8-1-2017

Physical Activity Modulates Corticospinal Excitability of the Lower Limb in Young and Old Adults

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Accepted version. *Journal of Applied Physiology*, Vol. 123, No. 2 (August 2017): 364-374. DOI.

Journal of Applied Physiology, Vol. 123, No. 2 (2017): 364-374. DOI.

Physical activity modulates corticospinal excitability of the lower limb in young and old adults

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Abstract

Aging is associated with reduced neuromuscular function, which may be due in part to altered corticospinal excitability. Regular physical activity (PA) may ameliorate these age-related declines, but the influence of PA on corticospinal excitability is unknown. The purpose of this study was to determine the influence of age, sex, and PA on corticospinal excitability by comparing the stimulus-response curves of motor evoked potentials (MEP) in 28 young (22.4 ± 2.2 yr; 14 women and 14 men) and 50 old adults (70.2 ± 6.1 yr; 22 women and 28 men) who varied in activity levels. Transcranial magnetic stimulation was used to elicit MEPs in the active vastus lateralis muscle (10% maximal voluntary contraction) with 5% increments in stimulator intensity until the maximum MEP amplitude. Stimulus-response curves of MEP amplitudes were fit with a four-parameter sigmoidal curve and the maximal slope calculated ($slope_{max}$). Habitual PA was assessed with tri-axial accelerometry and participants categorized into either those meeting the recommended PA guidelines for optimal health benefits (>10,000 steps/day, high-PA; $n = 21$) or those not meeting the guidelines (<10,000 steps/day, low-PA; $n = 41$). The MEP amplitudes and $slope_{max}$ were greater in the low-PA compared with the high-PA group ($P < 0.05$). Neither age nor sex influenced the stimulus-response curve parameters ($P > 0.05$), suggesting that habitual PA influenced the excitability of the corticospinal tract projecting to the lower limb similarly in both young and old adults. These findings provide evidence that achieving the recommended PA guidelines for optimal health may mediate its effects on the nervous system by decreasing corticospinal excitability.

NEW & NOTEWORTHY Transcranial magnetic stimulation was used to determine whether achieving the recommended 10,000 steps/day for optimal health influenced the excitability of the corticospinal tract projecting to the knee extensor muscles. Irrespective of age and sex, individuals who achieved >10,000 steps/day had lower corticospinal excitability than those who performed <10,000 steps/day, possibly representing greater control of inhibitory and excitatory networks. Physical activity involving >10,000 steps/day may mediate its effects on the nervous system by decreasing corticospinal excitability.

Human aging is accompanied by multiple impairments of the neuromuscular system that are associated with a decline in motor function, a reduced ability to perform daily activities, and a decreased quality of life.[18,22,24,49] The mechanisms for the age-related neuromuscular deficits occur as a result of impairments within both the skeletal muscle and the nervous system. For example, within the nervous system, aging is associated with cortical atrophy, including the precentral gyrus,[46] reduced brain white matter and myelinated nerve fiber length,[33] altered brain neurochemistry,[38,48] reduced motor unit number,[40,43] and instability of neuromuscular transmission.[22] Ultimately, these age-related changes may be reflected by alterations in the excitability of the corticospinal tract.[12] Corticospinal excitability can be investigated noninvasively by delivering increasing intensities of transcranial magnetic stimulation (TMS) to the primary motor cortex and measuring the elicited motor evoked potentials (MEPs) at the muscle with surface electromyography (EMG). This experimental approach generates a stimulus-response curve that is usually sigmoidal, with the curve characteristics representative of the net excitatory and inhibitory synaptic inputs of the cortical and spinal motoneurons.[6,16,50] Despite the sensitivity of this technique to pharmacological perturbations that alter corticospinal excitability,[4] studies investigating the stimulus-response characteristics with aging are limited and have been conducted primarily on quiescent muscles of the upper limb,[42,54] with few studies conducted on active muscles[58] or muscles of the lower limb.[56]

The findings from the few studies that have investigated the effects of aging on corticospinal excitability when assessed by the stimulus-response curve are equivocal, with some studies reporting lower excitability with age[39,42,47,58] and others finding no differences.[53,54,56] The explanation for the disparities between studies is not clear but may be due to multiple factors, including the muscle group studied, the level of muscle activation during the measurements, the biological sex, and/or the physical activity (PA) level of the participants. For example, studies on the first dorsal interosseous (FDI) muscle demonstrated that an age-related decrease in corticospinal excitability may be exclusive to old women[42,54] or when the muscle is activated.[58] In contrast, no age or sex differences were observed in the vastus lateralis muscle; however, all the young and old participants in this study reported engaging in regular PA.[56] Taken together, these data suggest that the age-related decrease in corticospinal excitability may be limited to muscles of the upper limb and/or can be ameliorated by regular engagement in PA.

The benefits of regular PA and exercise training with increased age include increases in brain volume via increased gray and white matter density and improved motor and cognitive performance.[1,13,14,19,62] Indeed, studies employing noninvasive brain stimulation and imaging techniques show that high levels of PA may reduce age-related loss of interhemispheric inhibition.[35,36] Additionally, other studies, albeit in young adults, indicate that high levels of PA can enhance the response to an excitatory paradigm that is thought to be regulated by long-term potentiation-like mechanisms.[10] Furthermore, even a single acute bout of aerobic exercise was shown to modulate the excitability of several motor intracortical inhibitory networks.[37,51,52] Despite this emerging evidence, the influence of age, sex, and PA levels on the excitability of the corticospinal tract projecting to the lower limb remains unknown.

The purpose of this study was to determine the influence of age, sex, and PA levels on the corticospinal excitability of a lower limb muscle by comparing the stimulus-response curves of MEPs during low-intensity activation. We specifically measured the stimulus-response curve during activation because it is functionally more relevant, and the excitability of the corticospinal tract differs in an active compared with rested state.[16] We targeted the knee extensor muscles because *1*) the knee extensors are highly susceptible to age-related losses in muscle function[5,25,27] *2*) knee extensor mechanical power output is closely associated with functional performance in old adults,[2,44] and *3*) the knee extensors are particularly sensitive to prolonged periods of disuse[15] and to lower physical activity levels that accompany aging.[28] Based on the previous research conducted on the FDI muscle that found an age-related decrease in corticospinal excitability in old women[42,54] or when the muscle was active,[58] we hypothesized that in the active knee extensor muscles, old adults would have lower corticospinal excitability compared with young adults and that the difference would be more pronounced in the old women. In addition, we hypothesized that, regardless of sex, the steepness of the stimulus-response curves would be greater in active young and old adults and that the age-related difference in corticospinal excitability would be less in the old adults who engaged regularly in high levels of PA (i.e., >10,000 steps/day). The cutoff of 10,000 steps/day was chosen based on the PA guidelines that recommend ~10,000 as the minimum number of steps required for health benefits in young and old men and women.[59–61]

MATERIALS AND METHODS

Subjects.

