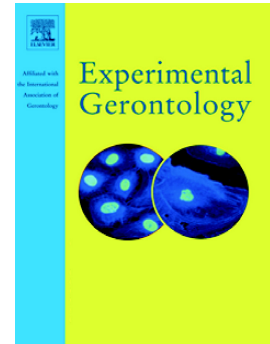


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Sex-independent and dependent effects of older age on cycle-to-cycle variability of muscle activation during gait

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Abstract

Background: Older age is associated with higher stride time variability in female and male gait, which may have a neuromuscular origin. We sought to determine how older age and sex affect muscle activation variability during gait, and how these patterns relate to stride time variability.

Methods: Ninety-three adults (51 females; aged 20-82 years) completed six gait trials at their self-selected speed. Cycle-to-cycle variabilities (CCVs) were calculated for stride time, and for amplitude of electromyography (EMG) of the rectus femoris (RF), tibialis anterior (TA), and gastrocnemius lateralis (GL) recorded over different gait phases. Statistical models tested for Age x Sex x Muscle effects and for relationships between EMG CCVs and stride time CCVs.

Results: Significant Age and Age x Muscle effects on EMG CCV were observed in several phases of gait ($p < 0.05$), where each year of age was associated with 0.11-0.18% *higher* EMG CCV, generally in the RF. A significant Age x Sex x Muscle effect on EMG CCV at mid-stance ($p < .05$) indicated that, in females, each year of age was associated with 0.11% *lower* GL CCV. Significant but low strength correlations ($\rho = .298-.351$) were found between EMG CCV and stride time CCV.

Conclusions: Associations between older age and higher muscle activation variability were generally sex-independent. A sex-dependency in GL activation variability may contribute to gait instability in aging females. Individual variabilities of muscle activation were not strongly related to stride time variability.

Keywords: normal aging, sex differences, gait, motor variability, electromyography

1. Introduction

With older age, adults are more likely to suffer a fall. Around 30-60% of adults aged 65+ years fall each year, with 20% of these falls resulting in injury, hospitalization and/or death [1]. Risk of falling is also sex-dependent, where risk is higher in older females than older males [2]. One frequently cited factor linked to the risk of falling in older age is high gait variability; more specifically, the cycle-to-cycle variability of stride time during gait [3]. This is supported by evidence that older age is associated with higher stride time variability [4, 5], most strongly in females [4], and by a cohort study that followed older adults for one year and found that those with higher stride time variability were more likely to fall [6]. Taken together, these results suggest that stride time variability has a role in age-related falls, particularly for females.

As it is speculated that stride time variability is a factor of the collective inputs and feedback from the central and peripheral nervous systems [6], its association with age may be a consequence of age-related changes to neuromuscular structure and physiology. Older age is associated with neural alterations to both central [7] and peripheral [8, 9] structures, including to motor units (MUs). It is believed that with older age, the MUs of adults increase in size with motor neuron death, such that surviving motor neurons innervate a larger number of muscle fibres [8, 9]. In addition, the discharge behaviour of these MUs change. In the upper limb, Laidlaw et al [10] found that MU discharge rate was more variable in older adults than young adults. Similar findings have been reported in muscles that produce gait. Hourigan et al [11] and Piasecki et al [12] each reported higher near-fibre jiggle, a measure reflecting variations in individual MU potential shape, in older adults than young adults. Further, Negro et al [13] showed that MU discharge rate variability can explain up to 70% of the variance in force output during isometric contractions. Thus, Christou [14] argues that the more variable activation of

MUs is responsible for the lower steadiness (higher variability) of motor output found with older age.

The variability of motor output in the gait of older adults may then be better understood by investigating how older age affects the activation of individual muscles, as measured by electromyography (EMG). In fact, several studies have carried out such investigations [15–17]. Schmitz et al [15] measured muscle activations in young and older adults walking at their preferred-speed, grouping males and females together. They found that older adults had higher normalized muscle activation than young adults during loading for the gastrocnemius lateralis (GL), and at mid-stance for the vastus lateralis, soleus, tibialis anterior (TA), and rectus femoris (RF). Marques et al [16] also observed the same age difference in TA activation during stance. In contrast, they identified lower RF activation in older adults than young adults, which may have been due to investigating females only. Thus, our recent study [17] grouped males and females separately, finding that, though older age was generally associated with higher ankle muscle activation during the gait cycle, there were sex-dependent age associations with higher RF activation (in males) and higher GL activation (in females). Together, these studies highlight that the muscle activations involved in the gait of aging males and females can differ; perhaps the activation of knee extensors is more relevant in aging males and the activation of ankle plantar flexors is more relevant in aging females. However, the extent to which older age and sex influence the *variability* of these muscular activations (i.e. EMG), from gait cycle to gait cycle, is uncertain and may ultimately lead to variability in the motor output of gait.

