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ORIGINAL ARTICLE

Neuromuscular performance in the hip joint of elderly fallers and non-fallers

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Abstract

Backgrounds Low strength and neuromuscular activation of the lower limbs have been associated with falls making it an important predictor of functional status in the elderly.

Aim To compare the rate of neuromuscular activation, rate of torque development, peak torque and reaction time between young and elderly fallers and non-fallers for hip flexion and extension.

Methods We evaluated 44 elderly people who were divided into two groups: elderly fallers ($n = 20$) and elderly non-fallers ($n = 24$); and 18 young people. The subjects performed three isometric hip flexion and extension contractions. Electromyography data were collected for the rectus femoris, gluteus maximus and biceps femoris muscles.

Results The elderly had 49 % lower peak torque and 68 % lower rate of torque development for hip extension, 28 % lower rate of neuromuscular activation for gluteus maximus and 38 % lower rate of neuromuscular activation for biceps femoris than the young ($p < 0.05$). Furthermore, the elderly had 42 % lower peak torque and 62 % lower rate of torque development for hip flexion and 48 % lower rate of neuromuscular for rectus femoris than the young ($p < 0.05$). The elderly fallers showed consistent trend toward a lower rate of torque development than elderly non-fallers for hip extension at 50 ms (29 %, $p = 0.298$, $d = 0.76$) and 100 ms (26 %, $p = 0.452$, $d = 0.68$).The motor time was 30 % slower for gluteus maximus, 42 % slower for rectus femoris and 50 % slower for biceps femoris in the elderly than in the young.

Discussion Impaired capacity of the elderly, especially fallers, may be explained by neural and morphological aspects of the muscles.

Conclusion The process of senescence affects the muscle function of the hip flexion and extension, and falls may be related to lower rate of torque development and slower motor time of biceps femoris.

Keywords Accidental falls · Muscle strength · Dynamometer · Electromyography · Aging

Introduction

Falls are the main cause of morbidity, mortality and elevated costs of public health services in older adults and they are the result of complex interactions between various risk factors including alterations in visual, vestibular and proprioceptive systems, lower strength and reduced muscle activation [1]. An important risk factor for falls in the elderly is the decline in muscle function of the lower limbs that has been related to loss of mobility and functional independence in activities of daily living [1, 2].

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The primary strategy to restore balance and avoid falls is the activation of the ankle muscles. When this initial strategy fails, the secondary strategy requires hip joint [3, 4]. The hip strategy is generally adopted by the elderly due to insufficient production of ankle torque, making the strength and rate of torque development (RTD) of the hip muscles especially important in this population [5].

The hip flexors and hip extensor muscles are important for functional activities, including walking, standing and climbing [1, 4]. For example, the hip flexors are responsible for generating 40 % of the power produced in the preswing phase of walking [6] and have been associated with increased odds of requiring multiple steps during imbalance [7]. Activities such as walking and rising from a chair produce high external torques in the flexion direction, so it is important that the hip extensor muscles have the capacity to elicit adequate extensor torques to produce movement [8]. The elderly need to produce 89 % of maximum isometric hip extensor torque to ascend stairs; for walking this percentage is lower, but still represents a high percentage of maximal strength in older adults [9, 10].

Despite the important function of the hip muscles in maintaining mobility and preventing falls in older adults, there are relatively few studies investigating the strength and RTD of the hip joint in this demographic [7, 11, 12]. These studies primarily focused on mobility, the effect of age on reduced muscle strength, use of a multiple or single step during imbalance, or on hip adduction and abduction performance [7, 11, 12]. Our investigation differs because it focuses on the association of hip flexor and extensor performance to fall history by examining the time course of muscle activation and the rate of torque development, in addition to strength.

An important neural factor associated with strength and RTD is the rate of neuromuscular activation (RNA). Recently, it has been found that a slow RNA may indicate a deficit in nervous activation of muscle and inefficiency in the transmission of impulses to the periphery [13]. The loss of motor responsiveness has been shown to increase reaction time, increase the latency of muscle activation (premotor time), increase antagonist coactivation as well as reduce recruitment of motor neurons, possibly more so in elderly fallers [3, 14].