Seventy-eight individuals participated in this study: 28 young adults (19–28 yr; 14 men and 14 women) and 50 old adults (61–87 yr; 28 men and 22 women). All participants provided written informed

consent and underwent a general health screening, which included body composition assessment via dual X-ray absorptiometry (GE Lunar Prodigy densitometer, Madison, WI). Each participant was apparently healthy and free of any neurological conditions. All experimental procedures were approved by the Marquette University Institutional Review Board and conformed to the principles of the Declaration of Helsinki.

Experimental protocol.

Participants reported to the laboratory on two occasions, once for a familiarization session and once for an experimental session to measure the stimulus-response characteristics of the corticospinal tract to the knee extensor muscles. Testing was performed on the limb the participant reported was their preferred kicking leg (dominant leg), except where the participant reported a previous surgical procedure of the dominant leg (4 old, 3 women; 1 young woman). During the familiarization session, 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 voluntary contractions (MVCs) and submaximal isometric contractions. For both sessions, participants were seated upright in the high Fowler's position with their knee fixed at 90° flexion in a Biodex System 4 Dynamometer (Biodex Medical, Shirley, NY). The position of the ergometer was adjusted so that the axis of rotation of the ergometer's lever arm was aligned with the axis of rotation of the participant's knee. The length of the ergometer'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 participants to the seat with the ergometer's four-point restraint system at the shoulders and hips. Participants were prohibited from grasping any part of the ergometer with their hands to ensure that the measured torques were generated primarily by the knee extensor muscles.

The experimental session began with electrical stimulation of the femoral nerve at rest to quantify the maximum peak-to-peak compound muscle action potential [maximum M-wave (M_{max})] of the vastus lateralis muscle. Following electrical stimulation, participants performed a minimum of three brief (2–3 s) MVCs to obtain the maximum voluntary isometric torque of the knee extensors. Participants were provided strong verbal encouragement and visual feedback on their performance with a 56-cm computer monitor mounted 1–1.5 m directly in front of their line of vision. Each MVC was interspersed with ≥60 s of rest, and MVC attempts were continued until the two highest values were within 5% of each other. Torque output from the ergometer's transducer was digitized at 500 Hz with a Power 1401 A/D converter and stored online using Spike 2 software [Cambridge Electronics Design (CED), Cambridge, UK]. The highest isometric torque output was considered the individual's MVC and was used to calculate the 10% MVC target torque to acquire the active stimulus-response curves.

Physical activity assessment.

Physical activity was quantified for each participant with a triaxial accelerometer (GT3X; ActiGraph, Pensacola, FL) worn around the waist for ≥4 days (2 weekdays and 2 weekend days). Participants were provided a PA log and standardized instructions to wear the ActiGraph monitor throughout the course of the day, excluding periods of bathing or sleeping, for a minimum of 4 days. Data were exported at 60-s epochs and analyzed with the Actilife version 6 software (ActiGraph). Wear time validation was compared with the self-reported activity logs, and the data were included in the analyses if the accelerometer was worn for a minimum of 8 h for ≥3 days. To test the influence of PA on the corticospinal excitability of the active knee extensor muscles, participants were dichotomized into two

categories of either high (≥10,000 steps/day) or low PA (<10,000 steps/day) based on the upper end of the current PA guidelines (60, 61).

Electrical stimulation and electromyographic recordings.

Surface Ag/AgCl electromyographic (EMG) electrodes (Grass Products; Natus Neurology, Warwick, RI) were adhered to the skin in a bipolar arrangement overlying the muscle bellies of the vastus lateralis, vastus medialis, rectus femoris, and bicep 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 (CED).

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 vastus lateralis, vastus medialis and rectus femoris. 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 at 100–400 V. Stimulus intensity began at 50 mA and was increased incrementally by 50 mA until a plateau was reached in both the unpotentiated resting twitch torque (Q_{tw}) and the M_{max} for all three quadriceps muscles. The intensity was then increased by an additional 20% to ensure that the stimuli were supramaximal (range 120–720 mA). Three stimuli were delivered at the supramaximal intensity to the resting muscle and averaged to obtain M_{max} for the vastus lateralis muscle.

Transcranial magnetic stimulation.

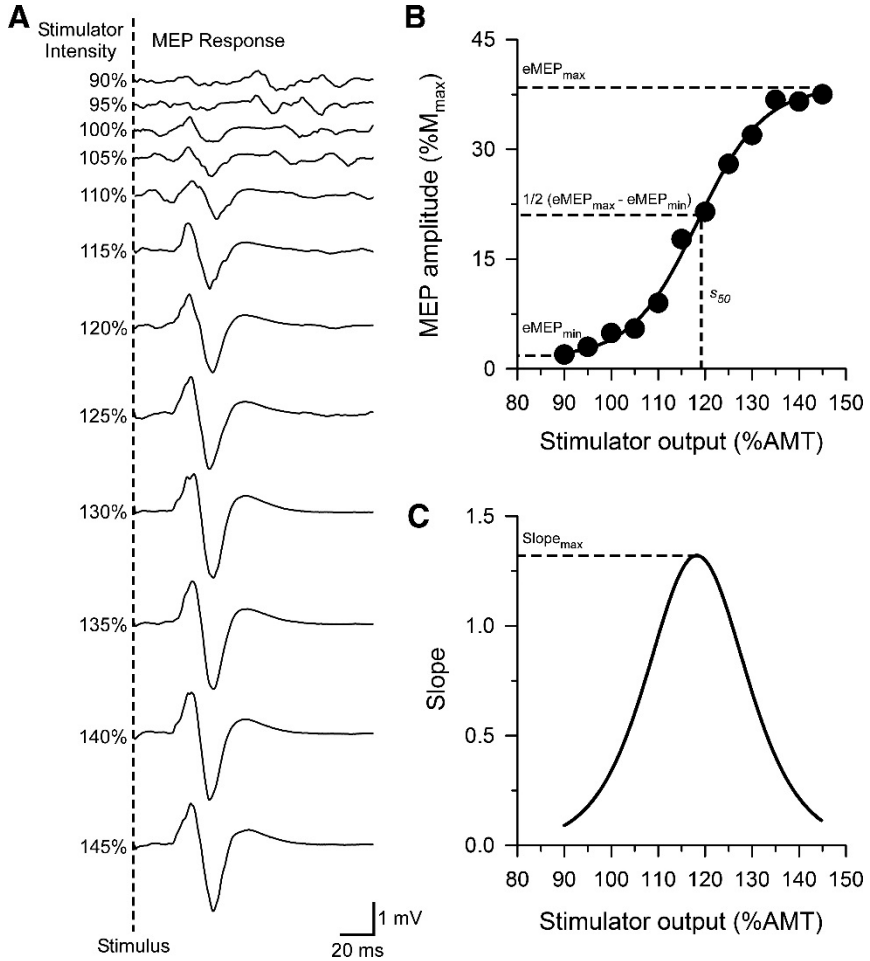
The motor cortex was stimulated by delivering a 1-ms duration magnetic pulse with a concave double-cone coil (110-mm diameter: maximum output 1.4 T) connected to a monophasic magnetic stimulator (Magstim 200^2; Magstim, Whitland, UK). The coil was initially positioned with the center of the coil ~1 cm 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 (EEG) cap and was determined as the location that elicited the greatest MEP in the vastus lateralis while the subject contracted at 10% MVC. This position was marked on the EEG cap to ensure repeatable placement of the coil for the remainder of the experiment.