Therefore, this study investigated the effects of older age and sex on muscle activation variability during gait and explored the relationships between the activation variability of individual muscles and the stride time variability of males and females. We hypothesized that

older age would be associated with higher stride time variability and higher muscle activation variability in males and females, and that relationships between muscle activation variability and stride time variability would be stronger in females than in males.

2. Methods

2.1 Participants

Healthy adults aged 20 years and older (N = 93, 51 females) were recruited by the Laboratory of Biomechanics and Industrial Ergonomics of the University of Cagliari (Italy) among students and individuals who regularly attend the University of the Third Age of Quartu S. Elena (Italy) through convenience sampling. Participants were excluded if they had any neurologic or orthopedic condition that severely impaired gait, balance, and muscular strength; borderline cases were examined by a physician. All participants provided informed consent to participate in the study, which was given ethics clearance from the institutional ethics board.

2.2 Procedure

On arrival, participants were instrumented for EMG gait analysis. Wireless surface EMG sensors (1000 Hz; FreeEMG, BTS Bioengineering, Italy) were placed over the RF, TA, and GL of each lower limb according to SENIAM guidelines [18], as described in Bailey et al [17]. The reader is referred to Bailey et al [17] for a detailed explanation on why these muscles were selected. Briefly, the RF was located as the midpoint on the line between the anterior superior iliac spine and the superior aspect of the patella, the TA was located as one-third distal on the line between the fibular head and the medial malleolus, and the GL was located as one-third distal on the line between the fibular head and the heel. Reflective markers (14 mm diameter) were placed on the participant's trunk and lower limbs according to the Davis protocol [19] and

gait kinematics were analyzed using an optoelectronic eight-camera system (120 Hz; Smart-D, BTS Bioengineering, Italy). After instrumentation, participants practiced walking forwards at their preferred speed over a 10 m platform, then completed six trials of gait with 30 s of rest between trials. Participants self-initiated their gait for each trial. Marker position data and EMG data from forward, straight-walking and constant-speed strides of each leg were subsequently analyzed.

2.3 Data analysis

2.3.1 Calculation of gait speed and stride time variability. Marker position data were used to identify the first and second heel strike events using Smart Analyzer (BTS Bioengineering, Italy). The time between these events defined the stride time for each gait cycle. Gait cycles were analyzed from the middle four metres of the platform to extract straight-walking and constant-speed cycles. Thus, cycles within the acceleration and deceleration phases were not studied. From six trials of gait, this resulted in analyzing five to eight gait cycles from each leg for each participant. From each cycle, gait speed was extracted as the quotient of stride length (heel marker displacement between heel strike events) and stride time and was averaged across gait cycles for each leg. Stride time variability was extracted as the cycle-to-cycle variability (CCV) using the coefficient of variation and reflects the variability between steps. Stride time CCV was calculated independently for each leg. A preliminary analysis confirmed that there were no significant between-leg differences in gait speed or stride time CCV; therefore we calculated mean values across legs and used these for all subsequent analyses.

2.3.2 Calculation of muscle activation variability. EMG cycles were located with respect to the gait cycles identified above. EMG cycles were filtered to remove the DC bias, bandpass filtered using a dual-pass Butterworth filter (2nd order, 20-450 Hz), then linear

enveloped with a dual low-pass Butterworth filter (4th order, 6 Hz cut-off) [15, 17]. The enveloped EMG cycles were time-normalized to 101 points relative to the gait cycle (0-100%), with 0% and 100% representing the first and second heel strike events that define a gait cycle, and amplitude-normalized to the peak value of the gait cycle [20, 21]. These normalized EMG cycles were subdivided into the loading (0-10%), mid-stance (10-30%), terminal stance (30-60%), initial swing (60-73%), mid-swing (73-87%), and terminal swing (87-100%) phases [15, 17]. Root mean square values were extracted for each combination of muscle, phase, and leg within each gait cycle. For each combination, EMG CCV was then calculated as the cycle-to-cycle coefficient of variation of the root mean squares. Like with gait speed and stride time CCV, there were no between-leg differences in EMG CCV and so mean EMG CCV values were calculated between legs and subsequently analyzed for each muscle and phase.