Thus, the present study aimed to compare the parameters of RNA, RTD and reaction time of the hip flexor and extensor muscles, between young, elderly non-fallers and elderly fallers. The hypothesis of the present study was that elderly fallers would have a lower RNA, lower strength and RTD, and slower reaction times than elderly non-fallers, and elderly non-fallers would exhibit performance closer to young adults.

Materials and methods

Subjects

Data from 44 elderly women, aged 60–85 years, and 18 young female university students aged 18–25 years were considered for this study. The older participants were divided into two groups: elderly fallers ($n = 20$) and elderly non-fallers ($n = 24$) based on whether the participant had fallen or not in the year before the evaluation. Similar to previous research [1, 15, 16], a fall was considered an unintentional event in which the subject ended on the ground and was investigated through a survey developed specifically for this study. Table 1 shows the descriptive characteristics of the sample. Individuals who had pain, fracture, a serious soft tissue injury in the 6 months previous to the study, a history of neurological, cardiovascular or respiratory disease, or vestibular or peripheral neuropathy were excluded [15, 16].

This study was approved by the ethics committee for the use of human subjects and all participants signed an informed consent form.

Procedures

The protocol for data collection consisted of a dynamometric (System 4 PRO, Biodex®, New York, USA) synchronized with an electromyographic telemetry system (TM900, Noraxon®, Phoenix, USA) for assessment of the hip flexor and extensor muscles in the dominant limb. All data were recorded at a sampling frequency of 2000 Hz.

Strength assessment

The volunteers performed a 5-min warmup walking on a motorized treadmill (Millennium Super ATL, INBRAMED®, Gravataí, BRA) at their preferred walking speed and were familiarized with the strength assessment that included practicing three submaximal isometric contractions [15, 16].

During strength testing of the hip, the volunteers were placed in a supine position and the rotational axis of the dynamometer was aligned with the axis of the hip. The lever of the dynamometer was positioned 5 cm above the upper border of the patella of the dominant limb. The waist and the contralateral limb were fixed in position by straps [16]. The hip joint was positioned at 60° of flexion for both movements. The assessment protocol included three maximal isometric contractions of hip flexion and extension that were held for 5 s with a rest interval of 30 s between them.

Table 1 Subject characteristics

Variable	Young ($n = 18$)	ENF ($n = 24$)	EF ($n = 20$)
Age (years)	21.8 ± 2.1	65.5 ± 6.16	68.9 ± 6.5
Mass (kg)	61.4 ± 7.5	65.2 ± 13.1	65.6 ± 10.2
Height (m)	1.62 ± 0.05	1.55 ± 0.06	1.52 ± 0.05
Body mass index ($kg \cdot m^{-2}$)	23.3 ± 3.30	27.1 ± 4.8	28.3 ± 3.9
Number of falls	–	–	1.9 ± 1.1

EF elderly faller, *ENF* elderly non-faller

Neuromuscular activation assessment

The electromyogram signals were collected using Ag/AgCl disc electrodes (Miotec®, Porto Alegre, Brazil) with an active area of 1 cm^2 and inter-electrode distance of 2 cm arranged in bipolar configuration. The electrodes were placed over the rectus femoris (RF) muscle, over the biceps femoris (BF) and over the gluteus maximus (GM) according to the guidelines of the Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles project (SENIAM) [17].

The volunteers were cued to begin contraction with a light stimulus that also provided a signal to the data collection software to mark the onset of the stimulus. During data analysis, the onset of torque was defined as the value equal to or greater than 5 % of peak torque and the onset of EMG was defined as the value equal to or greater than 5 % of peak EMG [15].