Stimulus-response protocol.

The active motor threshold (AMT) of the vastus lateralis was determined during brief isometric contractions at 10% MVC. The required torque output for each participant was provided visually, with a horizontal bar superimposed on the torque displayed on the computer monitor. For both the determination of the AMT and obtaining the stimulus-response curve, participants were verbally cued to contract for 2–3 s once every 10 s (7–8 s of relaxation between contractions). Single-pulse TMS was delivered to the contralateral motor cortex after the participant had sustained the 10% MVC for 2 s. An EMG response to TMS that was at least twofold greater than the prestimulus EMG was considered an MEP. The AMT was determined as the minimum stimulator intensity eliciting MEPs in at least four of eight trials in the vastus lateralis. Once the AMT was acquired, 11 stimulator intensities ranging from 90 to 140% of the AMT in 5% increments were used to obtain each participant's stimulus-response curve of the MEP amplitudes evoked in the active vastus lateralis (Fig. 1). The 11 stimulator intensities were administered in a randomized order, with 10 stimuli delivered at each intensity. Each set of 10

contractions was separated by ≥60 s of rest. Peak-to-peak MEP amplitudes for each stimulus were measured online with custom software (Spike 2; CED), and the average of the 10 stimuli was calculated. The average MEP amplitudes were plotted against the stimulus intensity to visualize the stimulus-response curve. If a clear plateau in MEP amplitude was not present after the initial 11 intensities (37 old, 17 women; 17 young, 10 women), additional intensities were administered in 5% increments starting at 145% of the AMT either until *1*) a plateau in the MEP amplitude was observed or *2*) the maximum stimulator output was reached (10 old, 7 women; 1 young woman) (42, 54). A plateau in the stimulus-response curve was obtained if the MEP amplitude showed little to no increase for a minimum of three incremental stimulator intensities. The highest average peak-to-peak MEP amplitude recorded for a sequence of 10 stimuli was considered the individual specific maximal MEP amplitude (MEP_{max}).

Fig. 1. *A*: raw motor evoked potential (MEP) responses from the vastus lateralis evoked with transcranial magnetic stimulation (TMS) intensities ranging from 90 to 145% of the active motor threshold (%AMT) for a representative young woman. *B*: the average peak-to-peak MEP amplitude of the 10 stimulations for each



intensity expressed relative to M_{max} (%M_{max}) was plotted against the stimulator intensities to obtain the stimulus-response curve. The curve was fit with a 4-parameter sigmoidal function that generated an estimate of the minimum ($eMEP_{min}$) and maximum ($eMEP_{max}$) MEP amplitudes as well as the stimulator intensity (s_{50}), eliciting an MEP response midway between $eMEP_{max}$ and $eMEP_{min}$. *C*: to quantify the maximum slope ($slope_{max}$) of the stimulus-response curve, the derivative of *Eq. 1* was calculated and plotted against the stimulator intensity.

Data analysis.

To account for the differences in the vastus lateralis (VL) M_{max} amplitudes between participants (Table 1), all MEP amplitudes were expressed relative to the individual specific M_{max}. The average peak-to-peak MEP amplitude of the 10 stimulations at each intensity was then plotted against the stimulator intensities expressed relative to the AMT to obtain the stimulus-response curve (Fig. 1). Each participant's stimulus-response curve was fit with a four-parameter sigmoidal function (42, 54). The four-parameter iterative procedure (Sigmaplot 12.5; Systat Software, San Jose, CA) used the Levenberg-Marquard algorithm to minimize the residuals using the following equation (*Eq. 1*):

$$\mathrm{MEP}(s) = \mathrm{eMEP}_{\mathrm{min}} + \frac{\mathrm{eMEP}_{\mathrm{max}} - \mathrm{eMEP}_{\mathrm{min}}}{1 + e^{-(\frac{s - s_{50}}{b})}}$$

Table 1. Anthropometrics and stimulus-response curve characteristics for all participants

	Men		**Women**		***P* Value**		
Variable	**Young (14)**	**Old (28)**	**Young (14)**	**Old (22)**	**Age**	**Sex**	**Age × Sex**
Age, yr	22.4 ± 0.6	70.1 ± 1.1	22.3 ± 0.5	70.4 ± 1.4	<**0.001**	0.958	0.897
Height, cm	176.1 ± 2.2	177.0 ± 1.7	164.6 ± 1.5	161.3 ± 0.9	0.475	<**0.001**	0.208
Weight, kg	74.5 ± 2.7	83.6 ± 2.2	63.8 ± 2.3	65.1 ± 2.5	0.052	<**0.001**	0.146
BMI, kg/m^{-2}	23.9 ± 0.4	26.7 ± 0.6	23.5 ± 0.7	25.1 ± 0.9	**0.009**	0.210	0.458
Body fat, %	17.3 ± 0.8	29.2 ± 1.1	28.9 ± 1.8	38.0 ± 1.5	<**0.001**	<**0.001**	0.331
MVC torque, Nm	273.5 ± 17.1	185.1 ± 10.2	184.5 ± 11.6	107.6 ± 5.3	<**0.001**	<**0.001**	0.616
AMT (%SO)	42.9 ± 1.8	52.8 ± 2.0	44.5 ± 2.5	53.0 ± 2.7	<**0.001**	0.701	0.780
M_{max}, mV	18.6 ± 0.8	9.5 ± 0.8	11.4 ± 0.8	6.9 ± 0.5	<**0.001**	<**0.001**	**0.004**
MEP_{max}, mV	5.1 ± 0.7	2.5 ± 0.2 (24)	3.5 ± 0.4 (12)	2.1 ± 0.2 (19)	<**0.001**	**0.006**	0.122
MEP_{max} (%M_{max})	28.6 ± 4.3	29.2 ± 2.7 (24)	32.7 ± 4.1 (12)	33.0 ± 3.1 (19)	0.893	0.268	0.957
$Slope_{max}$	0.95 ± 0.17	0.72 ± 0.06 (24)	0.92 ± 0.11 (12)	0.89 ± 0.11 (19)	0.261	0.543	0.401

