



# Reductions in Motor Unit Firing are Associated with Clinically Meaningful Leg Extensor Weakness in Older Adults

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Received: 31 January 2023 / Accepted: 2 August 2023 / Published online: 21 August 2023  
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## Abstract

Weakness, one of the key characteristics of sarcopenia, is a significant risk factor for functional limitations and disability in older adults. It has long been suspected that reductions in motor unit firing rates (MUFs) are one of the mechanistic causes of age-related weakness. However, prior work has not investigated the extent to which MUFs is associated with clinically meaningful weakness in older adults. Forty-three community-dwelling older adults (mean: 75.4 ± 7.4 years; 46.5% female) and 24 young adults (mean: 22.0 ± 1.8 years; 58.3% female) performed torque matching tasks at varying submaximal intensities with their non-dominant leg extensors. Decomposed surface electromyographic recordings were used to quantify MUFs from the vastus lateralis muscle. Computational modeling was subsequently used to *independently* predict how slowed MUFs would negatively impact strength in older adults. Bivariate correlations between MUFs and indices of lean mass, voluntary activation, and physical function/mobility were also assessed in older adults. *Weak* older adults ( $n = 14$ ) exhibited an approximate 1.5 and 3 Hz reduction in MUFs relative to *non-weak* older adults ( $n = 29$ ) at 50% and 80% MVC, respectively. Older adults also exhibited an approximate 3 Hz reduction in MUFs relative to young adults at 80% MVC only. Our model predicted that a 3 Hz reduction in MUFs results in a strength decrement of 11–26%. Additionally, significant correlations were found between slower MUFs and poorer neuromuscular quality, voluntary activation, chair rise time performance, and stair climb power ( $r$ 's = 0.31 to 0.43). These findings provide evidence that slowed MUFs are mechanistically linked with clinically meaningful leg extensor weakness in older adults.

**Keywords** Dynapenia · Muscle strength · Motoneuron · Motor unit · Older adults · Sarcopenia

## Introduction

More than 40% of older adults in the U.S. have at least one functional limitation while performing daily tasks considered essential for maintaining independence [1]. In fact, approximately 30% of older adult women and

approximately 15% of older adult men report an inability to lift or carry 10 pounds [2]. Most notably, weakness, the key characteristic of the most recent sarcopenia definitions [3], has long been recognized as a major determinant of

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physical limitations and poor health in older adults [4–9]. Thus, the preservation of strength, and ultimately physical function/mobility, in older adults continues to be a major public health priority as it drastically reduces healthcare costs and improves quality of life [10].

The mechanisms of age-related weakness are multifactorial, with neurologic and skeletal muscle factors being key contributors to strength [11–15]. Regarding neural factors, skeletal muscle force is determined via recruitment of adequate numbers of alpha-motoneurons (MNs) and the modulation of their firing rates (i.e., rate coding). It has long been postulated that reductions in motor unit (a single MN and the muscle fibers it innervates) firing rate (MUFR) are one of the mechanistic causes of age-related weakness [16–22]. However, there are discrepancies in the literature regarding age-related changes in MUFR with some authors reporting slower MUFRs for older adults relative to young adults [18, 21–25], while others report similar MUFRs between older adults and young adults [17, 26–31]. For instance, Roos et al. [26] and Kirk et al. [31] reported that MUFRs were similar between older adults and young adults for the vastus medialis and gastrocnemii muscles, and Dalton et al. [27] reported that slower MUFRs were only observed in older adults during low-to-moderate contraction intensities (i.e.,  $\leq 50\%$  of maximal strength) for the soleus muscle. In contrast, Connelly et al. [23], Kamen and Knight [21], Christie and Kamen [18], and Piasecki et al. [22] reported that older adults have slower MUFRs relative to young adults at all contraction intensities for the vastus lateralis and tibialis anterior muscles. These discrepancies likely arise for several reasons, ranging from differences in skeletal muscle group(s) examined, contraction intensities performed, participant sample sizes, instrumentation, analytical aspects of the motor unit recordings, etc. In addition, prior work has simply compared older adults to young adults, with little attention given to whether MUFRs differ between older adults with low muscle function (e.g., weakness) relative to those who are higher functioning.

In this work, we sought to use an interdisciplinary approach (human recordings and computational modeling) to investigate the extent to which MUFRs are associated with clinically meaningful weakness in older adults (weakness classifications were determined using the cut points suggested by Manini et al. [4] as part of the Health, Aging, and Body Composition Study). Specifically, the purpose of this study was to determine MUFRs between older adults with clinically meaningful leg extensor weakness [3, 4] relative to older adults without leg extensor weakness via decomposed surface electromyographic recordings. Our a priori hypothesis was that, for the vastus lateralis muscle, *weak* older adults would have slower MUFRs relative

to *non-weak* older adults at moderate-to-high contraction intensities (i.e.,  $\geq 50\%$  of maximal strength).

## Methods

A full, detailed description of the methodology, along with corresponding citations, are provided as supplemental material (insert link to supplemental material). Here, due to space limitations, we present an abbreviated overview of our methodology.

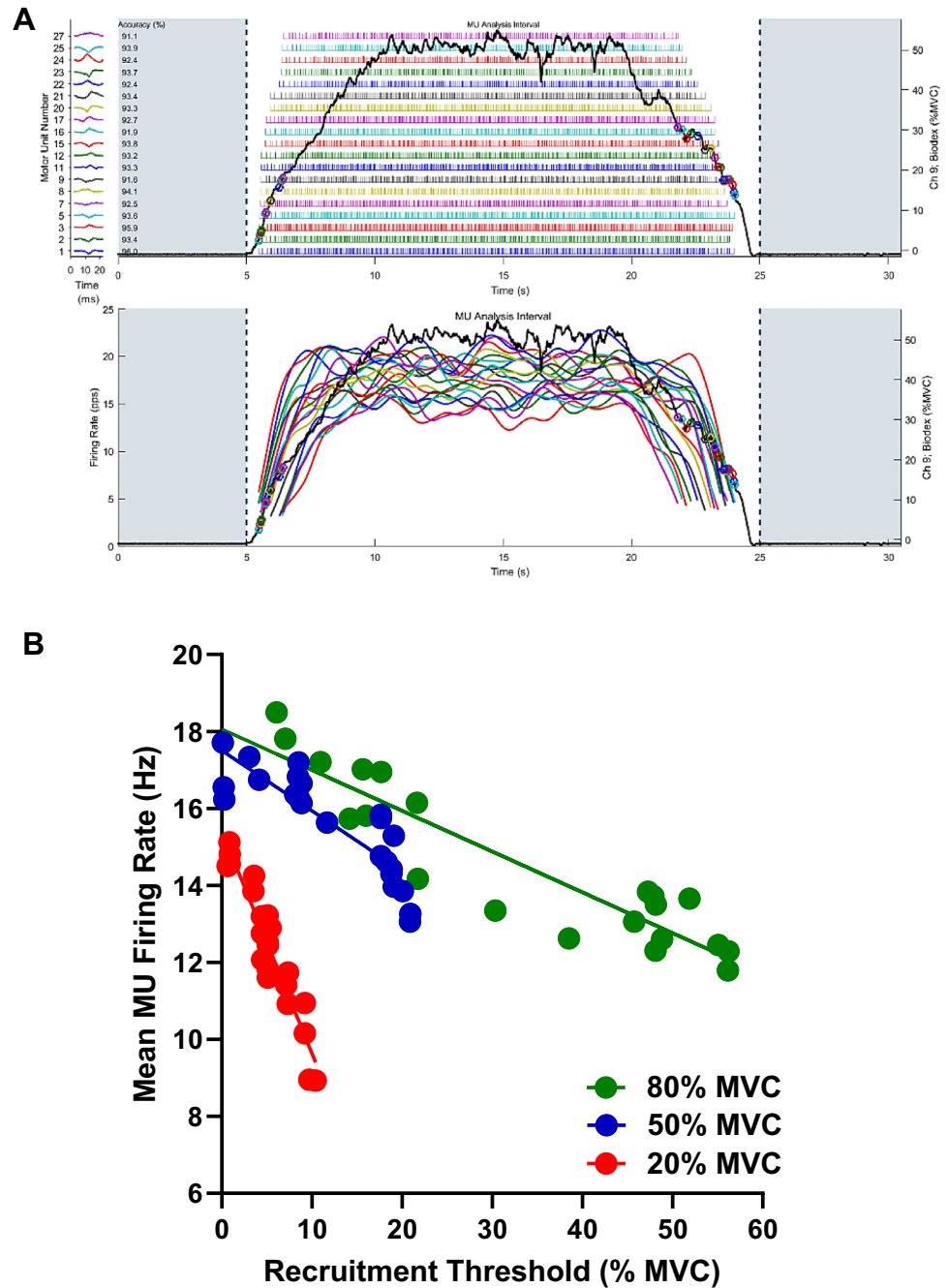
### General Overview

Forty-three older adults and 24 young adults participated in this study. Participants had their non-dominant isokinetic and isometric leg extensor strength, as well as their handgrip strength, assessed. Participants also performed physical function/mobility tests, body composition assessments via dual-energy x-ray absorptiometry (DXA), and completed trapezoidal, target torque matching tasks at contraction intensities of 20%, 50%, and 80% of their maximal volitional contraction (MVC). Decomposed surface electromyographic (EMG) recordings were used to estimate MUFRs from the non-dominant vastus lateralis muscle during these target torque matching tasks. The primary outcome of interest was the y-intercept calculated from the mean MUFR versus the recruitment threshold scatterplot fit with a linear regression (calculated on a subject-by-subject basis at each contraction intensity; see Fig. 1). The y-intercept method, as opposed to using simply MUFRs at given contraction intensities, was preferred because it acts as a normalization process adjusting for the inherent influence of each motor unit's recruitment threshold on its respective firing rate (see supplemental methods for further details). For clarity, henceforth the y-intercept data will be referred to as 'normalized MUFR'. Subsequently, a multi-scale, high-fidelity, anatomically-detailed computational model was used to *independently* predict how reductions in normalized MUFR would negatively impact strength in older adults. Additionally, associations between normalized MUFRs and indices of lean mass, voluntary activation, and physical function/mobility were performed via bivariate correlations.