Data analysis

Torque signal

Data analysis was performed using specific routines developed for the MATLAB environment. The torque signals were processed with a fourth-order Butterworth filter set to a cutoff frequency of 3 Hz [15]. Peak torque was defined as the maximal joint torque obtained during the three contractions performed. RTD was obtained using the following equation [3, 15]:

$$\mathrm{RTD}_{(1,100)} = \frac{(\mathrm{Torque}_{n=100} - \mathrm{Torque}_{n=1})}{(100\,\mathrm{samples}/2000\,\mathrm{Hz})}.$$

The RTD was analyzed at 50, 100, 150 and 200 ms from the onset of torque similar to the methods of Aagaard et al. [18]. The torques data were normalized to body mass as done previously [3].

EMG signal

The EMG signal was filtered with a Butterworth bandpass filter of 20–500 Hz. Then, the EMG signal was rectified and smoothed using a low-pass, fourth-order filter with a cutoff frequency of 3 Hz. The cutoff frequencies of the filters used to analyze the torque and EMG signals were determined using the residual analysis method [15, 19]. Using the filtered EMG signal, we analyzed the RNA during isometric contractions using the following equation:

$$\mathrm{RNA}_{(1,100)} = \frac{(\mathrm{EMG}_{n=100} - \mathrm{EMG}_{n=1})}{(100\,\mathrm{samples}/2000\,\mathrm{Hz})}.$$

The RNA was analyzed at 50 and 100 ms from the onset of EMG and normalized to peak EMG [20].

Temporal variables

Reaction time (RT) was defined as the time between the onset of the light to the onset of torque. The reaction time was fractionated into two components: premotor time (PMT) and motor time (MT). PMT was measured by calculating the time from the onset of the light to the onset of EMG. The MT was measured as the time between the EMG onset and the onset of torque as done previously (Fig. 1) [3, 15].

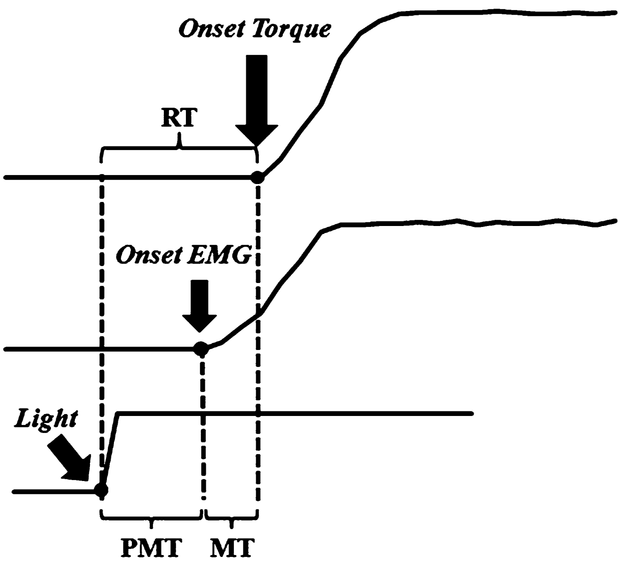

Fig. 1 Calculations of RT (reaction time), PMT (premotor time) and MT (motor time) were made using a light timing mark, the onset of EMG and the onset of torque

Statistical analysis

PASW 18.0 (SPSS Inc., Chicago, USA) was used for all statistical analyses. Multivariate analysis of variance (MANOVA) was used to compare the dependent variables between groups followed by Gabrieĺs post hoc test when appropriate. Standardized effect sizes (*d*) comparing fallers and non-fallers were calculated for all variables according to the equation:

$$d = \frac{\left(\mathrm{Mean}^{\mathrm{NF}} - \mathrm{Mean}^{\mathrm{F}}\right)}{S},$$

where $\mathrm{Mean}^{\mathrm{NF}}$ is the mean value of non-fallers, $\mathrm{Mean}^{\mathrm{F}}$ is the mean value of fallers and S is the pooled standard deviation. d values between 0.20 and 0.49 were considered as a small effect, d values between 0.50 and 0.79 as medium effect and d values $\geq$0.80 as a large effect [21].

Results

The multivariate analysis revealed that differences in neuromuscular performance existed between groups ($F = 5.62$, $p < 0.001$).