Values are means ± SE. BMI, body mass index; MVC, maximal voluntary contraction; AMT, active motor threshold; MEP_{max}, motor evoked potential maximum; $slope_{max}$, maximal slope of the stimulus-response curve. MVC torque was the highest 0.5-s average isometric torque output. The percentage of the stimulator output (%SO) that elicited an MEP in at least 4 of 8 stimulations during the 10% MVC contractions was considered the AMT. M_{max} was the average peak-to-peak maximal compound muscle action potential amplitude, or M-wave, from the 3 supramaximal electrical stimuli delivered to the femoral nerve in the unpotentiated, rested state. MEP_{max} was the highest average peak-to-peak MEP amplitude recorded for a sequence of 10 stimuli (reported in absolute mV) and relative to M_{max} (%M_{max}). The greatest rate of change in the MEP amplitude for a given increase in stimulator intensity was considered the $slope_{max}$. MEP_{max} and $slope_{max}$ were reported only for those participants with a 4-paramater sigmoidal stimulus-response curve, and the sample sizes are presented as the numbers within the parentheses. Boldfaced *P* values highlight statistical significance at $P < 0.05$.

where MEP (*s*) is the MEP amplitude for a given stimulator intensity *s*, $eMEP_{max}$ and $eMEP_{min}$ are the maximum and minimum MEP amplitudes, respectively, estimated by the function, s_{50} is the stimulator intensity eliciting an MEP response that is midway between $eMEP_{max}$ and $eMEP_{min}$, *b* is the slope of the curve at s_{50}, and *e* is the base of the natural logarithm. To quantify the maximum slope of the curve ($slope_{max}$), we calculated the derivative of *Eq. 1* and plotted it against the stimulator intensities expressed as a %AMT (Fig. 1).

Data were excluded from the analysis either if a plateau in the stimulus-response curve was not obtained or if the number of iterations (algorithmic attempts to fit the data) to obtain a four-parameter sigmoidal curve fit was exceeded. As a result, data from seven old participants were removed; i.e., two men and one woman did not reach a plateau in their stimulus-response curve, and two men and two women exceeded the number of iterations to enable a four-parameter sigmoidal curve fit. Data from two young participants were also removed; i.e., one woman did not reach a plateau in the curve, and another woman exceeded the number of iterations to enable a sigmoidal curve fit. Thus, 69 participants were included in the analysis of the four-parameter sigmoidal curve (Table 1 and Fig. 2). In addition, the minimum wear times for PA assessment were not met by three old men, two old women, and two young men and were excluded from the PA analyses. Therefore, of the 78 participants, full data sets, including both PA assessment and four-parameter sigmoidal curve fits, were obtained from 62 participants (Table 2): 38 old adults (70.5 ± 6.7 yr; 17 women) and 24 young adults (23.1 ± 3.2; 12 women). The number of participants included in each analysis is reflected by the degrees of freedom from the statistical tests and as the sample size in the relevant figures and tables.

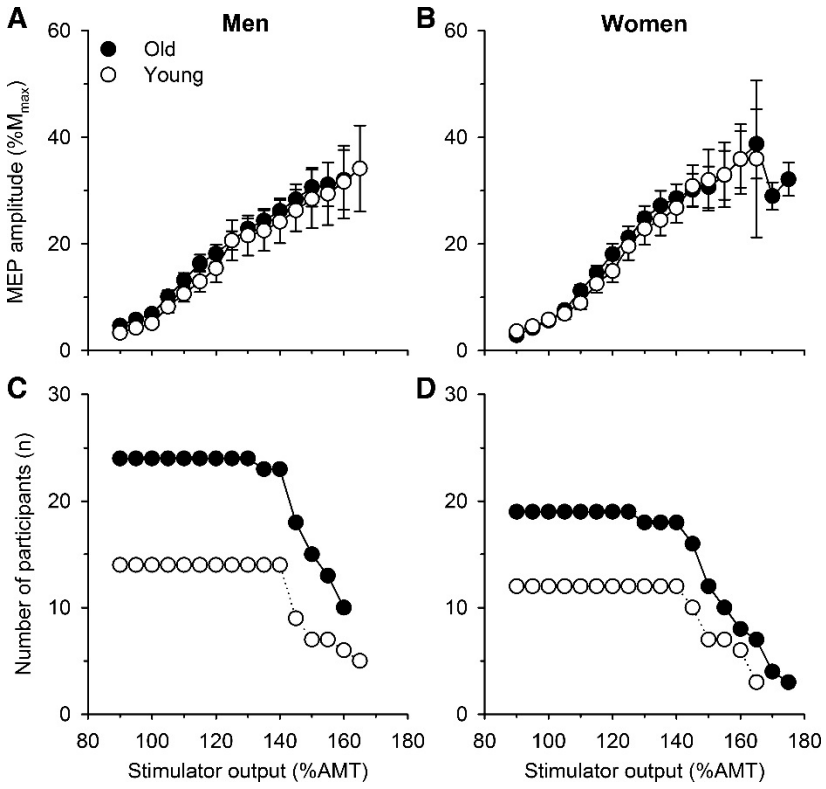


Fig. 2. Stimulus-response curves from the active vastus lateralis muscle were similar between the young and old for both men (*A*) and women (*B*), with no difference between the sexes. The no. of participants included for each intensity decreased at the higher stimulator intensities for young and old men (*C*) and women (*D*) because *1*) a clear plateau in the MEP amplitude had already occurred and/or *2*) the maximum stimulator output was

reached. Of the initial 78 participants, 4-parameter sigmoidal stimulus-response curves were obtained from 69 participants.

Table 2. Anthropometrics and stimulus-response curve characteristics for the PA participants

	Young		Old		*P* Value		
Variable	**High PA [10 (5 W)]**	**Low PA [14 (7 W)]**	**High PA [11 (5 W)]**	**Low PA [27 (12 W)]**	**Age**	**PA**	**Age × PA**
Age, yr	21.8 ± 0.8	23.2 ± 0.5	71.1 ± 2.4	70.3 ± 1.2	<**0.001**	0.829	0.448
Height, cm	167.1 ± 1.8	172.1 ± 3.1	169.1 ± 0.4	170.1 ± 0.2	0.998	0.296	0.487
Weight, kg	66.6 ± 3.1	70.8 ± 3.4	69.2 ± 5.1	78.2 ± 2.7	0.187	0.086	0.528
BMI, kg/m^{-2}	23.8 ± 0.9	23.7 ± 0.6	23.9 ± 1.1	27.0 ± 0.8	0.080	0.119	0.107
Body fat, %	23.2 ± 3.0	23.8 ± 2.0	28.3 ± 1.7	35.3 ± 1.6	<**0.001**	0.082	0.142
PA, steps/day	12,326 ± 815	7,133 ± 494	13,565 ± 1,174	6,381 ± 402	0.725	<**0.001**	0.154
MVC torque, Nm	213.4 ± 20.6	234.0 ± 21.7	150.1 ± 18.8	155.1 ± 12.1	<**0.001**	0.492	0.673
AMT (%SO)	42.3 ± 1.9	43.6 ± 2.6	50.5 ± 1.4	50.0 ± 1.9	**0.003**	0.854	0.717
M_{max}, mV	15.7 ± 1.7	14.6 ± 1.2	9.1 ± 1.4	7.6 ± 0.5	<**0.001**	0.242	0.855
MEP_{max}, mV	4.1 ± 0.6	4.4 ± 0.6	2.3 ± 0.3	2.4 ± 0.2	<**0.001**	0.623	0.768
MEP_{max} ($\%M_{max}$)	26.6 ± 3.5	32.4 ± 4.2	28.9 ± 3.0	33.5 ± 2.8	0.652	0.163	0.873
$Slope_{max}$	0.71 ± 0.11	0.99 ± 0.09	0.60 ± 0.10	0.88 ± 0.08	0.294	**0.010**	0.996

Values are means ± SE. PA, physical activity; W, women. Participants were dichotomized based on PA levels assessed with a triaxial accelerometer worn around the waist as either high PA (>10,000 steps/day) or low PA (<10,000 steps/day). Data for each variable were reported for only the participants with a 4-parameter sigmoidal stimulus-response curve and PA assessment for a minimum of 8 h for ≥3 days. The sample sizes for each group, including the number of women, are reported in parentheses. Boldfaced *P* values highlight statistical significance at $P < 0.05$.