2.4 Statistical analyses

Mixed effect models tested for effects and interactions of Age (as a continuous variable) and Sex (male, female) on participant height, mass, gait speed, and stride time variability. Age-based effects on stride time variability were further explored in a linear regression model by computing the β -coefficient to estimate the year-by-year rate of change. Gait speed was entered as a covariate in this model since there is evidence that gait speed influences age-related differences in gait variability [22].

EMG CCVs were then analyzed for each gait phase using mixed effect models, testing for effects of Age, Sex, and Muscle (RF, TA, GL), as well as Age-based two- and three-way interactions. Model residuals were visually inspected and confirmed to approximate a normal distribution. Age-based effects were also explored in linear regression models to estimate year-

by-year rates of change. These regression models included Bonferroni corrections to mitigate type I error and gait speed as a covariate.

EMG CCVs with a significant Age-based effect were then compared to stride time variability in correlation analyses. Since some muscle and phase combinations of EMG CCV were not normally distributed (Shapiro-Wilks $p < .05$), Spearman ρ coefficients were computed to test for relationships between the variabilities of individual muscle activations and the motor output of gait. The strength of coefficients was categorized as negligible (.00-.29), low (.30-.49), moderate (.50-.69), high (.70-.89) or very high (.90-1.00) [23]. Correlations analyses were performed for three scenarios: sexes pooled, males only, and females only. Statistical significance for all analyses was set at $p < .05$.

3. Results

3.1 Participant characteristics

Sample sizes of males and females in each decade are summarized in Table 1 and characteristics of participants are summarized in Table 2. Males were taller and heavier than females ($ps < .05$). Age was associated with shorter height, slower gait speeds, and higher variability in stride time ($ps < .05$). With gait speed as a covariate, the association between Age and stride time variability did not reach significance ($\beta = .012$, $p = .175$). No Age x Sex effects were observed in participant characteristics.

Table 1. Sample sizes of males and females for each decade.

Decade	Males (N)	Females (N)	Total (N)
20-29 years	13	6	19
30-39 years	1	0	1
40-49 years	3	0	3
50-59 years	2	4	6
60-69 years	13	30	43

70-79 years	10	9	19
80-89 years	0	2	2

Table 2. Effects of Age and Sex on participant characteristics. Values are means (standard deviations). Age was statistically analyzed as a continuous variable and is only stratified here to show the Age-based effects. Variability characteristics are calculated as cycle-to-cycle coefficients of variation.

Characteristic	20-29 years		60-69 years		70-79 years	
	Males	Females	Males	Females	Males	Females
Height (cm) † ‡	173 (6)	161 (6)	173 (5)	156 (6)	170 (6)	158 (5)
Mass (kg) ‡	70.2 (8.1)	52.9 (4.8)	73.4 (10.9)	57.2 (10.8)	75.2 (10.4)	61.1 (12.5)
Gait speed (m/s) †	1.21 (0.14)	1.22 (0.14)	1.15 (0.25)	1.12 (0.16)	1.10 (0.23)	1.10 (0.22)
Stride time variability (%) †	3.4 (2.1)	2.2 (0.6)	4.2 (2.2)	3.2 (1.2)	3.5 (1.4)	3.5 (1.6)

† significant Age effect ($p < .05$)

‡ significant Sex effect ($p < .05$)

3.2 Muscle activation variability

Significant Age, Age x Muscle, and Age x Sex x Muscle effects were found and are summarized below for each gait phase (see also supplementary material 1). No significant Sex or Age x Sex effects were observed in any model (supplementary material 1).

3.2.1 Loading

There was an Age x Muscle interaction on CCV ($p = .036$), where each year was associated with 0.11% higher RF CCV ($\beta = .112$, $p = .041$) (Figure 1A).

3.2.2 Mid-stance

There was an Age x Muscle x Sex interaction on CCV ($p = .033$), where each year was associated with 0.18% lower GL CCV in females ($\beta = -.179$, $p = .017$) but no change in males ($\beta = -.056$, $p = .473$) (Figure 2).

3.2.3 Terminal stance

There was an Age effect on CCV ($p = .002$), but no two-way or three-way interactions. Each year was associated with 0.11% higher CCV ($\beta = .111$, $p = .019$). Post-hoc inspections of muscle-specific regressions revealed that the Age effect was driven mainly by higher RF CCV ($\beta = .184$, $p = .003$) (Figure 1B).