Rate of torque development

When comparing young subjects with the two elderly groups, post hoc tests revealed significant differences for RTD at 50 ms (RTD_50), 100 ms (RTD_100), 150 ms (RTD_150), 200 ms (RTD_200) and peak torque (PT) during both hip extension and flexion ($p < 0.001$ in all comparisons, Table 2). The elderly had 68 % lower rate of torque development and 49 % lower peak torque for hip extension than the young; and for hip flexion the elderly had 62 % lower rate of torque development and 42 % lower peak torque than the young. Moreover, the peak torque in the elderly was 42 % lower than young subjects for hip flexion and 49 % lower for hip extension. There were no statistical differences between elderly fallers and elderly non-fallers for any of the parameters related to the production of hip torque (Table 2) despite a consistently lower RTD in elderly fallers.

Medium effect sizes for differences between elderly fallers and non-fallers were observed for RTD_50 and RTD_100 (Fig. 2) during hip extension, but no statistical significance were observed ($p = 0.298$ and $p = 0.452$, respectively). Elderly fallers showed a consistently lower RTD that was −23 % below the elderly non-fallers across the four measurements.

Rate of neuromuscular activation

During isometric extension of the hip, the young had 32 % higher RNA of GM at 100 ms (RNA_100_GM), 41 % for BF at 50 ms (RNA_50_BF) and 38 % for BF at 100 ms (RNA_100_BF) ($p < 0.05$) than elderly fallers; when compared with elderly non-fallers, 36 % for the RNA of GM at 50 ms (RNA_50_GM), 35 % for RNA_50_BF and 39 % for RNA_100_BF ($p < 0.05$) (Table 3). For isometric flexion of the hip, the young differed significantly from both the other groups (elderly fallers and non-fallers) including 48 % higher RNA of RF at 50 ms (RNA_50_RF) and 47 % for RNA of RF at 100 ms (RNA_100_RF) ($p < 0.05$) (Table 3). There were no statistical differences between elderly fallers and elderly non-fallers for the rate of neuromuscular activation for any analyzed muscle.

Reaction time

Univariate analysis revealed no significant differences between groups for RT (Table 2). However, the young were 31 % faster than the elderly fallers and 28 % than non-fallers for the MT of GM muscle during hip extension (Table 3). When comparing elderly fallers and the young, the young were 59 % faster than elderly fallers for the MT of BF during hip extension and 43 % for the MT of RF during hip flexion. No significant differences existed between elderly fallers and non-fallers for MT, but the standardized effect size for MT of BF was medium, showing that fallers had 37 % slower MT for BF than non-fallers.

Discussion

This study found important differences in RTD, RNA and MT during hip flexion and extension between the young and elderly which indicate that the process of senescence affects the rapid activation of the hip musculature and the production of hip torque. Prior study showed that the elderly had 22 and 31 % lower maximum mean isometric strength than the young in hip flexion and extension, respectively, and produced 16 % slower hip flexion and extension [22]. These data are similar to those observed in this study, showing large differences in hip extension and impairments in ability to produce strength and to produce strength quickly in the elderly. Furthermore, some differences, such as the RNA of GM and MT of BF and RF muscles, were observed only between young and elderly fallers.