Statistical analysis.

Individual univariate analyses of variance (ANOVA) were performed between the anthropometric and stimulus-response curve characteristics and age (young or old), sex (male or female), and/or PA [high PA (>10,000 steps/day) or low PA (<10,000 steps/day)] as the grouping variables (Tables 1 and 2). In addition, stepwise linear regressions were performed with the stimulus-response curve characteristics as the dependent variables [AMT (%SO), MEP_{max} ($\%M_{max}$), or $slope_{max}$] and age, height, weight, BMI, PA, and MVC torque as the independent (predictor) variables. For clarity, only the significant regression analyses are presented, whereas all individual univariate ANOVA statistics are reported (Tables 1 and 2).

The VL MEP amplitude data (expressed relative to M_{max}) were analyzed with individual two- or three-factor repeated-measures ANOVAs. The within-subject factors were intensity (11 levels: 90–140% AMT in 5% increments), and the between-subject factors were age group (2 levels: young or old) and sex (2 levels: male or female) or PA (2 levels: high PA or low PA). Because the sample sizes decreased at the higher stimulator intensities (e.g., Figs. 2 and 4), we also analyzed the VL MEP amplitude data with a linear mixed-model approach, with the stimulator intensity used as the repeated measure (18 levels: 90–175% AMT in 5% increments). The dependent variable for the mixed-model design was the average

peak-to-peak MEP amplitude from the 10 stimuli delivered at each intensity, and the fixed effects were the stimulator intensity (18 levels: 90–175% AMT in 5% increments), age group (2 levels: young or old), and sex (2 levels: male or female) or physical activity (2 levels: high PA or low PA). The results from the repeated-measures ANOVAs and the linear mixed-model analyses were similar. Thus, for simplicity, we report only the statistical outcomes from the repeated-measures ANOVAs.

Normal distribution and the homogeneity of variance of the data were assessed using the Kolmogorov-Smirnov test and Levene's statistic, respectively. For the repeated-measures ANOVAs, if the assumptions of sphericity were violated, the critical value for F was adjusted using the Greenhouse-Geisser epsilon correction from the Mauchly test of sphericity. All significance levels were set at $P < 0.05$, and all statistics were performed using the IBM SPSS Statistics 24 for Windows statistical software package (IBM, Chicago, IL). Data are presented as means ± SD in the text and means ± SE in the tables and figures.

RESULTS

Table 1 shows the anthropometric and stimulus-response curve characteristics for all of the participants in this study. Young adults had ~45% higher MVC (229.0 ± 70.2 Nm) than old adults [158.0 ± 58.3 Nm; $F_{(1, 74)} = 53.19$, $P < 0.001$] and men (214.6 ± 70.8 Nm) were ~56% stronger than women [137.5 ± 50.1 Nm; $F_{(1, 74)} = 53.98$, $P < 0.001$]. Furthermore, there was no difference in knee extensor MVC between the high- (180.2 ± 70.0 Nm) and low-PA groups [182.0 ± 78.4 Nm; $F_{(1, 54)} = 0.79$, $P = 0.377$], with no interactions for age [$F_{(1, 54)} = 0.33$, $P = 0.567$] or sex [$F_{(1, 54)} = 0.04$, $P = 0.850$].

Influence of age and sex.

The VL M_{max} amplitude was ~79% higher in the young (15.0 ± 4.7 mV) compared with the old [8.4 ± 3.6 mV; $F_{(1, 74)} = 78.59$, $P < 0.001$] and ~45% higher in the men (12.6 ± 5.7 mV) than in the women [8.7 ± 3.4 mV; $F_{(1, 74)} = 41.02$, $P < 0.001$]. When expressed in absolute terms, the MEP_{max} amplitudes were ~91% greater in the young (4.4 ± 2.2 mV) compared with the old [2.3 ± 0.9 mV; $F_{(1, 65)} = 30.33$, $P < 0.001$] and ~35% greater in the men (3.5 ± 2.1 mV) than in the women [2.6 ± 1.3 mV; $F_{(1, 65)} = 8.20$, $P = 0.006$]. However, if the MEP_{max} amplitudes were expressed relative to M_{max}, the MEP_{max} did not differ between the young (30.5 ± 15.1% M_{max}) and old [30.9 ± 13.3% M_{max}; $F_{(1, 65)} = 0.02$, $P = 0.893$] nor between the men (29.0 ± 14.1% M_{max}) and women [32.9 ± 13.6% M_{max}; $F_{(1, 65)} = 1.25$, $P = 0.268$].

Of the 78 participants, four-parameter sigmoidal stimulus-response curves were obtained from 69 participants: 26 young adults ($r^2 = 0.96 \pm 0.04$) and 43 old adults ($r^2 = 0.97 \pm 0.03$). The mean group stimulus-response curves for the young and old men and women are shown in Fig. 2. As expected, the MEP amplitude (%M_{max}) increased with each increase in stimulator intensity [$F_{(1.5, 95.4)} = 171.89$, $P < 0.001$]. There were, however, no effects of age [$F_{(1.5, 95.4)} = 0.56$, $P = 0.527$] or sex [$F_{(1.5, 95.4)} = 1.38$, $P = 0.253$], nor was there any interaction [$F_{(1.5, 95.4)} = 0.35$, $P = 0.643$] for the stimulus-response curves. In addition, analysis of the $slope_{max}$ of the stimulus-response curve (Table 1) revealed no effect of age [$F_{(1, 65)} = 1.29$, $P = 0.261$] or sex [$F_{(1, 65)} = 0.37$, $P = 0.543$] nor any interaction [$F_{(1, 65)} = 0.71$, $P = 0.401$].

To test for potential differences in activation strategies between the knee extensor muscles and across the groups, MEP amplitudes were also expressed relative to the root mean squared prestimulus EMG (%RMS) of the VL and then compared across age and sex. Similar to the results expressed relative to M_{max}, MEP amplitude increased with each stimulator intensity [$F_{(1.5, 86.1)} = 133.03$, $P < 0.001$], and there were no effects of age [$F_{(1.5, 86.1)} = 0.40$, $P = 0.611$] or sex [$F_{(1.5, 86.1)} = 1.24$, $P = 0.286$], nor was there any interaction [$F_{(1.5, 86.1)} = 2.48$, $P = 0.105$].