### Participants

Forty-three community-dwelling older adults (63–90 years, mean:  $75.4 \pm 7.4$  years; 46.5% female) and 24 young adults (19–25 years, mean:  $22.0 \pm 1.8$  years; 58.3% female) were included in the primary analysis (see

**Fig. 1** Example of motor unit firing rate profile (a) and linear regression of the motor unit firing rate vs. recruitment threshold plot used to calculate the y-intercept (b) for one older adult participant. **a** Torque-matching task and the identification of the associated motor units during a 50% MVC. Torque is in black and MUs are illustrated in color (e.g., circles on the torque trace indicate derecruitment time points and each colored line indicates a particular motor unit). *Top*: Individual motor unit firing rates underlie the target torque tracing. *Bottom*: Individual motor unit firing rates were smoothed by low-pass filtering each motor unit's impulse train with a 1-s Hanning window. **b** The mean firing rate for each motor unit was plotted against its recruitment threshold at each contraction intensity (i.e., 20%, 50%, 80% MVC). Linear regression was applied to calculate the slope and y-intercept at each intensity level. To control for the inherent influence of larger-threshold motor units firing slower than lower-threshold motor units, we used the y-intercept value as our primary variable of interest (referred to as 'normalized MUFR' in the manuscript). *Hz* Hertz, *MU* motor unit, *MVC* maximal voluntary isometric contraction



Tables 1 and 2). Participants were living independently and were free of overt musculoskeletal and neurological disease. The Ohio University Institutional Review Board approved this study, and all study participants provided written informed consent in accordance with the Declaration of Helsinki.

To characterize the older adults, we measured their (1) six-minute walk gait speed, (2) short physical performance battery test, and (3) comorbidities via the Charlson

Comorbidity Index. In all participants, we assessed body composition (including appendicular and thigh lean mass) via DXA (Hologic Discovery QDR model Series, Waltham, MA, USA), time of moderate-to-vigorous intensity physical activity via accelerometry (ActiGraph, wgt3x-bt, Pensacola, FL), and neuropsychological status via the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). See Tables 1 and 2.

**Table 1** Descriptive characteristics of study population (mean  $\pm$  SD)

	Age-related weakness		Older adult phenotypic weakness	
	Young adults N = 24	Older adults N = 43	Non-weak n = 29	Weak n = 14
	Age (years)	22.0 $\pm$ 1.8	75.4 $\pm$ 7.4 <sup>a</sup>	72.8 $\pm$ 6.4
Women (%)	58.3	46.5	44.8	50
Height (cm)	170.4 $\pm$ 8.8	166.1 $\pm$ 11.1 <sup>a</sup>	167.6 $\pm$ 8.7	162.9 $\pm$ 14.7
Weight (kg)	71.3 $\pm$ 12.6	76.0 $\pm$ 15.9	75.0 $\pm$ 16.4	78.0 $\pm$ 15.2
Body mass Index (kg/m <sup>2</sup> )	24.3 $\pm$ 2.9	27.4 $\pm$ 4.8 <sup>a</sup>	26.5 $\pm$ 4.8	29.3 $\pm$ 4.5
Body fat (%)	27.8 $\pm$ 7.1	33.1 $\pm$ 7.3 <sup>a</sup>	31.7 $\pm$ 7.8	36.0 $\pm$ 5.5 <sup>a</sup>
Thigh lean mass (kg)	5.6 $\pm$ 2.0	5.1 $\pm$ 1.0 <sup>a</sup>	5.2 $\pm$ 1.1	4.9 $\pm$ 1.0
Appendicular lean mass/ height <sup>2</sup>	7.4 $\pm$ 1.6	7.0 $\pm$ 1.2	7.1 $\pm$ 1.3	6.9 $\pm$ 1.2
Isometric LE strength (N-m)	156.1 $\pm$ 55.7	85.2 $\pm$ 36.0 <sup>a</sup>	94.7 $\pm$ 36.0	65.5 $\pm$ 27.8 <sup>a</sup>
Relative isokinetic LE strength (N-m/kg)	2.5 $\pm$ 0.4	1.3 $\pm$ 0.4 <sup>a</sup>	1.5 $\pm$ 0.4	0.8 $\pm$ 0.2 <sup>a</sup>
Neuromuscular quality (N-m/kg)	31.4 $\pm$ 4.4	19.0 $\pm$ 5.1 <sup>a</sup>	21.6 $\pm$ 3.9	13.7 $\pm$ 2.7 <sup>a</sup>
Handgrip strength (kg)	36.3 $\pm$ 10.8	27.9 $\pm$ 9.7 <sup>a</sup>	30.1 $\pm$ 9.3	23.2 $\pm$ 9.1 <sup>a</sup>
Moderate-to-vigorous activity (mins/week)	171.5 $\pm$ 51.9	112.5 $\pm$ 57.9 <sup>a</sup>	125.5 $\pm$ 54.3	85.7 $\pm$ 57.5 <sup>a</sup>
Chair rise time (s)	–	11.0 $\pm$ 3.5	9.4 $\pm$ 2.3	14.2 $\pm$ 3.5 <sup>a</sup>
Stair climb power (watts)	415.2 $\pm$ 107.1	265.6 $\pm$ 96.7 <sup>a</sup>	294.2 $\pm$ 93.0	206.3 $\pm$ 77.3 <sup>a</sup>
Six-min walk gait speed (m/s)	–	1.4 $\pm$ 0.3	1.5 $\pm$ 0.2	1.1 $\pm$ 0.3 <sup>a</sup>
Charlson index (% 10-year survival)	–	50.9 $\pm$ 21.7	54.8 $\pm$ 22.6	42.9 $\pm$ 17.9
SPPB score	–	11.0 $\pm$ 1.3	11.6 $\pm$ 0.7	9.8 $\pm$ 1.4 <sup>a</sup>
RBANS score	102.5 $\pm$ 10.5	105.5 $\pm$ 13.0	107.2 $\pm$ 12.2	102.1 $\pm$ 14.5
Voluntary activation (%)	94.0 $\pm$ 3.5	89.5 $\pm$ 8.8 <sup>a</sup>	90.8 $\pm$ 7.5	86.7 $\pm$ 10.7

For the voluntary activation measurement the sample sizes were  $n = 22$  for young adults,  $n = 33$  for older adults,  $n = 22$  for non-weak older adults, and  $n = 11$  for weak older adults

<sup>a</sup>Significant difference relative to the comparison group (e.g., young vs. older adults)