Table 2 Rate of torque development, peak torque and reaction time

Variable/joint movement	Groups			*p* value	*d*
	Young	ENF	EF		
Hip extension					
RTD_50 (N m s^{-1} kg^{-1})	6.53 ± 2.13*	2.36 ± 0.96	1.68 ± 0.82	<0.001	0.76
RTD_100 (N m s^{-1} kg^{-1})	7.16 ± 2.38*	2.58 ± 1.01	1.91 ± 0.95	<0.001	0.68
RTD_150 (N m s^{-1} kg^{-1})	6.33 ± 2.07*	2.38 ± 0.87	1.89 ± 0.92	<0.001	0.55
RTD_200 (N m s^{-1} kg^{-1})	4.75 ± 1.53*	1.95 ± 0.63	1.70 ± 0.81	<0.001	0.34
PT (N m kg^{-1})	2.61 ± 0.58*	1.41 ± 0.35	1.23 ± 0.46	<0.001	0.44
RT (s)	0.39 ± 0.12	0.46 ± 0.22	0.43 ± 0.17	0.396	0.15
Hip flexion					
RTD_50 (N m s^{-1} kg^{-1})	4.70 ± 1.53*	1.80 ± 0.56	1.71 ± 0.90	<0.001	0.12
RTD_100 (N m s^{-1} kg^{-1})	4.93 ± 1.36*	1.97 ± 0.61	1.75 ± 0.88	<0.001	0.29
RTD_150 (N m s^{-1} kg^{-1})	3.83 ± 0.85*	1.77 ± 0.52	1.43 ± 0. 72	<0.001	0.54
RTD_200 (N m s^{-1} kg^{-1})	2.25 ± 0.87*	1.36 ± 0.41	1.06 ± 0.44	<0.001	0.70
PT (N m kg^{-1})	1.24 ± 0.15*	0.76 ± 0.11	0.69 ± 0.17	<0.001	0.49
RT (s)	0.36 ± 0.09	0.47 ± 0.32	0.53 ± 0.34	0.203	0.18

EF elderly faller, *ENF* elderly non-faller

* $p < 0.05$ in relation to ENF and EF

** $p < 0.05$ in relation to EF

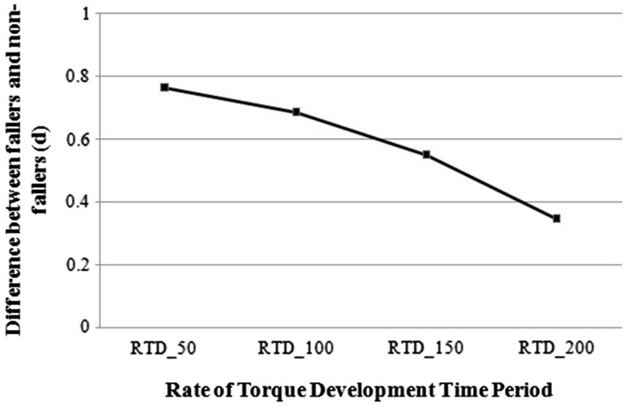

Fig. 2 Standardized effect sizes (*d*) for the differences in hip extension rate of torque development between elderly fallers and non-fallers are compared at 50 ms (RTD_50), 100 ms (RTD_100), 150 ms (RTD_150) and 200 ms (RTD_200) after torque onset

Our study did not demonstrate differences in neuromuscular performance between elderly fallers and non-fallers great enough to achieve statistical significance. This, in part, may be due to the complex etiology of falling that includes muscle weakness, gait performance, balance, visual acuity, cognitive function and environmental factors [1]. In addition to traditional hypothesis testing, it is also important to consider the standardized effect size which has been used to detect meaningful differences in performance more useful to practice [21]. Moderate standardized effect sizes indicated that there was a general trend for fallers to have lower hip extension RTD than non-fallers and slower MT of BF during hip extension, but results were not statistically significant. The hypothesis that elderly fallers would exhibit lower RTD than elderly non-fallers was therefore only partially supported.

Torque and rate of torque development

The decrease in muscular performance in the elderly may result from neuromuscular dysfunction inherent to the process of aging that involves both neural (the magnitude of efferent motor neuron activity in the initial phase of contraction, the firing rate and recruitment of motor neurons) and morphological aspects (muscle size, relative area of fast fibers, the composition of myosin heavy chain isoforms and distribution of muscle fibers) [18, 23]. These physiological parameters can differentially affect the RTD depending on the phase of contraction. During very early in the time course of muscle contraction (near 50 ms), the RTD may be influenced by intrinsic properties of the muscle and maximal muscle strength, and RTD later in the time course (after 100 ms) may be more closely related only to maximal muscle strength [24]. The differences in RTD between the young and elderly were greatest at the initiation of contraction, suggesting impaired neuromuscular activation with aging.

