or sex (P = 0.339; η_p^2 = 0.01) (Fig. 4). Because no age or sex differences were observed in recovery, we restricted our analyses of the mechanistic measurements to those performed immediately following the fatiguing exercise (Post 1).

Voluntary Activation

Baseline voluntary activation from the five sets of MVC-60–80% contractions and calculated with the estimated resting twitch (Eq. 1) were not different between the age groups (young = 98 ± 2%, old = 97 ± 3%, and very old = 95 ± 7%; P = 0.317; η_p^2 = 0.02) or between men (97 ± 3%) and women (97 ± 4%; P = 0.835; η_p^2 = 0.00) (Fig. 5 and Table 2). Of the initial 103 participants with baseline voluntary activation measurements, 7 participants (1 young woman, 3 old women, and 3 old men) were excluded from the postfatiguing exercise comparisons due to an inability to obtain a reliable estimated resting twitch (i.e., the 3-point regression R^2 was < 0.80). As a result, the calculations from Eq. 2 were used to test whether voluntary activation changed immediately following the fatiguing dynamic exercise.
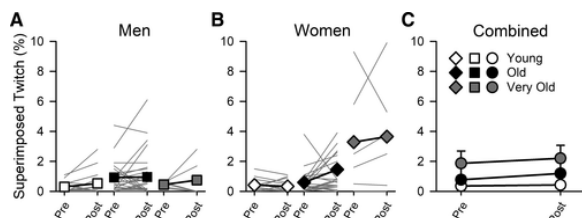
Fig. 5. Voluntary activation from the motor cortex before and immediately after the high-velocity fatiguing exercise. *A* and *B*: voluntary activation (Eq. 2) measured with TMS delivered to the motor cortex before (Pre) and immediately following the fatiguing exercise (Post 1) for men (*A*) and women (*B*). Group means for each age cohort are depicted by the black outlined symbols, while the individual data are depicted by the gray lines. *C*: because voluntary activation did not differ between men and women (P > 0.05), the men and women were combined for the young, old, and very old. Baseline voluntary activation did not differ between the age groups (P > 0.05) and did not change compared with baseline immediately following the fatiguing exercise (Post 1). Values for each group are means ± SE. Error bars are omitted in *A* and *B* and are obscured by the symbols for the young and old in *C*.

Similar to the findings from Eq. 1, the baseline voluntary activation calculated with the superimposed twitch (Eq. 2) did not differ between the age groups (P = 0.052; η_p^2 = 0.06) nor between men (0.7 ± 1.0%) and women (0.9 ± 1.6%; P = 0.761; η_p^2 = 0.00) (Table 2). The ability to voluntarily activate the muscle immediately following the fatiguing exercise (Post 1) did not change compared with baseline in the young men (P = 0.564; η_p^2 = 0.02) or women (P = 0.109; η_p^2 = 0.18), very old men (P = 0.414; η_p^2 = 0.13) or women (P = 0.655; η_p^2 = 0.04), or the old men (P = 1.000; η_p^2 = 0.00). However, the superimposed twitch (%) increased compared with baseline in the old women (P = 0.023; η_p^2 = 0.18), indicating a reduction in the ability to volitionally activate the muscle. Regression analyses with all participants included revealed no association between the relative reductions in power and the changes in voluntary activation (r = 0.191, P = 0.053).

M Waves and MEPs

Baseline M-wave peak-to-peak amplitudes (M_{max}) and areas for the VL, VM, and RF are presented in Table 2. Because the changes in the VL, VM, and RF M-wave areas and M_{max} following the fatiguing exercise were similar, we only report the data for the VL. The VL M-wave area immediately following the fatiguing exercise (Post 1)