**Table 2** Sex-specific descriptive characteristics of study population (mean  $\pm$  SD)

	Age-related weakness				Older adult phenotypic weakness			
	Young adults <i>N</i> = 24		Older adults <i>N</i> = 43		Non-weak <i>n</i> = 29		Weak <i>n</i> = 14	
	Males <i>n</i> = 10	Females <i>n</i> = 14	Males <i>n</i> = 23	Females <i>n</i> = 20	Males <i>n</i> = 16	Females <i>n</i> = 13	Males <i>n</i> = 7	Females <i>n</i> = 7
Age (years)	22.9 $\pm$ 1.4	21.4 $\pm$ 1.8	75.4 $\pm$ 6.4 <sup>b</sup>	75.4 $\pm$ 8.5 <sup>b</sup>	73.5 $\pm$ 5.5	71.9 $\pm$ 7.4	79.9 $\pm$ 6.6 <sup>b</sup>	81.9 $\pm$ 6.7 <sup>b</sup>
Height (cm)	178.2 $\pm$ 6.9	164.9 $\pm$ 5.0 <sup>a</sup>	173.6 $\pm$ 7.6	157.4 $\pm$ 7.5 <sup>a,b</sup>	173.2 $\pm$ 6.6	160.8 $\pm$ 5.6 <sup>a</sup>	174.7 $\pm$ 10.1	151.1 $\pm$ 6.6 <sup>a,b</sup>
Weight (kg)	83.1 $\pm$ 6.9	62.9 $\pm$ 7.9 <sup>a</sup>	84.1 $\pm$ 12.9	66.6 $\pm$ 14.1 <sup>a</sup>	82.4 $\pm$ 13.5	65.8 $\pm$ 15.4 <sup>a</sup>	88.0 $\pm$ 11.2	68.1 $\pm$ 12.1 <sup>a</sup>
Body mass index (kg/ m <sup>2</sup> )	26.0 $\pm$ 2.1	23.1 $\pm$ 2.8	27.9 $\pm$ 4.0	26.9 $\pm$ 5.7 <sup>b</sup>	27.5 $\pm$ 4.4	25.3 $\pm$ 5.1	28.8 $\pm$ 2.8	29.9 $\pm$ 5.9 <sup>b</sup>
Body fat (%)	22.0 $\pm$ 4.8	32.7 $\pm$ 4.4 <sup>a</sup>	29.2 $\pm$ 5.7 <sup>b</sup>	37.5 $\pm$ 6.5 <sup>a,b</sup>	27.7 $\pm$ 6.0	36.6 $\pm$ 7.0 <sup>a</sup>	32.8 $\pm$ 3.0	39.1 $\pm$ 5.8 <sup>a</sup>
Thigh lean mass (kg)	7.2 $\pm$ 0.6	4.9 $\pm$ 0.7 <sup>a</sup>	5.8 $\pm$ 0.7 <sup>b</sup>	4.2 $\pm$ 0.6 <sup>a,b</sup>	5.9 $\pm$ 0.7	4.3 $\pm$ 0.7 <sup>a</sup>	5.6 $\pm$ 0.5	4.1 $\pm$ 0.5 <sup>a</sup>
Appendicular lean mass/height <sup>2</sup>	8.8 $\pm$ 1.1	6.3 $\pm$ 0.8 <sup>a</sup>	7.8 $\pm$ 0.8 <sup>b</sup>	6.1 $\pm$ 1.0 <sup>a</sup>	8.0 $\pm$ 0.8	6.0 $\pm$ 0.8 <sup>a</sup>	7.5 $\pm$ 0.6	6.3 $\pm$ 1.3 <sup>a</sup>
Isometric LE strength (N-m)	213.2 $\pm$ 34.0	115.3 $\pm$ 19.6 <sup>a</sup>	109.6 $\pm$ 32.3 <sup>b</sup>	63.5 $\pm$ 17.9 <sup>a,b</sup>	118.6 $\pm$ 33.2	70.5 $\pm$ 17.4 <sup>a</sup>	89.0 $\pm$ 18.9 <sup>b</sup>	50.5 $\pm$ 10.2 <sup>a</sup>
Relative isokinetic LE strength (N-m/kg)	2.8 $\pm$ 0.4	2.3 $\pm$ 0.3 <sup>a</sup>	1.4 $\pm$ 0.5 <sup>b</sup>	1.1 $\pm$ 0.4 <sup>a,b</sup>	1.6 $\pm$ 0.4	1.3 $\pm$ 0.3 <sup>a</sup>	0.9 $\pm$ 0.1 <sup>b</sup>	0.8 $\pm$ 0.2 <sup>b</sup>
Neuromuscular qual- ity (N-m/kg)	32.6 $\pm$ 4.4	30.4 $\pm$ 4.3 <sup>a</sup>	20.2 $\pm$ 5.4 <sup>b</sup>	17.7 $\pm$ 4.6 <sup>a,b</sup>	22.7 $\pm$ 4.2	20.3 $\pm$ 3.1	14.4 $\pm$ 2.8 <sup>b</sup>	13.0 $\pm$ 2.7 <sup>b</sup>
Handgrip strength (kg)	47.0 $\pm$ 4.6	27.4 $\pm$ 4.2 <sup>a</sup>	34.2 $\pm$ 8.4 <sup>b</sup>	20.6 $\pm$ 4.6 <sup>a,b</sup>	35.8 $\pm$ 8.8	23.2 $\pm$ 2.9 <sup>a</sup>	30.5 $\pm$ 6.7	15.9 $\pm$ 3.3 <sup>a,b</sup>
Moderate-to-vigorous activity (mins/week)	133.3 $\pm$ 70.4	202.7 $\pm$ 193.6	120.8 $\pm$ 56.5	103.1 $\pm$ 59.4 <sup>b</sup>	124.6 $\pm$ 52.8	126.6 $\pm$ 58.2	112.0 $\pm$ 67.9	59.4 $\pm$ 30.9 <sup>b</sup>
Chair rise time (s)	–	–	10.6 $\pm$ 2.3	11.4 $\pm$ 4.6 <sup>a</sup>	10.0 $\pm$ 2.1	8.7 $\pm$ 2.4	12.2 $\pm$ 1.8 <sup>b</sup>	16.2 $\pm$ 3.7 <sup>a,b</sup>
Stair climb power (watts)	506.5 $\pm$ 72.8	339.1 $\pm$ 60.5 <sup>a</sup>	313.2 $\pm$ 84.0 <sup>b</sup>	210.8 $\pm$ 81.4 <sup>a,b</sup>	330.6 $\pm$ 93.0	249.4 $\pm$ 73.7 <sup>a</sup>	273.5 $\pm$ 39.3	139.0 $\pm$ 29.1 <sup>a,b</sup>
Six-min walk gait speed (m/s)	–	–	1.4 $\pm$ 0.2	1.2 $\pm$ 0.4 <sup>a</sup>	1.5 $\pm$ 0.2	1.5 $\pm$ 0.2	1.3 $\pm$ 0.2	0.8 $\pm$ 0.2 <sup>a,b</sup>
Charlson index (% 10-year survival)	–	–	50.0 $\pm$ 22.3	51.9 $\pm$ 21.5	55.7 $\pm$ 20.7	53.6 $\pm$ 25.5	36.9 $\pm$ 21.5	48.8 $\pm$ 12.1
SPPB score	–	–	11.3 $\pm$ 1.0	10.7 $\pm$ 1.6	11.5 $\pm$ 0.9	11.7 $\pm$ 0.5	10.7 $\pm$ 1.0 <sup>b</sup>	8.9 $\pm$ 1.1 <sup>a,b</sup>
RBANS score	102.9 $\pm$ 15.9	102.2 $\pm$ 4.5	102.6 $\pm$ 13.2	108.8 $\pm$ 12.3	104.1 $\pm$ 11.7	110.8 $\pm$ 12.2	99.1 $\pm$ 16.7	105.1 $\pm$ 12.5
Voluntary activation (%)	92.7 $\pm$ 3.4	94.8 $\pm$ 3.4	88.7 $\pm$ 9.7	90.1 $\pm$ 8.0 <sup>b</sup>	90.1 $\pm$ 7.5	91.4 $\pm$ 7.8	86.4 $\pm$ 13.1	87.0 $\pm$ 8.6

For the voluntary activation measurement the sample sizes were *n* = 22 for young adults (8 males and 14 females), *n* = 33 for older adults (16 males and 17 females), *n* = 22 for non-weak older adults (10 males and 12 females), and *n* = 11 for weak older adults (6 males and 5 females)

<sup>a</sup>Significant difference relative to the sex-specific comparison within sub-groups (e.g., young adult males vs. females)

<sup>b</sup>Significant difference relative to the sex-specific comparison between groups (e.g., young vs. older adult males)

## Leg Extension and Handgrip Strength

Non-dominant, isometric and isokinetic leg extension strength measures were recorded via a dynamometer (Biodex System 4 Dynamometer, Biodex Medical Systems Inc., Shirley, NY). Maximal handgrip strength was assessed using a portable JAMAR® Hydraulic Hand Dynamometer; (Model 5030J1; Lafayette Instrument Co.; Lafayette, Indiana).

## Clinically Meaningful Weakness Classifications

As stated above, older adult clinically meaningful weakness phenotypes were determined using the same isokinetic leg extension strength protocol that was performed in the Health, Aging, and Body Composition Study from which the leg extensor weakness thresholds were derived [4]. Specifically, older adults were classified as ‘weak’ for the leg extensors if their non-dominant isokinetic (60°/sec) strength relative to their body weight was  $\leq 1.12$  Nm/kg for men and  $\leq 1.00$  Nm/kg for women. This weakness classification represents the 1st & 3rd decile (for men & women,

respectively) of sex-specific, relative strength values, which has been shown to be predictive of non-disabled older adults subsequently developing severe mobility limitations [4]. These cut points were derived from a cohort of 1355 men and 1429 women (mean:  $73.6 \pm 2.85$  years) that were tracked over  $\sim 5.9$  years [4]. Older adults with values above these sex-specific cut points were classified as ‘non-weak’. Of note, all young adults were classified as ‘non-weak’ as their values were above the sex-specific 1st & 3rd decile cut points. All assessments conducted were performed by lab personnel blinded to weakness classification.

## Neuromuscular Quality

Isokinetic leg extensor strength of the non-dominant leg was expressed relative to thigh lean mass, obtained via whole body DXA scans (Hologic Discovery QDR model Series, Waltham, MA, USA). Calculation of whole-body, appendicular, and non-dominant thigh lean tissue mass were performed using the analysis package (Hologic APEX, ver. 4.0.2).

## Voluntary Activation

Here, the doublet interpolation technique was performed. Specifically, the participant was asked to perform one-to-two 5-s isometric MVCs while a 100-Hz supramaximal doublet was delivered during the peak force output followed by a second doublet delivered to the resting muscle. The increase in force immediately following the stimulation was expressed relative to a potentiated response evoked by the same doublet applied at rest (i.e., one-to-two seconds after the supramaximal doublet stimulation during the MVC). We should also mention that 10 older adults and two young adults were unable to complete the voluntary activation testing due to discomfort associated with the stimulation.

## Motor Unit Recordings and Analyses

Trapezoidal, isometric contractions were performed using the same mechanical setup as described for the leg extension strength measures at 20%, 50%, and 80% MVC. Target torque matching templates contained, in the following order, a(n) (1) quiescent period (4 s), (2) ascending phase (10% MVC/s), (3) steady-state, plateau phase (10 s for the 20% and 50% MVC, and 8 s for the 80% MVC), (4) descending phase (10% MVC/sec), and (5) additional quiescent period (4 s). All torque matching templates were completed twice with 1 min of rest between bouts.