Although there were no age or sex differences in the stimulus-response curves of MEPs (Fig. 2), the percent stimulator output (%SO) that elicited the AMT was higher in the old (52.9 ± 11.4 %SO) compared with the young [43.7 ± 7.9 %SO; $F_{(1, 74)} = 14.00$, $P < 0.001$], with no differences between men (49.5 ± 10.6 %SO) and women [49.7 ± 12.0 %SO; $F_{(1, 74)} = 0.15$, $P = 0.701$] or any interaction [$F_{(1, 74)} = 0.08$, $P = 0.780$] (Table 1). Regression analyses revealed that the only significant predictor of the AMT was age (yr), with a Pearson's correlation coefficient of 0.40 ($P < 0.001$).

Influence of physical activity.

To test the influence of PA on the stimulus-response curve, only participants with both a four-parameter sigmoidal curve and PA assessment for a minimum of 8 h for ≥3 days were included in the analyses (Table 2). As a result, 21 participants met the recommended PA guidelines (high PA: >10,000 steps/day[1]), and 41 participants did not (low PA: <10,000 steps/day) (Fig. 3). The average steps per day were significantly greater for the old adults meeting PA guidelines [13,565 ± 3,894 steps/day; $n = 11$ (5 women)] compared with those who did not meet the guidelines [6,381 ± 2,087 steps/day; $n = 27$ (12 women)] [$t_{(36)} = 7.4$, $P < 0.001$] and young adults who met the guidelines [12,326 ± 2,577 steps/day; $n = 10$ (5 women)] compared with those who did not [7,133 ± 1,847 steps/day; $n = 14$ (7 women)] [$t_{(22)} = 5.8$, $P < 0.001$] (Fig. 3*A*). There were no significant differences between the average number of steps per day between old and young participants who were meeting guidelines [$t_{(19)} = 0.9$, $P = 0.41$] or between the old and young participants who were not meeting PA guidelines [$t_{(39)} = -1.1$, $P = 0.26$].

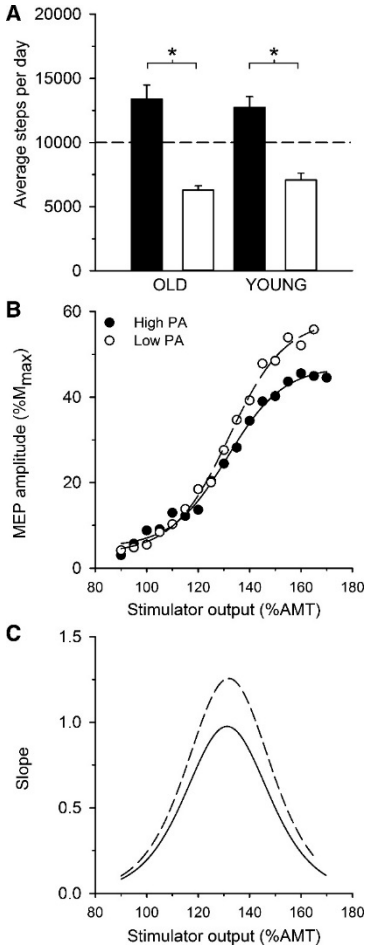


Fig. 3. *A*: the average steps/day were ~2-fold greater in the high- (>10,000 steps/day) compared with low-physical activity (PA) participants (<10,000 steps/day) but were not different between the groups for the young and old (*significant difference at $P < 0.001$). *B*: stimulus-response curves from 2 representative young female participants reveal that the MEP amplitudes at a similar relative stimulator intensity were larger in the low- compared with the high-PA participant. *C*: additionally, although both participants had similar active motor thresholds and $slope_{max}$ occurred at the same %AMT, the low-PA participant had a steeper $slope_{max}$.

The influence of habitual PA on the stimulus-response curve of the VL is shown in Fig. 4. As expected, MEP amplitude (%M_{max}) significantly increased with each increase in stimulator intensity [$F_{(1.62, 85.6)}$ = 149.42, $P < 0.001$]. There was also a significant intensity × PA interaction [$F_{(1.61, 85.6)}$ = 3.49, $P = 0.045$], which was due to larger MEP amplitudes in the low-PA compared with the high-PA group at the same relative stimulator intensity (%AMT) (Fig. 4). Neither age [$F_{(1.61, 85.6)}$ = 0.16, $P = 0.810$] or sex [$F_{(1.61, 85.6)}$ = 0.77, $P = 0.772$] nor any other interactions altered the intensity × PA interaction. Consistent with these findings, the $slope_{max}$ of the stimulus-response curve (Table 2 and Fig. 4) was ~40% lower for the high-PA group (0.66 ± 0.34) compared with the low-PA group [0.92 ± 0.34; $F_{(1, 54)}$ = 8.09, $P = 0.006$], with no effect of age [$F_{(1, 54)}$ = 0.02, $P = 0.897$], sex [$F_{(1, 54)}$ = 0.58, $P = 0.451$], nor any other interactions. Regression analyses supported these findings by revealing that the only significant predictor of $slope_{max}$ was PA (steps/day), with a Pearson's correlation coefficient of −0.40 ($P = 0.001$).