increased compared with baseline for the young (Pre = 84.2 ± 17.8 mV·ms and Post = 86.1 ± 17.9 mV·ms; P = 0.003; η_p^2 = 0.27), old (Pre = 58.5 ± 19.4 mV·ms and Post = 62.9 ± 19.5 mV·ms; P < 0.001; η_p^2 = 0.40), and very old (Pre = 42.3 ± 17.5 mV·ms and Post = 45.1 ± 18.2 mV·ms; P = 0.021; η_p^2 = 0.40) (Fig. 6), but the relative changes did not differ between the age groups (P = 0.103; η_p^2 = 0.05) or between the sexes (P = 0.785; η_p^2 = 0.00). The VL M_{max} immediately following the fatiguing exercise increased compared with baseline in the old (Pre = 8.9 ± 3.8 mV and Post = 9.5 ± 3.7 mV; P < 0.001; η_p^2 = 0.30) but did not change in the young (Pre = 15.0 ± 3.9 mV and Post = 15.0 ± 3.8 mV; P = 0.738; η_p^2 = 0.00) or very old adults (Pre = 5.8 ± 2.9 mV and Post = 5.9 ± 2.7 mV; P = 0.490; η_p^2 = 0.04). Accordingly, the relative change in the VL M_{max} showed a main effect of age (P = 0.024; η_p^2 = 0.07) and was greater in the old (8.3 ± 14.5%) compared with the young (0.4 ± 4.5%; P= 0.016) but did not differ between the young and very old (4.0 ± 15.2%; P = 0.691), the old and very old (P = 0.515), or the men (4.1 ± 2.1%) and women (4.2 ± 2.2%; P = 0.965; η_p^2 = 0.00). Regression analyses revealed no association between the relative reductions in power and the changes in the VL M-wave area (r = 0.168, P = 0.089) or M_{max} (r = 0.122, P = 0.216).
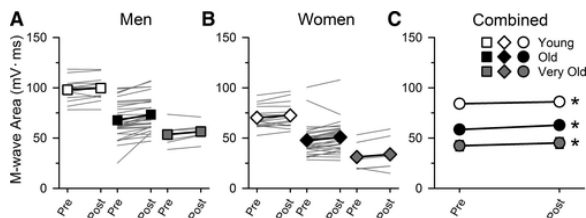
Fig. 6. Compound muscle action potentials before and immediately after the high-velocity fatiguing exercise. *A* and *B*: the compound muscle action potential areas (M-wave area) of the vastus lateralis measured before (Pre) and immediately following the fatiguing exercise (Post 1) for men (*A*) and women (*B*). Group means for each age cohort are depicted by the black outlined symbols, while the individual data are depicted by the gray lines. *C*: because the changes in the M-wave area elicited by the fatiguing exercise did not differ between the men and women in any of the three age cohorts (P > 0.05), the men and women were combined for the young, old, and very old. The baseline M-wave areas were greater in the young (84.2 ± 17.8 mV·ms) compared with the old (58.5 ± 19.4 mV·ms; P < 0.001) and greater in the old compared with the very old (42.3 ± 17.5 mV·ms; P = 0.003), but the relative increase in the M-wave area following the fatiguing exercise (*P < 0.05) did not differ among the young, old, and very old (P > 0.05). Values for each group are means ± SE. Error bars are omitted in *A* and *B* and are obscured by the symbols in *C*.

Baseline MEP data for the VL, VM, and RF are presented in Table 2. The peak-to-peak MEP amplitudes (%M_{max}) immediately following the fatiguing exercise (Post 1) did not change compared with baseline for the VL (Pre = 31 ± 11% and Post = 34 ± 12%; P = 0.054; η_p^2 = 0.04), VM (Pre = 35 ± 13% and Post = 36 ± 14%; P = 0.915; η_p^2 = 0.00), or RF (Pre = 44 ± 12% and Post = 45 ± 14%; P = 0.668; η_p^2 = 0.00).