Surface EMG signals were recorded from the non-dominant vastus lateralis muscle via a Bagnoli Desktop system (Delsys, Inc., Natick, MA). Signals were detected with a

5-pin array EMG sensor (Delsys, Inc., Natick, MA). Filtered EMG signals served as the input to the Precision Decomposition III algorithm, which was utilized via dEMG Analysis software (ver. 1.1, Delsys, Inc., Natick, MA). Decomposition-Synthesize-Decomposition-Compare testing was used to remove motor units with detection accuracy  $< 90.0\%$ . Before analysis, all MUFR curves were smoothed by low-pass filtering each motor unit’s impulse train with a 1-s Hanning window.

The mean MUFRs data was individually normalized at each contraction intensity (see supplemental material for further details). Here, the y-intercept from the mean MUFR versus recruitment threshold scatterplot fit with a linear regression was used as this approach theoretically controls for the ‘onion skin phenomenon’ of MUFRs (i.e., larger-threshold motor units fire slower than lower-threshold motor units). Specifically, for each participant at each contraction intensity (20%, 50%, and 80% MVC) the coefficients of the linear regression (y-intercept and slope) were calculated for the mean MUFRs versus the recruitment threshold scatterplot. Thus, the y-intercepts (our index for ‘normalized MUFR’) and slopes were determined for each participant in a particular group, at each contraction intensity, before averaging the aggregated data by group and subsequently disaggregating the data by sex.

## Computational Modeling

Developed with NEURON simulation environment (ver. 7.6). Simulations were run on the Neuroscience Gateway Stampede2 KNL Super-computer with NEURON 7.6 tool. Analysis was performed in *MATLAB* (ver. 9.9.0 [R2020b]). For the vastus lateralis computer model employed herein, we incorporated several features of biological variability (i.e., heterogeneity and overlap in cell properties of different MN types), which simulates MN recruitment, firing rates, and force generation more accurately than computer models missing those features. *See below for further details.*

### Vastus Lateralis Motoneuron Pool Model—Individual Cells

Cat MNs, as opposed to rodents, were used as the foundation of our human cell models as their electrical and firing properties, as well as force production, are very similar to humans. Specifically, the reconstructed morphologies of identified slow (S), fatigue-resistant (FR), and fast-fatiguing (FF) cat cells were used to represent the three-dimensional anatomy of model MNs (Fig. 4a). To mimic the physiological activation of MNs via synaptic inputs, excitatory synapses were distributed over the dendrites of each model



cell following the realistic distribution of Ia afferent-to-motoneuron contacts labeled intracellularly with horseradish peroxidase in type-identified cat MNs. Additionally, the model MNs include somatic and dendritic voltage-gated and calcium-activated ion channels that mediate transient and persistent inward/outward currents underlying nonlinear MN firing properties. Moreover, their membrane electrical passive and active properties matched those measured experimentally from cat MN (see Fig. 5a).

#### Vastus Lateralis Motoneuron Pool Model—Synaptic Input

Individual cells in the MN pool model were stimulated through synaptic inputs with trapezoidal activation waveforms, like in our human experimental protocols. The conductance of these synaptic inputs was determined using effective synaptic currents of pyramidal inputs to spinal MNs, in which large MNs received higher synaptic current than small MNs (Fig. 5d). The mean MUF<sub>R</sub> of recruited motor units was measured in the simulation via a 200 ms moving window (during the plateau phase of trapezoidal waveforms).

#### Vastus Lateralis Motoneuron Pool Model—Motor Unit Type Composition

The percentage of different cell types (S, FR, FF) in the vastus lateralis muscle MN pool was determined using previously published older adult fiber type data (see supplemental material for further details). Fifty-eight percent of motor units were identified as Type I (S), 25% were Type IIA (FR), and 17% were Type IIB (FF). The average innervation ratio was estimated to be: 1 (Type I): 2.5 (Type IIA): 5 (Type IIB). Thus, the innervation ratio for Type IIA is 2.5 times greater than the innervation ratio for Type I, while the innervation ratio for Type IIB is 5 times greater than the innervation ratio with Type I. Based on these muscle fiber types and innervation ratio data, the MN pool innervating the vastus lateralis muscle for older adults consisted of ~81% S-type, ~14% FR-type, and ~5% FF-type cells. As the vastus lateralis muscle MN pool was modeled with 189 model cells in the present study, 153 were S-type, 27 were FR-type, and 9 were FF-type cells (Fig. 4a).

#### Vastus Lateralis Motoneuron Pool Model—Force Generation

Here, spike trains were converted to twitch forces in each cell in the MN pool model, and all motor unit forces were summated to simulate the total vastus lateralis muscle force (Fig. 5a). To simulate the changes in force production with aging, we adjusted the motor unit force parameters (twitch force amplitude and duration) by the percent changes observed from young adults to older adults (i.e.,

twitch force amplitude was decreased by 36% and twitch force duration was increased by 15%). Then, the synaptic drive to the MNs was reduced to record the change in predicted strength (Fig. 5b, secondary y-axis).

#### Statistical Analysis

Repeated measures analysis of covariances (ANCOVAs) were performed to examine group differences in the respective dependent variable (e.g., normalized MUF<sub>R</sub>). Here, group (e.g., *weak* versus *non-weak* older adults) was a between-participant factor and contraction intensity were a within-participant factor (3 levels). Sidak post-hoc analyses were performed if a significant main effect of interaction was observed. One-way ANCOVAs were used for group wise comparisons. Sex was covaried in all aggregated data analyses. Additionally, directionally hypothesized bivariate correlations (i.e., one-tailed tests) were used to examine whether there were associations between slower normalized MUF<sub>R</sub>s and poorer neuromuscular quality, lower voluntary activation, and with reduced physical function/mobility in older adults. A p-value of  $\leq 0.05$  was required for statistical significance. Statistical Package for the Social Sciences (SPSS; version 25.0, Chicago, IL) was used for data analysis and presented as estimated marginal means  $\pm$  the standard error of mean (SEM). Effect sizes ( $\eta^2$ ) are also reported to aid in interpretation.

#### Results

##### 'Weak' versus 'Non-Weak' Older Adults

Older adult weakness phenotype group data was first aggregated for *weak* relative to *non-weak* older adults, covarying for sex. Next, this group data was disaggregated by sex to investigate sex-specific differences among and between weakness phenotypes. Of note, the disaggregated sex-specific data is likely underpowered, and thus should be interpreted with caution. These data are presented in Tables 3 and 4.

##### Aggregated Group Data by Older Adult Weakness Phenotypes

When compared to *non-weak* older adults, *weak* older adults exhibited 1.6 and 3.1 Hz slower (7.2% and 13.1% slower, respectively) normalized MUF<sub>R</sub>s at 50% and 80% MVCs, respectively (22.1 vs. 20.5 Hz and 23.7 vs. 20.6 Hz, respectively). Specifically, a significant group by contraction intensity interaction was observed for normalized MUF<sub>R</sub> (Fig. 2;  $p = 0.05$ ;  $\eta^2 = 0.07$ ). Post-hoc analyses indicated that *non-weak* older adults demonstrated a sequential increase

**Table 3** Mean and normalized (linear regression) motor unit firing rates of study population (EMM ± SEM)

	Age-related weakness		Older adult phenotypic weakness	
	Young adults N=24	Older adults N=43	Non-weak n=29	Weak n=14
<b>20% MVC</b>				
Motor unit number	14 ± 1.0	13.2 ± 0.7	12.9 ± 0.9	14.4 ± 1.3
Slope	- 0.70 ± 0.25	- 1.11 ± 0.19	- 1.2 ± 0.3	- 0.95 ± 0.4
Y-intercept (Hz)	19.5 ± 0.6	19.3 ± 0.5	19.5 ± 0.6	19 ± 0.9
R2 (%)	66.2 ± 4.3	57.7 ± 3.2	54.6 ± 4.1	64.6 ± 5.8
<b>50% MVC</b>				
Motor unit number	21.7 ± 1.2	15.6 ± 0.9 <sup>a</sup>	14.4 ± 1.1	18.5 ± 1.5
Slope	- 0.38 ± 0.06	- 0.47 ± 0.05	- 0.5 ± 0.07	- 0.42 ± 0.1
Y-intercept (Hz)	22.4 ± 0.5	21.6 ± 0.4	22.1 ± 0.4	20.5 ± 0.6 <sup>a</sup>
R2 (%)	76.4 ± 3.9	70.0 ± 2.9	70.5 ± 3.5	69.1 ± 5.0
<b>80% MVC</b>				
Motor unit number	18.9 ± 1.3	13.7 ± 1.0 <sup>a</sup>	13.5 ± 1.1	14.3 ± 1.6
Slope	- 0.29 ± 0.03	- 0.31 ± 0.02	- 0.33 ± 0.03	- 0.27 ± 0.05
(Hz)	25.5 ± 0.8	22.7 ± 0.6 <sup>a</sup>	23.7 ± 0.7	20.6 ± 0.9 <sup>a</sup>
R2 (%)	79.3 ± 3.6	71.3 ± 2.7	70.7 ± 3.8	72.3 ± 5.5