The RTD during hip extension showed medium standardized effect sizes (*d*) between elderly fallers and non-fallers, but no statistical significance was observed. In addition, Fig. 2 shows that this effect is time dependent,

Table 3 Rate of neuromuscular activation, motor time and premotor time

Variable/joint movement	Groups			p value	d
	Young	ENF	EF		
Extension hip					
RNA_50_GM (% pEMG s^{-1})	184 ± 90†	118 ± 80	150 ± 70	0.009	0.42
RNA_50_BF (% pEMG s^{-1})	234 ± 146*	151 ± 88	138 ± 84	0.017	0.15
RNA_100_GM (% pEMG s^{-1})	190 ± 101**	140 ± 84	129 ± 47	0.049	0.16
RNA_100_BF (% pEMG s^{-1})	240 ± 128*	147 ± 91	149 ± 84	0.030	0.02
PMT_GM (s)	0.24 ± 0.11	0.24 ± 0.09	0.25 ± 0.05	0.940	0.14
PMT_BF (s)	0.29 ± 0.11	0.30 ± 0.14	0.27 ± 0.11	0.999	0.24
MT_GM (s)	0.13 ± 0.08*	0.19 ± 0.11	0.18 ± 0.04	<0.001	0.12
MT_BF (s)	0.11 ± 0.02**	0.17 ± 0.19	0.27 ± 0.12	0.018	0.63
Flexion hip					
RNA_50_RF (% pEMG s^{-1})	261 ± 134*	125 ± 57	146 ± 88	0.012	0.28
RNA_100_RF (% pEMG s^{-1})	275 ± 145*	138 ± 73	152 ± 99	0.012	0.16
PMT_RF (s)	0.23 ± 0.08	0.25 ± 0.18	0.38 ± 0.32	0.102	0.50
MT_RF (s)	0.13 ± 0.04**	0.22 ± 0.19	0.23 ± 0.08	0.030	0.07

ENF elderly non-faller, *EF* elderly faller

* $p < 0.05$ in relation to ENF and EF

** $p < 0.05$ in relation to EF

† $p < 0.05$ in relation to ENF

i.e., the effect size was reduced as time of contraction increased. This suggests that there may be differences between elderly fallers and non-fallers for RTD and may likely occur at the onset of contraction. The reduced RTD very early in the contraction can be explained by reduction in nervous drive to the muscles or possibly better excitation contraction coupling in elderly non-fallers than fallers. The MT data for BF support this latter hypothesis given that fallers had slower MT for BF than non-fallers according to effect for this variable ($d = 0.63$).

Rate of neuromuscular activation

We also observed significantly greater RNA in the young in comparison to the elderly for the RF, GM and BF muscles that can contribute to the reduced responsiveness of the older subjects. Reduced activation of muscle, possibly due to diminished cortical function and decreased conduction of nerve impulses, can lead to a reduction in muscle strength and RTD and exacerbates the loss of function due to morphological changes at the muscle level [13, 20].

No significant differences in RNA were observed between the elderly groups that could explain the prevalence of falls. It is possible that this parameter of performance is not strongly related to fall history, or the lack of difference between elderly groups could be due to the way RNA was normalized. The difference in the absolute peak EMG amplitude (i.e., volts) between the fallers and non-fallers may have influenced the relative RNA (i.e., % peakEMG s^{-1}).

The RNA and RTD are diminished in older adults, which may result in increased susceptibility to falls with aging [15]. Rapid activation of the hip flexors is important in moving the stepping limb forward during imbalance, and rapid activation of the hip extensors is important to provide body support on the contralateral limb and accelerate the center of mass anteriorly. Carty et al. [13] demonstrated that both hip flexor and extensor weaknesses were associated with a twofold increased odds of requiring multiple steps to recover balance in a forward lean task.