Involuntary Contractile Properties

Baseline contractile properties from the electrical stimulation to the femoral nerve are presented in Table 2. The relative decrease in the amplitude of the potentiated resting twitch (Q_{tw}) immediately following the fatiguing exercise (Post 1) showed a main effect of age (P = 0.001; η_p^2 = 0.14) and was less in the young (−16 ± 17%) compared with the old (−30 ± 19%; P = 0.002) and very old (−35 ± 12%; P = 0.005) but did not differ between the old and very old (P = 0.562) nor between men (−28 ± 17%) and women (−24 ± 20%; P = 0.139; η_p^2 = 0.02) (Fig. 7). Similarly, the relative decrease in the rate of torque development of the Q_{tw} showed a main effect of age (P < 0.001; η_p^2 = 0.15) and was less in the young (−16 ± 18%) compared with the old (−33 ± 22%; P = 0.001) and very old (−39 ± 16%; P = 0.003) but did not differ between the old and very old (P = 0.550) nor between men

(−31 ± 20%) and women (−27 ± 23%; P = 0.185; η^2_p = 0.02). Of the initial 104 participants, 9 participants (6 old women, 2 old men, 1 very old woman) were unable to fully relax for the electrical stimulation immediately after the fatiguing exercise. For the remaining 95 participants (30 young, 54 old, and 11 very old), the relative increase in the half relaxation time showed a main effect of age (P = 0.001; η^2_p = 0.14) and was less in the young (29 ± 28%) compared with the old (67 ± 54%; P = 0.002) and very old (72 ± 59%; P = 0.034) but did not differ between the old and very old (P = 0.945) nor between men (53 ± 41%) and women (59 ± 60%; P = 0.998; η^2_p = 0.00).
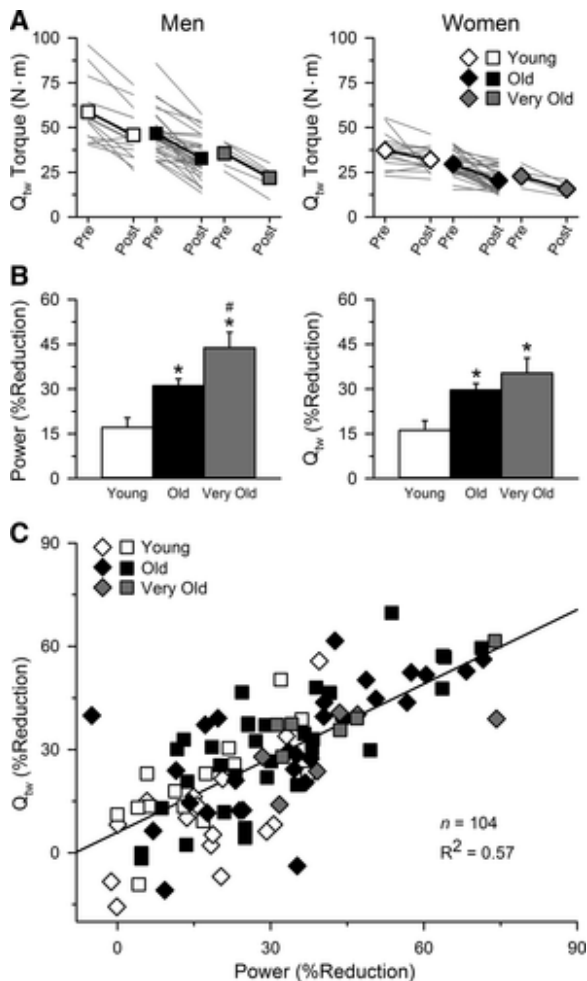
Fig. 7. Electrically evoked potentiated twitch amplitudes before and immediately after the high-velocity fatigue exercise. *A*: the potentiated twitch torque amplitude (Q_{tw}) measured before (Pre) and immediately after the fatiguing exercise (Post 1) for the young, old, and very old men and women. Group means for each age cohort are depicted by the black outlined symbols, while the individual data are depicted by the gray lines. *B*: the relative reductions in the Q_{tw} elicited by the fatiguing exercise showed a qualitatively similar trend with aging to the relative reductions in mechanical power. *C*: regression analyses revealed that the percent reductions in mechanical power were best predicted by the percent reductions in the Q_{tw}. Values are means ± SE. *P < 0.05, significantly different from young; #P < 0.05, significantly different from old.