EMM estimated marginal means, Hz Hertz, MVC maximal voluntary isometric contraction, SEM standard error of the mean

<sup>a</sup>Significant difference relative to the comparison group (e.g., young vs. older adults)

**Table 4** Sex-specific mean and normalized (linear regression) motor unit firing rates of study population (EMM ± SEM)

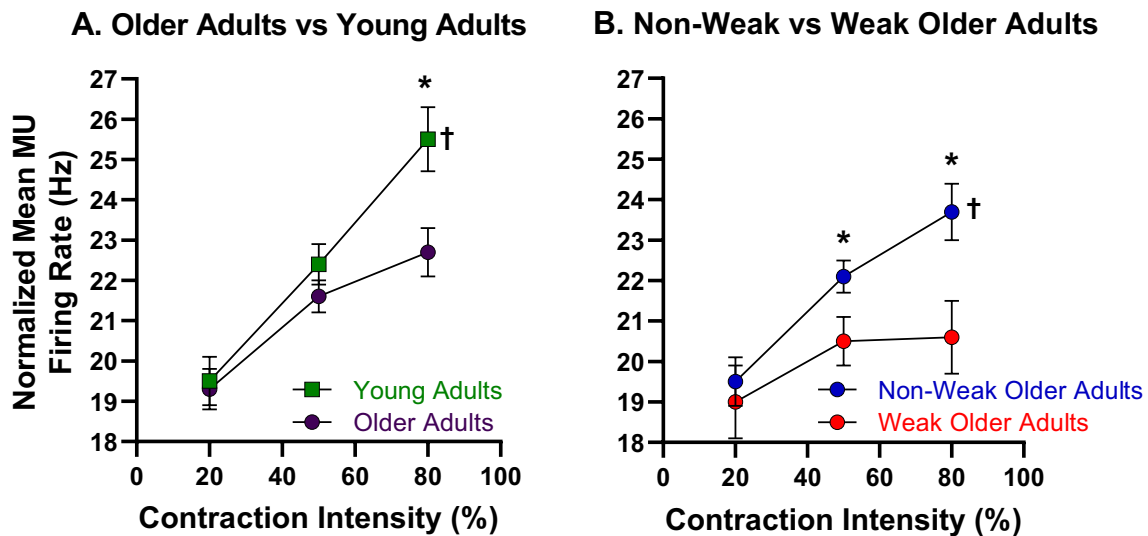
	Age-related weakness				Older adult phenotypic weakness			
	Young adults N=24		Older adults N=43		Non-weak n=29		Weak n=14	
	Males n=10	Females n=14	Males n=23	Females n=20	Males n=16	Females n=13	Males n=7	Females n=7
<b>20% MVC</b>								
Motor unit number	14.3 ± 1.7	13.2 ± 1.1	15.9 ± 1.1	10.5 ± 0.9 <sup>a</sup>	15.7 ± 1.3	9.6 ± 1.1 <sup>a</sup>	16.3 ± 2.0	12.1 ± 1.6
Slope	- 0.67 ± 0.37	- 0.70 ± 0.34	- 1.21 ± 0.25	- 1.01 ± 0.28	- 1.48 ± 0.3	- 0.85 ± 0.44	- 0.59 ± 0.51	- 1.29 ± 0.60
Y-intercept (Hz)	19.4 ± 1.1	19.4 ± 0.8	20.0 ± 0.7	18.6 ± 0.7	20.6 ± 0.6	18.2 ± 0.9 <sup>a</sup>	18.6 ± 1.2	19.3 ± 1.2
R2 (%)	59.4 ± 6.2	70.5 ± 6.5	63.5 ± 4.1	51.4 ± 4.6 <sup>a,b</sup>	58.7 ± 4.9	50.0 ± 6.7	74.4 ± 7.4	53.9 ± 9.2
<b>50% MVC</b>								
Motor unit number	22.4 ± 1.9	20.9 ± 1.7	17.1 ± 1.2 <sup>b</sup>	14.2 ± 1.4 <sup>b</sup>	15.4 ± 1.3	13.3 ± 1.8	21.0 ± 2.0 <sup>b</sup>	15.7 ± 2.4
Slope	- 0.40 ± 0.13	- 0.35 ± 0.05	- 0.55 ± 0.08	- 0.37 ± 0.04 <sup>a</sup>	- 0.61 ± 0.11	- 0.35 ± 0.06 <sup>a</sup>	- 0.42 ± 0.17	- 0.40 ± 0.08
Y-intercept (Hz)	22.2 ± 0.8	22.5 ± 0.6	21.4 ± 0.5	21.7 ± 0.5	22.2 ± 0.5	21.7 ± 0.6	19.6 ± 0.8 <sup>b</sup>	21.5 ± 0.8
R2 (%)	77.6 ± 3.9	75.0 ± 6.5	72.1 ± 2.5	67.7 ± 5.4	71.3 ± 3.1	69.7 ± 6.7	74.0 ± 4.6	63.9 ± 9.20
<b>80% MVC</b>								
Motor unit number	19.0 ± 1.9	18.6 ± 1.7	15.1 ± 1.3	12.2 ± 1.5 <sup>b</sup>	15.7 ± 1.5	10.9 ± 1.6 <sup>a</sup>	13.9 ± 2.3	14.8 ± 2.1
Slope	- 0.30 ± 0.05	- 0.28 ± 0.04	- 0.32 ± 0.04	- 0.31 ± 0.03	- 0.37 ± 0.04	- 0.30 ± 0.05	- 0.21 ± 0.06	- 0.33 ± 0.06
Y-intercept (Hz)	26.5 ± 1.5	24.7 ± 0.9	23.0 ± 1.0 <sup>b</sup>	22.3 ± 0.7	24.2 ± 1.0	23.1 ± 0.8	20.3 ± 1.6 <sup>b</sup>	20.7 ± 1.0
R2 (%)	77.8 ± 5.9	80.5 ± 4.4	71.3 ± 3.9	71.2 ± 3.7	73.6 ± 5.2	67.2 ± 5.6	66.1 ± 7.8	78.5 ± 7.6

EMM estimated marginal means, Hz Hertz, MVC maximal voluntary isometric contraction, SEM standard error of the mean

<sup>a</sup>Significant difference relative to the sex-specific comparison within sub-groups (e.g., young adult males vs. females)

<sup>b</sup>Significant difference relative to the sex-specific comparison between groups (e.g., young vs. older adult males)





**Fig. 2** Older adults with clinically meaningful leg extensor weakness exhibit slowing in motor unit firing rates during higher intensity contractions. During moderate-to-high contraction intensities (i.e.,  $\geq 50\%$  MVC) normalized motor unit firing rates were lower in older adults (purple circle; **a**) relative to young adults (green squares; **a**), as well

as in older adults with clinically meaningful leg extensor weakness (*weak*- red circles; *non-weak*- blue circles; **b**). Asterisks and daggers represent significant differences between groups and intensities, respectively. *Hz* Hertz

in normalized MUFRR at each contraction intensity level ( $p$ 's=0.01 to 0.03), whereas *weak* older adults did not exhibit any difference between contraction intensities ( $p$ 's=0.09 to 1.0). Additionally, *weak* older adults exhibited slower normalized MUFRRs relative to *non-weak* older adults at 50% and 80% MVCs ( $p$ 's=0.03 and 0.01, respectively), but not at 20% MVC ( $p=0.65$ ). Moreover, a group by contraction intensity interaction was not observed for the linear regression slope ( $p=0.88$ ;  $\eta^2 < 0.01$ ), nor for a group ( $p=0.45$ ;  $\eta^2=0.02$ ), or a contraction intensity main effect ( $p=0.21$ ;  $\eta^2=0.04$ ). These data are presented in Table 3 and Fig. 2b.

**Disaggregated Sex-Specific Group Data by Weakness Phenotypes**

When compared to *non-weak* older adult males, *weak* older adult males exhibited 2.6 and 3.5 Hz slower (11.7% and 16.1% slower, respectively) normalized MUFRRs at 50% and 80% MVCs (22.2 vs. 19.6 Hz and 24.2 vs. 20.3 Hz, respectively). Specifically, a significant group by contraction intensity interaction was observed for normalized MUFRR ( $p=0.05$ ;  $\eta^2=0.07$ ). Post-hoc analysis indicated that *non-weak* older adult males demonstrated a sequential increase in normalized MUFRR at each contraction intensity level ( $p$ 's < 0.01 to 0.04), whereas *weak* older adult males did not exhibit any difference between contraction intensities ( $p$ 's=0.07 to 1.0). Additionally, *weak* older adult males exhibited slower normalized MUFRRs relative to *non-weak* older adult males at 50% and 80% MVCs ( $p$ 's=0.03 and 0.01, respectively), but not at 20% MVC ( $p=0.68$ ).

When compared to *non-weak* older adult females, *weak* older adult females did not exhibit any differences at any contraction intensity level ( $p=0.08$ ;  $\eta^2=0.13$ ). Moreover, when compared to *non-weak* older adult males, *non-weak* older adult females exhibited 2.4 Hz slower (i.e., 11.7% slower; 20.6 vs. 18.2 Hz) normalized MUFRRs at 20% MVC ( $p=0.05$ ), but not at 50% and 80% MVCs ( $p$ 's=0.68 and  $p=0.41$ , respectively). When *weak* older adult males and females were compared, there were no differences at any contraction intensity level ( $p$ 's=0.11 to 0.82). Subsequently, a sex by weakness by contraction intensity interaction was not observed for the linear regression slope ( $p=0.51$ ;  $\eta^2=0.04$ ), nor for sex by weakness ( $p=0.95$ ;  $\eta^2 < 0.01$ ) or for sex by contraction intensity ( $p=0.49$ ;  $\eta^2=0.04$ ). Furthermore, neither a main effect for weakness ( $p=0.45$ ;  $\eta^2=0.02$ ) nor sex ( $p=0.43$ ;  $\eta^2=0.02$ ) was observed. These data are presented in Table 4.