3.2.4 Initial swing

There was an Age x Muscle interaction on CCV ($p = .015$), where each year was associated with 0.17% higher RF CCV ($\beta = .174$, $p = .022$) (Figure 1C).

3.2.5 Mid-swing

There was an Age effect on CCV ($p = .010$), but no two-way or three-way interactions. Each year was associated with 0.16% higher CCV ($\beta = .160$, $p = .001$). Post-hoc inspections of muscle-specific regressions revealed that the Age effect was driven mainly by higher RF CCV ($\beta = .249$, $p = .009$) (Figure 1D).

3.2.6 Terminal swing

There were no significant Age-based effects or interactions on CCV ($p > .05$).

3.2.7 Full gait cycle

There were no significant Age-based effects or interactions on CCV ($p > .05$). There was a trend towards an Age x Muscle effect ($p = .068$) where higher age tended to be associated with higher RF CCV ($\beta = .050$, $p = .011$).

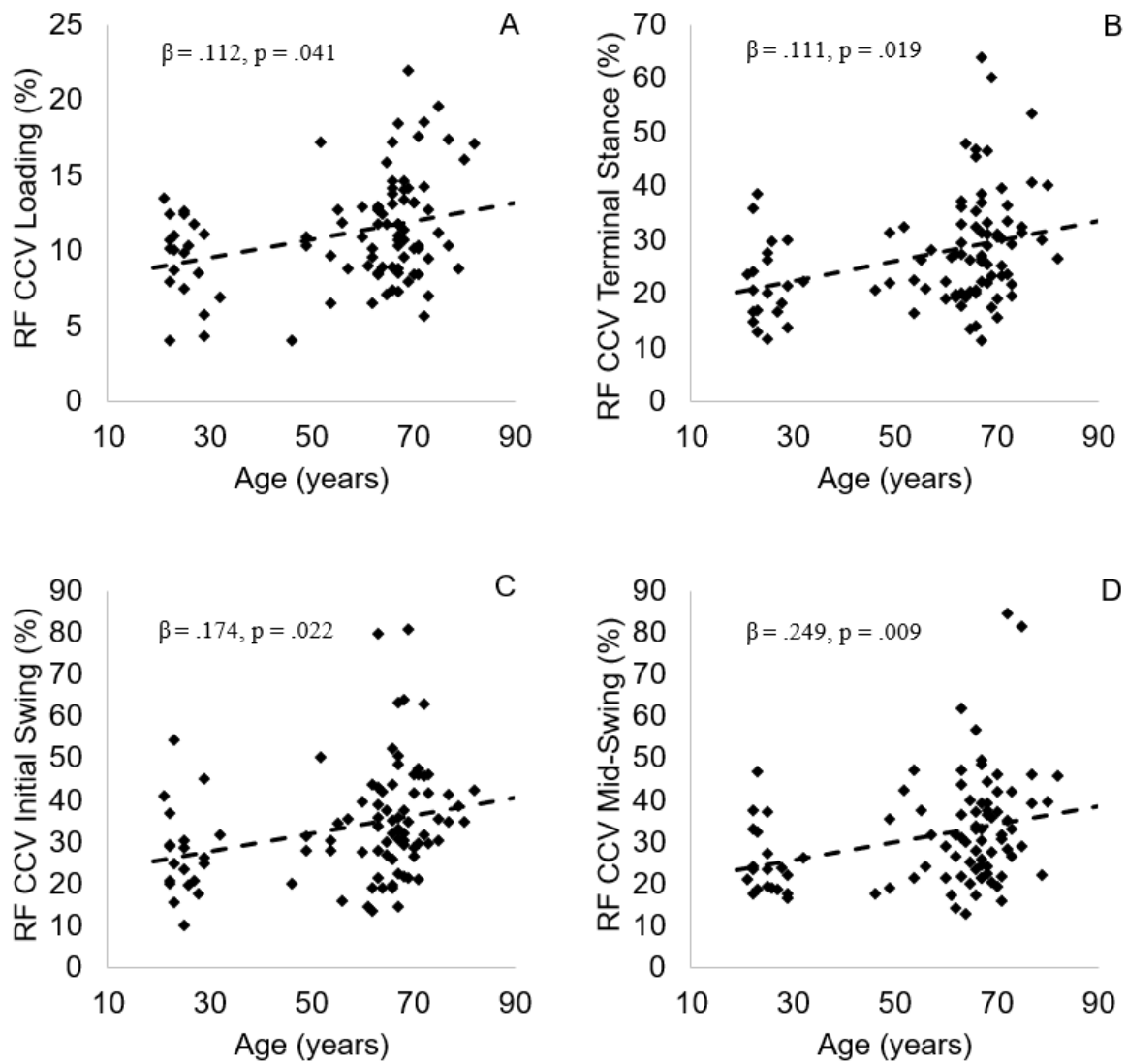


Figure 1. Rectus femoris activation variabilities (RF CCVs) during loading (A), terminal stance (B), initial swing (C), and mid-swing (D). Values are cycle-to-cycle coefficients of variation and males and females are pooled. The dashed lines illustrate the significant associations between age and RF CCV.

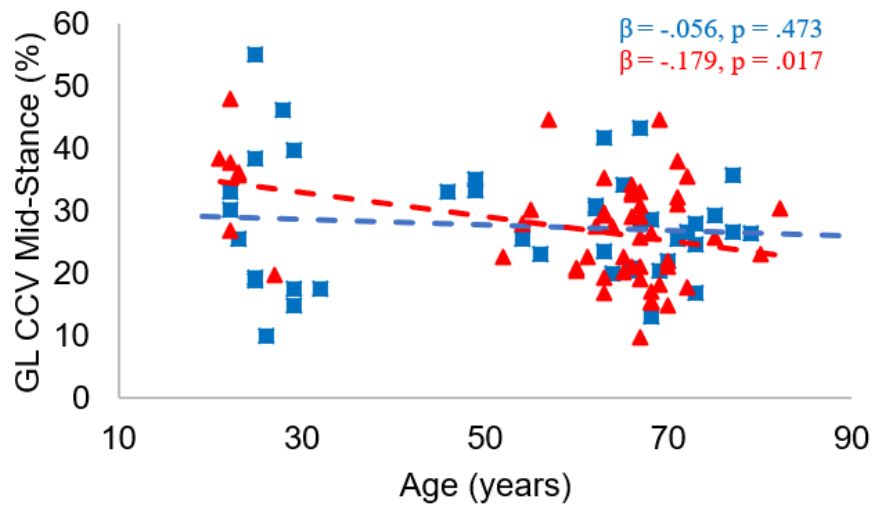


Figure 2. Gastrocnemius lateralis activation variability (GL CCV) during mid-stance. Values are cycle-to-cycle coefficients of variation for males (blue squares) and females (red triangles). The red dashed line illustrates the significant association between age and GL CCV in females.

3.3 Relationships between muscle activation variabilities and stride time variability