Baseline absolute (Nm/s) and normalized (s^{-1}) peak rates of torque relaxation from TMS to the motor cortex are also presented in Table 2. The relative decrease in the absolute peak rate of torque relaxation immediately following the fatiguing exercise (Post 1) showed a main effect of age (P < 0.001; η^2_p = 0.19) and was less in the young (−26 ± 11%) compared with the old (−40 ± 20%; P = 0.002) and very old (−53 ± 14%; P < 0.001) but did not differ between the old and very old (P = 0.051) nor between men (−36 ± 17%) and women (−29 ± 21%; P =

0.754; η_p^2 = 0.00). To account for the changes in the MVC torque outputs following the fatiguing exercise, the peak rates of torque relaxation were normalized to the MVC torque (MVC + SIT). Similar to the results from the absolute peak rate of torque relaxation, the relative decrease in the normalized peak rate of torque relaxation following the fatiguing exercise showed a main effect of age ($P < 0.001$; η_p^2 = 0.25) and was less in the young (−6 ± 9%) compared with the old (−24 ± 20%; $P < 0.001$) and very old (−35 ± 15%; $P < 0.001$) but did not differ between the old and very old ($P = 0.122$) nor between men (−19 ± 19%) and women (−22 ± 20%; $P = 0.760$; η_p^2 = 0.00).

Simple linear regression analyses revealed that the relative changes for all the contractile property measurements were significantly associated with the relative changes in mechanical power output during the fatiguing exercise: Q_{tw}($r = 0.75$; $P < 0.001$), rate of torque development of the Q_{tw} ($r = 0.74$; $P < 0.001$), half relaxation time ($r = −0.47$; $P < 0.001$), absolute peak rate of torque relaxation ($r = 0.64$; $P < 0.001$), and normalized peak rate of torque relaxation ($r = 0.54$; $P < 0.001$). However, the most closely associated variable with the reduction in mechanical power during the fatiguing exercise was the reduction in the Q_{tw} (Fig. 7).

DISCUSSION

This study determines the fatigability of the knee extensor muscles and identifies the primary mechanisms of fatigue in young, old, and very old men and women elicited by high-velocity concentric contractions. We show that aging of the neuromuscular system results in a progressive increase in the fatigability of the knee extensors during high-velocity contractions that is more pronounced in the very old adults (≥80 yr) and occurs similarly in both men and women (Fig. 3). We provide novel evidence that the neural drive from the motor cortex remains near optimal for the young, old, and very old adults but may play a minor role for the increased power loss of the knee extensors in old women (Fig. 5). Importantly, the age-related increase in power loss was strongly associated with the changes in electrically evoked contractile properties (Fig. 7), suggesting that the age-related increase in fatigability during high-velocity contractions was determined primarily by cellular mechanisms that disrupt excitation contraction coupling and/or cross-bridge function.

The Progressive Age-Related Increase in Fatigability of the Lower Limb Is Determined Primarily by Mechanisms Within the Muscle

In support of our hypotheses, we found that the power loss of the knee extensors performing a high-velocity fatiguing exercise progressively increased with age from a 17% loss in the young (23 yr) to a 31% loss in the old (71 yr) and a 44% loss in the very old adults (86 yr). Importantly, we also show that the age-related increase in fatigability was similar for both men and women (Fig. 3). A portion of the progressive increase in fatigability with aging may be the result of the decreased physical activity levels and increased sedentary behavior that is commonly observed in old compared with young adults (36). Indeed, the physical activity of the very old adults in our study was significantly lower than both the young and old (Table 1). However, because the old adults still experienced approximately twofold greater losses in relative mechanical power compared with the young (Fig. 3), despite having similar physical activity, it is unlikely that the age differences in fatigability observed here were due to differences in physical activity alone. Furthermore, our results likely underestimate the extent of the increased fatigability with aging, because in addition to the greater reductions in power, there were also greater decrements in the range of motion during the dynamic exercise for both the very old (~18°) and the old (~8°) compared with the young (~2°). The reduced range of motion would ultimately lead to a progressive decrease in the amount of mechanical work (J) performed per contraction throughout the exercise. Thus the incorporation of our findings with others (4, 9, 10, 37, 40, 45) reveals that age-related changes within the neuromuscular system result in an increased fatigability during high-velocity contractions that continually progresses into the latest stages of life and occurs similarly for both men and women (Fig. 3).