**Older Adults Versus Young Adults**

Age-related group data was first aggregated for older adults relative to young adults, covarying for sex. Next, this group data was disaggregated by sex to investigate sex-specific differences among and between age. These data are presented in Tables 3 and 4.

**Aggregated Group Data by Age**

When compared to young adults, older adults exhibited 2.8 Hz slower (11% slower) normalized MUFRRs at 80% MVC (25.5 vs. 22.7 Hz, respectively). Specifically, a significant group by

contraction intensity interaction was observed for normalized MUFR (Fig. 1;  $p=0.01$ ;  $\eta^2=0.07$ ). Post-hoc analyses indicated that young adults demonstrated a sequential increase in normalized MUFR at each contraction intensity level ( $p$ 's  $<0.01$ ), whereas older adults exhibited an increase between 20 and 50% MVCs ( $p <0.01$ ), and 20% and 80% MVCs ( $p <0.01$ ), but no difference between 50 and 80% MVCs ( $p=0.20$ ). Additionally, older adults exhibited slower normalized MUFRs relative to young adults at 80% MVC ( $p <0.01$ ), but not at 20% and 50% MVCs ( $p$ 's = 0.82 and 0.20, respectively). Moreover, a group by contraction intensity interaction was not observed for the linear regression slope ( $p=0.27$ ;  $\eta^2=0.02$ ), nor for a group ( $p=0.13$ ;  $\eta^2=0.04$ ), or a contraction intensity main effect ( $p=0.17$ ;  $\eta^2=0.03$ ). These data are presented in Table 3 and Fig. 2a.

### Disaggregated Sex-Specific Group Data by Age

When compared to young adult males, older adult males exhibited 3.5 Hz slower (13.2% slower) normalized MUFRs at 80% MVC (26.5 vs. 23.0 Hz, respectively). Specifically, a significant age by contraction intensity interaction was observed for normalized MUFR ( $p=0.02$ ;  $\eta^2=0.12$ ). Post-hoc analysis indicated that young adult males demonstrated a sequential increase in normalized MUFR at each contraction intensity level ( $p$ 's  $<0.01$ ), whereas older adult males exhibited an increase between 20 and 50% MVCs ( $p <0.01$ ), and 20% and 80% MVCs ( $p <0.01$ ), but no difference between 50 and 80% MVCs ( $p=0.19$ ). Additionally, older adult males exhibited slower normalized MUFRs relative to young adult males at 80% MVC ( $p=0.03$ ), but not at 20% and 50% MVCs ( $p=0.66$  and  $p=0.41$ , respectively).

When compared to young adult females, older adult females did not exhibit any differences at any contraction intensity level ( $p=0.32$ ;  $\eta^2=0.04$ ). Moreover, when young adult males and females were compared, there were no differences at any contraction intensity level ( $p$ 's = 0.31 to 0.95). Similarly, when older adult males and females were compared, there were no differences at any contraction intensity ( $p$ 's = 0.15 to 0.66). Subsequently, a sex by age by contraction intensity interaction was not observed for the linear regression slope ( $p=0.89$ ;  $\eta^2 <0.01$ ), nor for sex by intensity ( $p=0.93$ ;  $\eta^2 <0.01$ ), or for age by contraction intensity ( $p=0.27$ ;  $\eta^2=0.02$ ). Furthermore, neither a main effect for age ( $p=0.12$ ;  $\eta^2=0.04$ ) nor sex ( $p=0.51$ ;  $\eta^2 <0.01$ ) was observed. These data are presented in Table 4.

### Association Between Normalized Motor Unit Firing Rates and Indices of Muscle/Physical Function

The data presented herein is limited to older adults at 80% MVC. Significant associations were observed between

slower normalized MUFRs and poorer neuromuscular quality ( $r=0.43$ ;  $p <0.01$ ), as well as lower voluntary activation ( $r=0.33$ ;  $p=0.029$ ). Additionally, significant associations were observed between slower normalized MUFRs and reduced chair rise time performance ( $r=0.31$ ;  $p=0.020$ ), as well as diminished stair climb power ( $r=0.33$ ;  $p=0.015$ ). These data are presented in Fig. 3.

### Computational Modeling

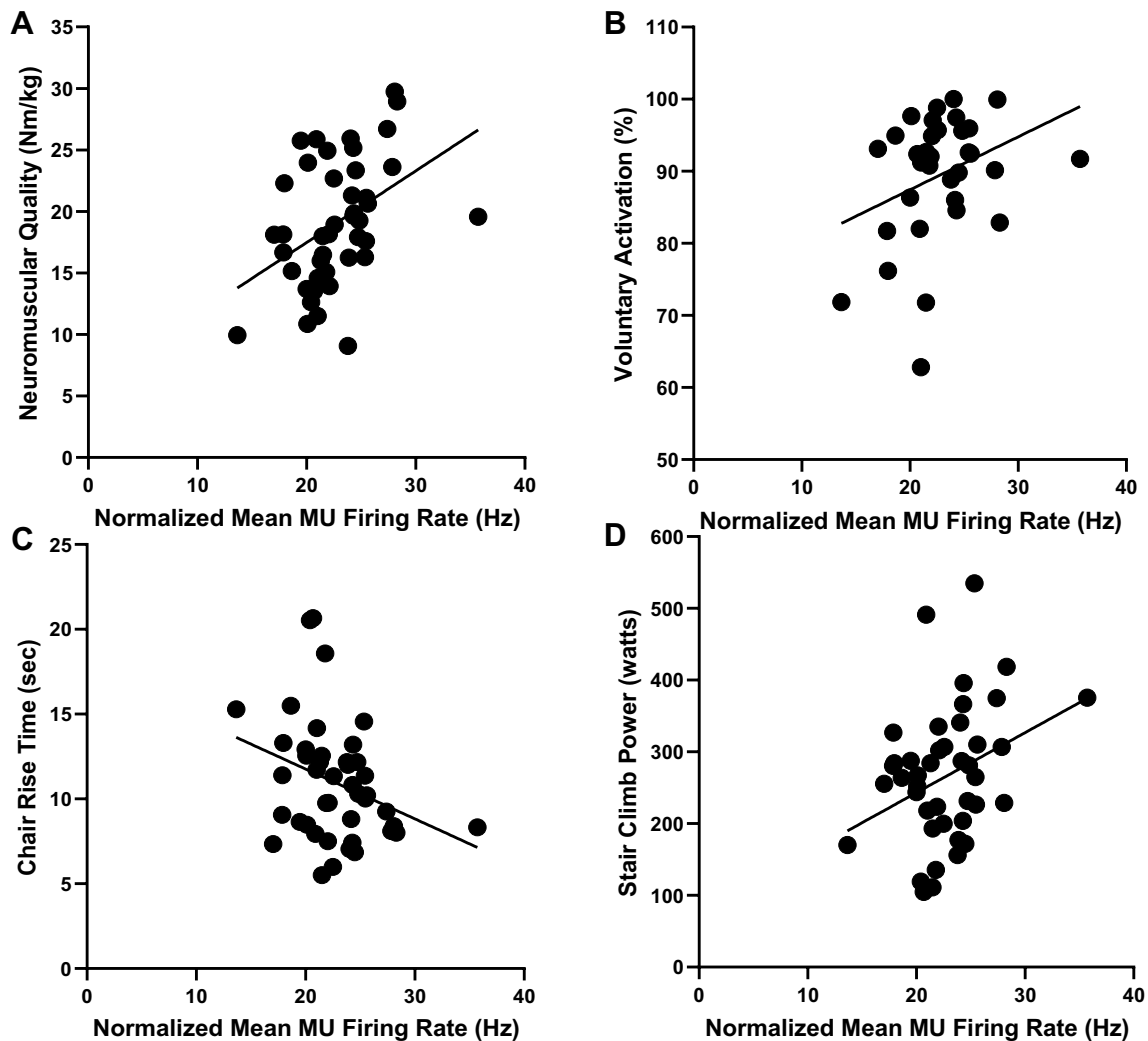
Using the vastus lateralis muscle MN pool model, we graded synaptic input to all cell types and calculated the generated vastus lateralis muscle force and mean MUFRs at each sub-maximal contraction intensity (between 50 and 90% MVC, Figs. 4, 5c). The mean MUFR at 80% MVC in the simulations was 24.92 Hz, which was slightly higher than our in vivo human recordings (see y-intercept data in Table 3). When a 3 Hz decrease in MUFR was calculated from the 80% MVC our simulations showed normalized MUFRs that were comparable to those recorded during a torque level between 54 and 69% MVC. In other words, a 3 Hz reduction in mean MUFR resulted in a strength decrement of 11–26%.