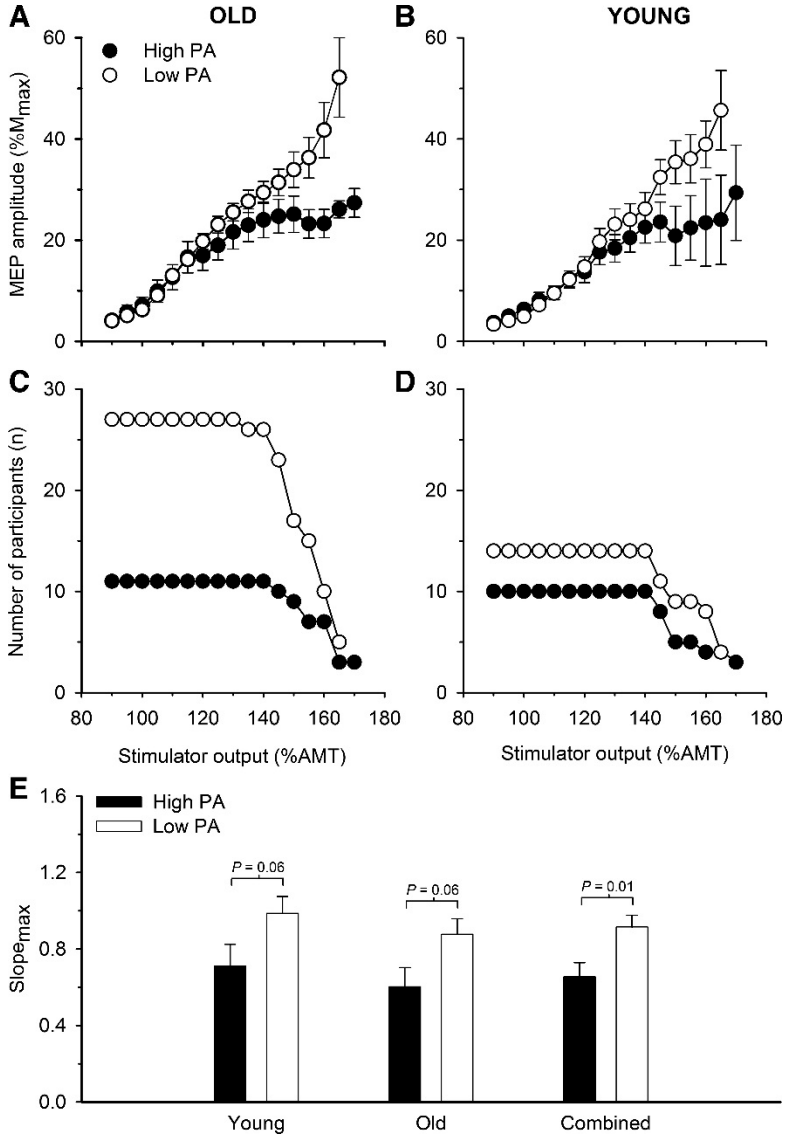


Fig. 4. *A* and *B*: the MEP amplitudes at a similar relative stimulator intensity (%AMT) were larger in the low- compared with the high-PA participants for both the old (*A*) and the young (*B*) but did not differ between the young and old. *C* and *D*: the no. of participants included for each intensity decreased at the higher stimulator intensities for the old (*C*) and young (*D*) because *1*) a clear plateau in the MEP amplitude had already occurred and/or *2*) the maximum stimulator output was reached. *E*: in addition to the larger MEP amplitudes, the low-PA participants also had a steeper $slope_{max}$ in the stimulus-response curve compared with the high-PA participants. Of the initial 78 participants, 4-parameter sigmoidal stimulus-response curves and complete PA assessment were obtained from 62 participants.

For the cohort of 62 adults who had a full data set, including both the stimulus-response curve and PA data, the AMT (%SO) remained higher in the old (50.2 ± 8.4% SO) compared with the young [43.0 ± 8.2% SO; $F_{(1, 54)} = 9.30$, $P = 0.004$], with no differences between men (48.0 ± 9.0% SO) and women [46.8 ± 9.0% SO; $F_{(1, 54)} = 0.05$, $P = 0.831$]. There were also no differences in the AMT (%SO) between the high-PA (46.6 ± 6.7% SO) and low-PA groups [47.8 ± 10.0% SO; $F_{(1, 54)} = 0.02$, $P = 0.882$], nor were there any other interactions.

DISCUSSION

The purpose of this study was to investigate the influence of aging and PA on corticospinal excitability in men and women during submaximal isometric contractions of the knee extensor muscles. Contrary to our first hypothesis, we found no age or sex differences in the stimulus-response curves in the active knee extensor muscle (Fig. 2). However, PA was associated with differences in the stimulus-response curve characteristics independent of age and sex (Figs. 3 and 4). Interestingly, in contrast to our second hypothesis, both active young and old participants (>10,000 steps/day) had smaller MEP amplitudes ($\%M_{max}$) and a more gradual increase in the MEP amplitude with each increase in stimulator intensity compared with the low-PA participants (<10,000 steps/day; Fig. 4). These data provide novel evidence that regular engagement in PA may modulate the excitability of the corticospinal tract to the lower limb and that the effect is similar in young and old men and women.

Age and sex did not modify the stimulus-response curve in the active lower limb.

We found no age or sex differences in the stimulus-response curve parameters for the active VL muscle (Fig. 2), including the $slope_{max}$ and MEP_{max} (Table 1). Notably, these findings persisted even when we accounted for potential differences in activation strategies across groups and between the knee extensor muscles by normalizing the MEP amplitude to the EMG activity immediately before the stimulus.[7] These data are consistent with other studies of the knee extensor muscles[56] but contrary to some targeting the intrinsic hand muscles.[42,58] The studies on the FDI suggest that age-related reductions in corticospinal excitability may be more prevalent in women[42] or when the muscle is activated.[58] For example, in the quiescent FDI muscle, old adults required a higher stimulator intensity to elicit both the MEP_{max} and the maximum slope of the stimulus-response curve compared with young adults.[42] However, in a similar study, but only in men, there were no age-related differences,[54] suggesting that the previously observed differences may have been determined by the older women.[42] In contrast, in the active FDI, corticospinal excitability appeared to be reduced with aging irrespective of sex.[58] Taken together with our findings on the active knee extensor muscle, age and sex may influence corticospinal excitability differently in upper and lower limb muscles.

Although the slope of the curve and the MEP amplitudes (%M_{max}) were similar between young and old adults (Fig. 2) and between men and women, the stimulator intensity needed to elicit the AMT (%SO) was higher in the old compared with the young adults (Table 1). The higher AMTs in the old adults may be interpreted as a decrease in corticospinal excitability and are consistent with others reporting higher resting motor thresholds with age.[3,41] Use of motor thresholds as an indicator for differences in corticospinal excitability should be interpreted with caution, however, because motor thresholds are strongly influenced by the distance between the stimulating coil and the underlying cortical tissue.[57] Accordingly, the cortex of old adults is typically atrophied compared with the young,[46] and skull thickness may also increase with age.[30] Thus, the distance between the stimulating coil and the cortical cells is likely greater in the old adults compared with the young, which would result in a higher motor threshold. However, it should also be considered that cortical cells may be less responsive with age, with either fewer cells activated at comparable stimulation intensities or, alternatively, the same number of cells activated but in a less synchronous way, leading to phase cancellation and resulting in a higher threshold.[31,32,42] Consequently, we are unable to determine whether the differences in the AMT between the young and old adults observed in this study were due to differences in corticospinal excitability per se, the distance from the coil to the underlying cortical cells, or the responsiveness of the cells themselves.

Corticospinal excitability differs with physical activity level.