In testing the mechanisms for the increased fatigability with age, we found that the ability of the motor cortex to volitionally activate the muscle was reduced following the dynamic exercise in only the old women (Fig. 5). The exercise-induced reduction in the ability to volitionally activate the muscle, when assessed by delivering TMS to the motor cortex, suggests that suboptimal neural drive from the cortical motor neurons may be contributing to the increased fatigability with age in women. However, this mechanism likely plays only a minor role, because *1*) the changes in voluntary activation were not associated with the relative reductions in power, and *2*) the change in voluntary activation following the exercise was highly variable between individuals (Fig. 5). There were also no changes in the MEP amplitudes (%M_{max}) when measured during the MVC immediately following the fatiguing exercise, suggesting that the excitability of the corticospinal tract projecting to the quadriceps muscles was unaltered in all three age cohorts. Additionally, our findings are supported by studies on the dorsiflexors (37), plantarflexors (9), and knee extensors (10) that found no differences in the ability to voluntarily activate the muscle following high-velocity fatiguing exercises between groups of young and old men. However, it is important to note that methodological limitations make it difficult to evaluate the mechanisms of fatigue during a dynamic contraction. Thus we must infer that the changes observed in the maximal isometric contractions following the dynamic exercise are an accurate reflection of the voluntary activation during the exercise. Clearly, the fatigue-induced reductions in the isometric MVC rarely coincide directly with the changes in mechanical power (9, 45). Accordingly, the reduction in MVC torque in our study was similar across all three age groups (Fig. 4) despite large differences between the groups in the loss of power (Fig. 3). Future studies that develop a reliable measurement to test the ability of the nervous system to voluntarily activate the muscle during a dynamic contraction will clarify whether the nervous system is contributing to the increased fatigability with age.

There is growing evidence that the aging process is accompanied by motor unit remodeling and instability of the neuromuscular junction that is exacerbated after the age of ~75–80 yr (19). Thus it is plausible that the progressive age-related increase in fatigability during high-velocity exercise is due to impairments in neuromuscular transmission and the excitability of the sarcolemma. To test this possibility, we quantified the changes in the compound muscle action potential (M wave) area and amplitude of the quadriceps muscles elicited by the high-velocity exercise. Our data, however, showed an increase in the M-wave area of the VL immediately following the exercise for all three age cohorts that occurred similarly for both the men and women (Fig. 6). The mechanisms for the potentiation of the M wave following a fatiguing exercise are unclear (42), but we observed no association between the changes in the M wave and the reductions in power, suggesting that the mechanism for the increased fatigability with age does not involve the neuromuscular junction or the ability of the action potential to propagate across the sarcolemma. These findings are in agreement with the increased M-wave area of the VM observed in young (~25 yr) and old men (~74 yr) following a high-velocity knee extension exercise (10), but in contrast to the decreased M-wave amplitude observed in the soleus of older men (~78 yr) following a high-velocity plantarflexor exercise (9). The explanation for the disparities between the studies is unclear but may involve the differential effect of aging on these two muscle groups (6).