Reaction time, pre-motor time and motor time

The present study did not observe any statistically significant differences between groups for reaction time during either hip flexion or extension. These data agree with previous studies where elderly fallers and non-fallers, and elderly with high and low levels of physical activity did not differ in RT of knee and ankle muscles [15, 20, 25]. Reaction time was fractioned into PMT and MT. PMT was not significantly different between groups, suggesting that aging does not affect the response time between recognition of the visual stimulus and activation of the muscle. PMT of knee and ankle muscles has previously been shown not to differ between the elderly and young or between elderly fallers and non-fallers [3, 15].

In contrast, the MT of GM was 30 % slower in the elderly groups in comparison to the young, but the MT of RF and BF muscles was 43 and 59 % slower (respectively) only in the elderly fallers compared to the young. The slower MT in the elderly may be due to lower recruitment of type II muscle fibers and reduced diameter of muscle fibers that affects nerve impulse conduction along the muscle fiber. Slowed excitation–contraction coupling in the elderly may also have resulted from a lower concentration of Ca^{2+} ions and lower activity of creatine kinase and actomyosin ATPase [2, 26, 27].

A medium standardized effect ($d = 0.63$) suggested that MT of BF was 37 % faster in the elderly non-fallers than fallers. This may have occurred due to alterations in excitation–contraction coupling previously mentioned as well as differences in musculotendinous stiffness [28]. Decreases in musculotendinous stiffness may hamper the transfer of force from the muscular system to the skeletal system and thereby increase the MT [3, 20, 29, 30]. Smaller cross-sectional area of muscles in the elderly, especially fallers, is likely to increase the compliance of the muscle. This increases the time between the development of muscle force and the transfer of that force to the skeletal system, resulting in a slower MT and RTD [28].

MT has been previously shown to be sensitive to changes in muscle function during aging that appears to be compromised in elderly fallers [25, 27]. We speculate that that elderly who have slow MT may have difficulty producing force quickly during imbalance which would increase their likelihood of falling. Future research should therefore investigate the underlying morphological and physiological mechanisms responsible for the slower MT seen in the elderly as well as determine if MT is modifiable by training.

In evaluating the statistical power of this study post hoc, the effect size for RTD at 50 ms during hip extension ($d = 0.76$) was used to determine the sample size that would have been needed to achieve statistical differences between the elderly groups. Results indicated that only three more subjects would have been needed in the elderly faller group to detect differences in RTD at 50 ms. This suggests that our study was simply underpowered to detect the moderate differences in hip performance that exists between elderly fallers and non-fallers. Future studies should therefore confirm these initial findings in a larger sample that includes both men and women.

Other studies in older adults have extensively investigated knee and ankle function [2, 13, 15], but have neglected the hip joint or are inconclusive regarding the importance of hip function and the risk of falls in elderly. However, the present study shows that the ability to develop hip extensor strength rapidly may be impaired in elderly fallers. In the elderly, RTD may be more closely related to fall risk than torque, because a loss of balance requires the prompt generation of extensor torques at the ankle, knee and hip to prevent a fall.

The results of the current study show that peak torque, rate of torque development and rate of neuromuscular activation of the hip extensors and flexors are compromised with aging. Standardized effect sizes suggest that there might be clinically important differences in hip strength and rate of torque development between fallers and non-fallers, but results were not statistically significant. A slowing of motor time in this sample of older adults suggests that impaired excitation–contraction coupling occurs with aging, which was greatest in the biceps femoris muscle of elderly fallers.

While it is evident that the hip musculature is important for functional tasks like walking and climbing stairs, the present research shows that hip strength is related to aging and suggests that there may be differences in hip strength between elderly fallers and non-fallers. In light of these findings, larger studies are needed to assess these differences and to recommend resistance training for hip muscles to reduce the risk of fall and the maintenance of mobility in the elderly.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding 467author states that there is no conflict of interest.

Ethical approval This study was approved by the ethics committee for the use of human subjects in compliance with ethical standards and (CEP 037/2012).

Informed consent All participants signed an informed consent form.

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