Our data provide evidence that the corticospinal excitability of the active VL muscle in the lower limb differs with PA levels. Specifically, corticospinal excitability was lower in the young and old men and women who met the PA guidelines (>10,000 steps/day, high PA) compared with those who did not (<10,000 steps/day, low PA) (Fig. 3 and Table 2). This finding is supported more specifically by the smaller MEP amplitudes and lower $slope_{max}$ of the stimulus-response curve in the high- compared with low-PA participants (Fig. 4) despite similar stimulator intensities to elicit the AMT. Although our results suggest that PA modulates corticospinal excitability, these effects were similar for the young and old men and women (Fig. 4). This finding is notable because the PA levels were well matched between the young and old adults who did or did not meet the PA guidelines. In addition, PA levels were approximately twofold greater in the high- compared with low-PA group, indicating a wide range of PA in our participants (Fig. 3). Interpreted together, our data suggest that PA likely plays a more prominent role in modulating corticospinal excitability of the lower limb than either age or sex. This is applicable because in the general population old adults are typically less active than young, and women are less active than men.[34]

There is limited knowledge about the impact of PA on corticospinal excitability. One study reported no differences in the stimulus-response curves of the quiescent adductor pollicis brevis muscle between groups of highly active or sedentary young adults that were categorized based on a self-reporting PA questionnaire.[10] However, following a paired-associative stimulation paradigm, which increases corticospinal excitability through long-term potentiation-like mechanisms, the highly active group showed a greater responsiveness to the stimulation paradigm compared with the sedentary group.[10] The discrepancies between the findings in this upper limb muscle and our findings in the lower limb may be the result of multiple factors. First, the number of corticospinal projections is greater in upper compared with lower limb muscles, and lower limb muscles are more directly involved in achieving high levels of PA and thus more susceptible to differences in PA levels.[28] Second, the data analysis differed between the two studies, with Cirillo et al.[10] reporting absolute MEP data (mV) from the middle section of the stimulus-response curves (110–140% RMT), whereas our current study quantified the curves with MEPs normalized to M_{max} from below the AMT until a plateau of the MEP was achieved

(MEP_{max}). Finally, the data presented in our study were obtained with low levels of muscle activation rather than in a rested state, which increases the net excitability of the motor system.[16] Thus, it is unclear what factors are responsible for the discrepancies between the studies, but it is possible that PA elicits differential responses in the corticospinal tract based on the muscle group and/or whether the muscle is activated or not.

There is a large body of literature demonstrating a shift in the balance between excitation and inhibition when the muscle is activated compared with at rest.[23,55,58] In the cortex, there is a reduction in inhibition of the muscles directly involved in the contraction, but cortical reciprocal inhibition remains in antagonist muscles.[23,55,58] This balance between inhibition and excitation is functionally important for the scaling of muscle activation and optimal targeted movements. Indeed, a decreased interhemispheric inhibition[58] and a loss of reciprocal inhibition during contraction[23] were reported in old adults compared with young. However, old adults are typically less active than young,[34] and these studies did not determine whether the PA levels differed between the two age cohorts. Thus, it is possible that the reported age-related differences in reciprocal[23] and interhemispheric inhibition[58] may actually be due to shifts in the balance of inhibition and excitation from differences in PA levels rather than aging per se.

Further insight into the differences in corticospinal excitability observed between the high- and low-PA participants can be gained by comparing our findings with studies on strength training and cast immobilization, which provide a continuum of different levels of sensorimotor activity. Consistent with our findings, there is an increase in corticospinal excitability in both upper-[11,63,64] and lower-limb[29,45] muscles in response to cast immobilization, although this is not always observed.[20] In addition, the excitability of the corticospinal tract was reduced for two muscles of the upper limb following 4 wk of strength training.[8,26] However, the findings from strength training and cast immobilization may also be influenced by changes in muscle strength, whereas our data suggest that the differences in corticospinal excitability between the low- and high-PA groups were independent of strength, because the MVC torque was similar between the groups (Table 1). The complementary data from our study compared with both cast immobilization[11,29,45,63,64] and strength training[8,26] reveal a potential paradigm that the excitability of the corticospinal tract increases in response to reduced sensorimotor activity. Whether the increased corticospinal excitability provides a beneficial adaptation for the motor system or is maladaptive remains unknown.

Multiple mechanisms may be responsible for the increased corticospinal excitability associated with low PA levels (Fig. 4). Because M_{max} was similar across the activity groups (Table 2) and the MEP was normalized to this value, the underlying mechanisms did not likely involve the neuromuscular junction or the ability of the action potential to propagate across the sarcolemma. In addition, normalizing the MEP amplitudes to M_{max} was necessary to control for other factors that are known to affect the EMG signal (e.g., subcutaneous adipose tissue, electrode placement, muscle architecture, etc.)[9,21] and often differ between individuals and populations. Therefore, the MEP amplitudes reported in this study represent the net excitatory and inhibitory synaptic transmissions terminating on both the cortical and spinal motor neurons.[17] As a result, we cannot conclusively identify where in the corticospinal pathway the differences between the PA groups occurred. However, several lines of evidence suggest that cortical mechanisms likely play a role in the differences observed with PA, although spinal mechanisms cannot be ruled out. For example, the increased corticospinal excitability following cast immobilization occurred in the absence of any detectable changes in spinal excitability, as measured by either the F-wave[63,64] or H-reflex.[11,29] In addition, a study using CNS-active drugs found that lorazepam, a positive allosteric modulator of $GABA_A$ receptors, and lamotrigine, an inhibitor of voltage-gated Na^+ and Ca^{2+}

channels, both depressed the MEP_{max} and stimulus-response curves without affecting the M-wave or F-wave.[4] If the mechanisms are similar for different PA levels, these studies in conjunction with our findings suggest that the decreased corticospinal excitability associated with high PA levels could be modulated in part by neural changes occurring within the cortex, presumably by alterations in the GABAergic system. This hypothesis is also supported by recent evidence that higher levels of physical fitness were positively associated with increased interhemispheric inhibition in middle-aged adults[35] and provides a foundation for future investigations.

Conclusions.

We provide evidence that low PA levels are associated with an increase in excitability of the cortiospinal tract projecting to an active muscle of the lower limb and that the effect is similar in young and old men and women. Importantly, these differences in corticospinal excitability with PA were observed in adults who achieved the recommended 10,000 steps/day for optimal health benefits compared with those who did not. Certainly 10,000 steps/day is achievable for most healthy community-dwelling adults. Future studies that localize the sites of change in excitability along the corticospinal tract will help advance our understanding of how PA affects the nervous system.

GRANTS

This work was supported by a National Institute of Aging grant (R21-AG-045766) to S. K. Hunter, an Australian National Health and Medical Research Council-Australian Research Council Dementia Research Development Fellowship (APP1097397) to A. E. Smith, and a National Institutes of Health Ruth L. Kirschstein Predoctoral Fellowship (F31-AG-052313) to C. W. Sundberg.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

H.H., C.W.S., A.E.S., and S.K.H. conceived and designed research; H.H., C.W.S., and A.K. performed experiments; H.H., C.W.S., A.E.S., A.K., and S.K.H. analyzed data; H.H., C.W.S., A.E.S., A.K., and S.K.H. interpreted results of experiments; H.H., C.W.S., A.E.S., and S.K.H. drafted manuscript; H.H., C.W.S., A.E.S., and S.K.H. edited and revised manuscript; H.H., C.W.S., A.E.S., A.K., and S.K.H. approved final version of manuscript; C.W.S., A.E.S., and S.K.H. prepared figures.

ACKNOWLEDGMENTS

We thank Bonnie Schlinder-Delap for assistance with scheduling participants, Jonathon Senefeld for helping develop the custom analysis routine, and Jordan Lemens for assisting with data analysis toward the final stages of the manuscript's preparation. We also thank the research participants for volunteering to make this study possible.

AUTHOR NOTES

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