In contrast to the limited involvement of the nervous system (Fig. 5) or neuromuscular propagation (Fig. 6) in explaining the increased fatigability with aging, we found strong support that the progressive age-related increase in power loss during high-velocity exercise was closely associated with mechanisms that disrupt contractile function within the muscle (Fig. 7). Specifically, the greater age-related reductions in mechanical power were closely associated with changes in the involuntary twitch properties elicited by electrical stimulation to the femoral nerve, as well as the peak rates of relaxation elicited by TMS to the motor cortex. The reduction in the electrically evoked twitch amplitude, for example, explained 57% of the variance for the reduction in power during the fatiguing exercise (Fig. 7). Although the specific cellular and molecular mechanisms cannot be identified by the changes in the involuntary contractile properties, these properties do provide valuable insight to the cellular processes that likely contribute to fatigue (13, 28). For example, the greater slowing of the

relaxation rates in the old compared with young adults following the exercise indicate that the mechanism likely involves factors that slow cross-bridge detachment and/or the uptake of Ca^{2+} back into the sarcoplasmic reticulum ([13]). Additionally, the greater reductions in the amplitude ([Fig. 7]) and the rates of torque development of the potentiated twitch in the old and very old adults compared with the young suggest that the mechanism also likely involves factors that either *1*) decrease the amplitude of the Ca^{2+} transient, *2*) reduce the number of cross bridges formed and/or the amount of force generated per cross bridge, and/or *3*) slow the transition step from the low- to high-force state of the cross-bridge cycle ([1], [11], [13]).

The leading cellular mechanisms purported to be responsible for the exercise-induced reductions in mechanical power within the muscle are an accumulation of metabolic by-products (i.e., H^+, P_i, and H_2PO-4H_2PO4-) that act to both directly inhibit cross-bridge function ([11]) and to impair excitation-contraction coupling ([1], [13]). Thus it is plausible that age-related changes within the muscle result in an increased production of metabolic by-products and/or an increased sensitivity of the muscle to a given concentration of metabolite accumulation during high-velocity exercise. However, preliminary data on single muscle fibers isolated from biopsies of the VL have shown that the decrements in peak fiber power elicited by a fatigue-mimicking condition (pH 6.2 + 30 mM P_i) were not different in fibers isolated from young (<35 yr) compared with old (>70 yr) adults ([11], [50]). These data suggest that the age-related increase in power loss during high-velocity exercise is not likely due to an increased sensitivity of the cross bridge to H^+ and P_i, at least not under saturating Ca^{2+} conditions ([50]). In contrast, there is evidence for an ~37% increase in the ATP cost of contraction during a dynamic plantarflexor exercise in old (~74 yr) compared with young (~22 yr) men and women ([33]). The greater ATP demand for a given amount of mechanical power output would presumably lead to a greater accumulation of metabolic by-products in old compared with young adults. Indeed, we are currently testing this hypothesis by measuring the accumulation of metabolic by-products (H^+, P_i, and H_2PO-4H_2PO4-) with phosphorus magnetic resonance spectroscopy (^{31}P-MRS) in the quadriceps of young and old men and women performing a high-velocity knee extension exercise in the magnet.

Concluding Remarks

Our data provide evidence that aging of the neuromuscular system results in an increased fatigability during high-velocity contractions of the knee extensors that continually progresses into the latest stages of life (≥80 yr) and occurs similarly for both men and women. By coupling noninvasive stimulation procedures to both the motor cortex and the peripheral nervous system with measures of surface EMG and torque output, we were able to localize the primary mechanism for the increased fatigability with aging to factors within the muscle. We conclude that the age-related increased power loss during high-velocity fatiguing exercise of the lower limb is determined primarily by cellular mechanisms that disrupt excitation contraction coupling and/or cross-bridge function and that the mechanisms are similar for both men and women.

GRANTS

DISCLOSURES

No conflicts of interests, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.W.S. and S.K.H. conceived and designed research; C.W.S., A.K., and H.H. performed experiments; C.W.S. and A.K. analyzed data; C.W.S. and S.K.H. interpreted results of experiments; C.W.S. prepared figures; C.W.S. drafted

manuscript; C.W.S. and S.K.H. edited and revised manuscript; C.W.S., A.K., H.H., and S.K.H. approved final version of manuscript.

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