As the global reduction of 3 Hz in mean firing rate of the MN pool would cause force reductions due to derecruitment of some cells, as well as the lowered firing rate of other cells, we quantified how much of the reduction in force was a result of each of these two mechanisms. Our simulations show that two-thirds of this force reduction is due to MN derecruitment, whereas one-third is due to the reduced cell firing rate. S-type MNs were derecruited the most (88–95% of the cells, mean of 91%), whereas FR- and FF-type MNs contributed more to force reduction due to the lowering of their firing (47–65% force reduction, mean of 56%).

### Discussion

The purpose of this study was to determine whether older adults with clinically meaningful leg extensor weakness had slower normalized MUFRs relative to older adults without leg extensor weakness via decomposed surface EMG recordings, along with linear regression analysis. Subsequently, we sought to *independently* predict how reductions in normalized MUFRs would affect force output via multi-scale, high-fidelity, anatomically-detailed computational modeling. Lastly, we examined the associations between normalized MUFRs and neuromuscular quality, voluntary activation, and with measures of physical function/mobility in older adults via directionally hypothesized bivariate correlation analysis.

Normalized MUFRs were found to be considerably slower in *weak* older adults relative to *non-weak* older adults at 50% and 80% MVC, whereas in older adults (in general) relative to young adults, considerably slower normalized MUFRs



**Fig. 3** Slower motor unit firing rates at 80% MVC is associated with poorer neuromuscular quality, lower voluntary activation, and reduced physical function/mobility in older adults. Normalized motor unit firing rates at 80% MVC were associated with neuromuscular quality

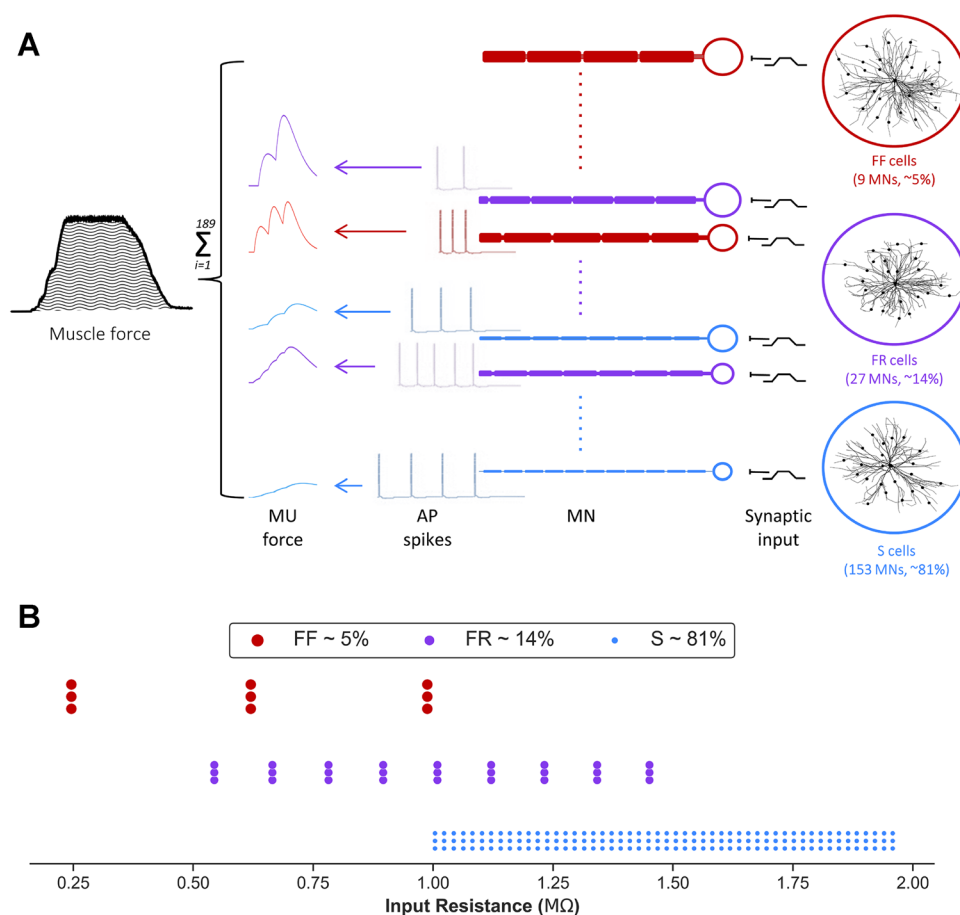
(a [top left];  $r=0.43$ ;  $p<0.01$ ), voluntary activation (b [top right];  $r=0.33$ ;  $p=0.03$ ), chair rise time (c [bottom left];  $r=0.31$ ;  $p=0.02$ ), and stair climb power (d [bottom right];  $r=0.33$ ;  $p=0.015$ ). *Hz* Hertz, *MVC* maximum voluntary isometric contraction, *MU* motor unit

were found only at 80% MVC. While these differences appeared to be more notable in males relative to females, the sex-specific data should be interpreted cautiously due to the small sample size. In addition, significant associations were noted between slower normalized MUFs and poorer neuromuscular quality, lower voluntary activation, reduced chair rise time, and diminished stair climb power. Interestingly, while females possessed a higher degree of voluntary activation, they generally had lower neuromuscular quality values, chair rise time performance (exception—*non-weak* females), and stair climb power. These novel findings extend prior work [20–23, 32–37], which described differences in MUF characteristics between older adults (in general) and young adults, to directly investigate to what extent MUFs are associated with clinically meaningful weakness in older adults. Notably, our findings not only link slowed MUFs

to clinically meaningful leg extensor weakness and impairments in physical function/mobility, but also provide valuable insights on how type-specific MNs and their respective MUs (classified based on anatomical, biophysical, firing, and force properties) contribute to clinically meaningful weakness.

Our findings of slowed normalized MUFs in older adults (in general) relative to young adults, during moderate-to-high intensity contractions (e.g.,  $\geq 50\%$  MVC) is largely consistent with the extant literature [20, 21, 23, 33, 34, 37]. Specifically, MUFs have been reported to be approximately 20–64% lower in older adults (in general) relative to young adults for the intrinsic hand muscles (first dorsal interosseus and abductor digiti minimi) [24, 66] and the lower extremity muscles (tibialis anterior and vastus lateralis) [20, 21, 23, 33, 34, 37]. One notable difference in our work, relative to many previous reports, is that we controlled for the influence

**Fig. 4** Schematic diagram of the vastus lateralis muscle's MN pool model structure and cells distribution. **a** Cell morphologies used for the development of S- (blue), FR- (purple), and FF- (red) type MN models. A total of 189 cells were included in the model, with 153 S-type MNs (~81%), 27 FR-type MNs (~14%), and 9 FF-type MNs (~5%). The model was stimulated with synaptic inputs of trapezoidal shape. The spike train of each MN were used to calculate the individual motor unit force; these were summed for the total vastus lateralis muscle force. **b** Distribution of MN type by input resistance within the pool, where each cell type was replicated three times with different spiking threshold to capture the biological variability observed experimentally. AP action potential, FF fast-fatiguing, FR fatigue-resistant, MN motoneuron, MU motor unit, S slow

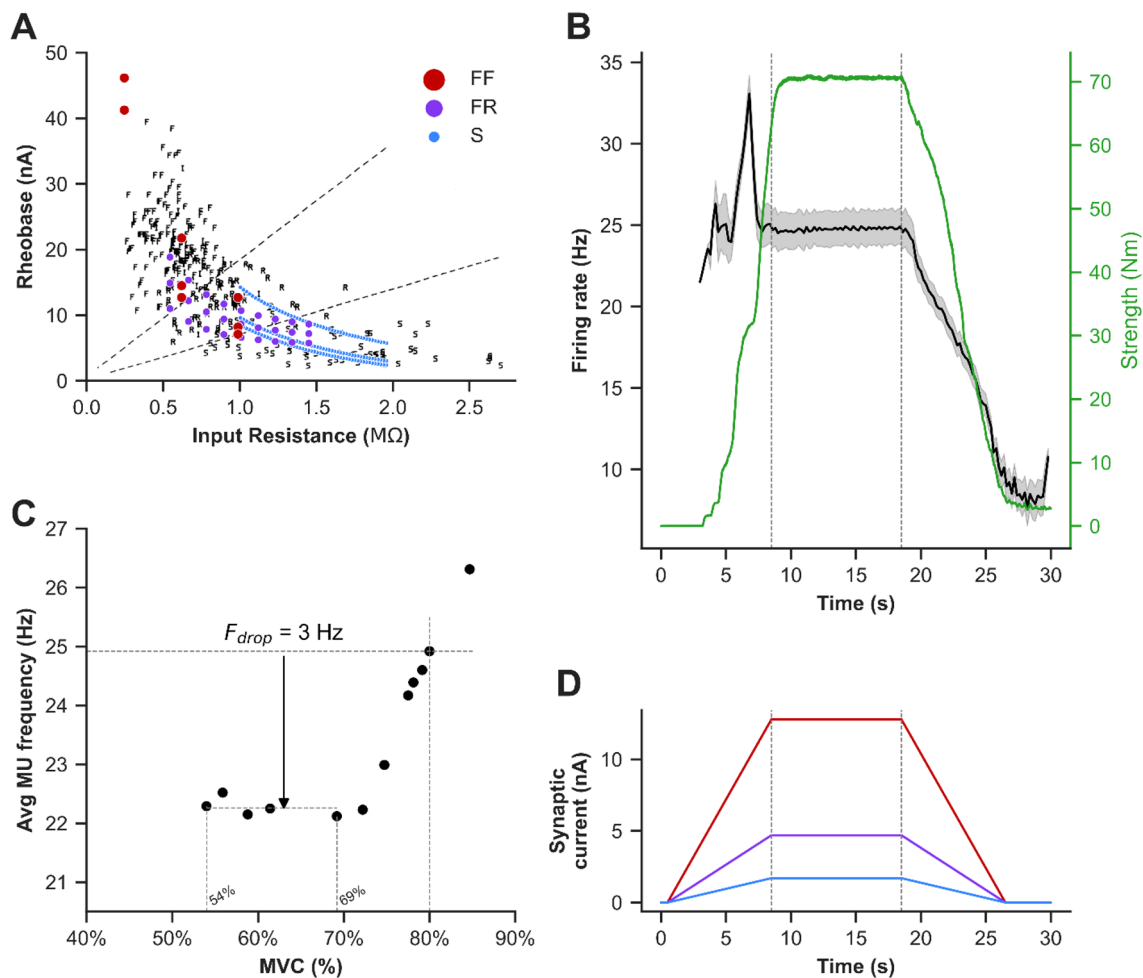


of the recruitment threshold on MUF<sub>R</sub> estimates. Utilizing the y-intercept of the linear regression, we were able to account for the influence of recruitment threshold on each motor unit's firing behavior. Thus, our observation of 12–14% slower MUF<sub>R</sub>s during moderate-to-high intensity contractions, which is slightly lower than some prior reports, may be attributed to our normalization procedure. Of note, our normalized MUF<sub>R</sub> values are similar to those found in prior work that utilized the same muscle and also the y-intercept method [38, 39].

The most crucial and novel finding of our work is the observation that older adults with clinically meaningful leg extensor weakness have significantly slower normalized MUF<sub>R</sub>s relative to older adults without leg extensor weakness, and that MUF<sub>R</sub>s are associated with indices of physical function and voluntary (neural) activation. These findings are consistent with prior studies assaying various amplitude characteristics of the interference EMG signal to estimate neural activation, which reported associations with reduced indices of mobility/physical function in older adults [40–46]. Thus, it is plausible that slower MUF<sub>R</sub>s underlies the findings from these prior studies. In fact, our computational model *independently* predicted that slower MUF<sub>R</sub>s result in notable strength reductions of 11–26%, with one-third being lost to a reduction

in MNFR and two-thirds being lost to MN derecruitment. In addition, slower normalized MUF<sub>R</sub>s were associated with not only poorer neuromuscular quality, but also with lower levels of voluntary activation and physical function. While the sex-specific findings are likely unpowered, it is interesting to note that the link between MUF<sub>R</sub> and indices of muscle/physical function appear strongest in men. However, the correlation with voluntary (neural) activation is stronger in women. Together, these novel findings provide evidence for impairments in MUF<sub>R</sub> being pathogenically linked to age-related weakness, which we postulate is potentially due to dysfunction of MN intrinsic excitability mechanisms.

From a broader perspective, we have previously assessed corticospinal excitability non-invasively using transcranial magnetic stimulation across older adult weakness phenotypes, wherein we reported that *weak* older adults exhibited lower indices of corticospinal excitability [47, 48]. While our prior work did not parse out cortical vs. MN excitability, others have suggested that older adults have lower indices of motor unit excitability relative to young adults. For instance, Christie and Kamen [18] reported that older adults had ~10% longer motor unit after-hyperpolarization durations, and very recently, Orsatto et al. [49] noted that older adults had a lower 'delta frequency', which is a variable thought to



**Fig. 5** Older adult vastus lateralis muscle MN pool simulations. **a** The rheobase versus input resistance relationship for the pool model (colored dots) overlaid over experimental data from Zengel et al. [52]. **b** MN firing rates (black) with 95% confidence interval (gray) and force (green) during the trapezoidal contraction of 80% MVC (illustrated in [D]). **c** Average motor unit firing rates against the relative voluntary contraction force. Firing rates at 80% MVC

is 24.92 Hz. The firing rates were reduced by 3 Hz, while force is reduced to 54–69% of MVC. **d** Effective synaptic input used to stimulate the pool where larger MNs receive higher synaptic currents (FF > FR > S). In panels **a**, **d**: blue, purple, and red refer to S-type MNs, FR-type MNs, and FF-type MNs, respectively. *FF* fast-fatiguing, *FR* fatigue-resistant, *Hz* Hertz, *MN* motoneuron, *MU* motor unit, *MVC* maximal voluntary isometric contraction, *S* slow

be reflective of a MN’s intrinsic excitability that is heavily influenced by the MN’s persistent inward current amplitude [50, 51]. Thus, one possible explanation for our noted slowed MUFrs in *weak* older adults is that these participants have reduced MN intrinsic excitability, which could be due to MN deterioration itself and/or an impaired monoaminergic system. However, there are other neurological related reasons for why we observed slowed MUFrs, such as reduced net excitatory drive to MN pool or reductions in afferent feedback. Yet, another potential reason may be due to age-related muscular changes (e.g., leftward shift in force-frequency relationship). Hence, further work is needed to better delineate the precise mechanism(s) responsible for slowed MUFrs in age-related weakness. Regardless, our findings suggest neurotherapeutic approaches that function to increase MUFrs may

have clinical utility for treating age-related weakness, with the most effective strategy likely depending on our depth of understanding the biological basis for slowed MUFrs patterns.

There are several limitations of our work that should be noted. First, this study was cross-sectional in nature. Thus, our results are not influenced by time (i.e., within-subject aging), and therefore, do not necessarily suggest cause-and-effect relationships. Second, our study population was community-dwelling older adults. Therefore, it is plausible that their MUFrs data may not be generalizable to institutionalized older adults. Third, we normalized strength data to thigh lean mass assessed via DXA for muscle quality assessment. Hence, this should be interpreted cautiously as this measure reflects not only the quadriceps muscle group, but also those of other muscles in the thigh region (e.g., adductors, biceps femoris, sartorius),



as well as other tissue components (e.g., connective tissue). Fourth, the MUFR work was only recorded from the vastus lateralis muscle, which only accounts for a partial amount the total force generation from the much larger quadriceps femoris muscle group. Thus, it is difficult to know if the other synergistic muscles involved in leg extension force displayed similar MUFR patterns. Fifth, some of our comparisons, particularly those where data were disaggregated by biological sex, were likely underpowered (i.e., *weak* older adults and with voluntary activation measures). Therefore, the multiple comparisons from these data should also be interpreted cautiously. Lastly, we would be remiss if we did not acknowledge that there is robust debate and legitimate concern over the physiological validity/accuracy of surface EMG-derived motor unit recordings. While this approach has advantages (e.g., non-invasive; hence, more feasible in certain populations, recording of higher threshold motor units, increased motor unit yield), the strategy used to evaluate the waveforms identified by a decomposition algorithm can limit the quality of the information and data must be interpreted within this context.

In conclusion, we presented evidence that *weak* older adults have significantly slower normalized MUFrs relative to *non-weak* older adults at moderate-to-high contraction intensities, and that this reduction in MUFR results in a significant strength decrement. Furthermore, we provided valuable insights on how MN types contribute differentially to clinically meaningful force loss. Additionally, we noted significant associations between slower normalized MUFrs and poorer neuromuscular quality, lower voluntary activation, reduced chair rise time, and diminished stair climb power. Taken together, these findings provide evidence that impairments in MUFR are mechanistically linked to clinically meaningful weakness, which provides rational support for the development of neurotherapeutic approaches that function to increase MUFrs to treat age-related weakness.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00223-023-01123-x>.

**Acknowledgements** This work was supported, in part, by grants from the National Institutes of Health (R01AG044424 to BC Clark; R01AG067758 to BC Clark, WD Arnold, and SM Elbasiouny; R01NS091836 to SM Elbasiouny; R01NS131816 to SM Elbasiouny; and F32AG069358 to NP Wages). The funder had no role in study design and conduct of the study; data collection, management, analysis, and interpretation; preparation, review, or approval of the manuscript; or in decision to submit the manuscript for publication.

**Author Contributions** All authors helped to conceptualize the study. NP Wages and BC Clark wrote the initial and final versions of the manuscript. NPW, LAC, and DT were responsible for data acquisition. NPW and LAC were responsible for data analysis. NPW and BCC performed the statistical operations. MHM and SME were responsible for the computational modeling work. All authors provided critical intellectual content. BCC also secured funding for the project that directly funded the work and served as PI on the work (R01AG044424). All authors revised the manuscript and gave final approval for this version of the article.

**Funding** BC Clark reported receiving grants from the National Institutes of Health during the conduct of the study; grants and personal fees from Regeneron Pharmaceuticals; grants from Astellas Global Development Inc, RTI Solutions, NMD Pharma, and OsteoDx Inc; and personal fees from Gerson Lehrman Group; and also reported serving as a co-founder and chief of aging research at OsteoDx Inc outside the submitted work. WD Arnold has served as a consultant for NMD Pharma A/S, Scholar Rock, Dyne therapeutics, Amplo Biotechnology, Argenx, Arrowhead Pharmaceuticals, Catalyst Pharmaceuticals, La Hoffmann Roche, and Novartis.

## Declarations

**Competing interest** Nathan P. Wages, Mohamed H. Mousa, Leatha A. Clark, Dallin Tavoian, W. David Arnold, Sherif M. Elbasiouny, Brian C. Clark have no competing interests.

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