Correlations analyses are summarized in Table 2. With sexes pooled, higher stride time variability was significantly correlated to higher RF CCV at terminal stance ($\rho = .342, p = .001$) and at initial swing ($\rho = .298, p = .004$). In males, a significant but low correlation was found between higher stride time variability and higher RF CCV at initial swing ($\rho = .328, p = .036$), and the correlations with RF CCV at terminal stance, mid-swing, and over the gait cycle approached significance ($\rho = .302-.305$). In females, significant but low correlations were found between higher stride time variability and higher RF CCV at loading ($\rho = .326, p = .018$), at terminal stance ($\rho = .351, p = .011$), and at initial swing ($\rho = .299, p = .031$).

Table 3. Spearman correlation coefficients (ρ) between stride time variability and muscle activation variability measures. Measures analyzed are those in which there was a significant Age-based effect or an Age-based trend.

Muscle activation variability measure	Sexes pooled (N = 93)	Males (N = 41)	Females (N = 52)
RF CCV at loading	.146	-.070	.326 *
GL CCV at mid-stance	.071	.035	.161
RF CCV at terminal stance	.342 **	.302 ^T	.351 *
RF CCV at initial swing	.298 **	.321 *	.299 *
RF CCV at mid-swing	.146	.302 ^T	.067
RF CCV over the gait cycle	.154	.305 ^T	.086

* significant correlation ($p < .05$)

** significant correlation ($p < .01$)

^T correlation trend ($.05 \leq p \leq .10$)

4. Discussion

This study represents the first known attempt to examine how older age and sex affect the cycle-to-cycle variability of muscle activation during gait, and how this variability relates to the variability of gait motor output. Our main findings are: 1) older age is associated with higher RF muscle activation variability during several phases of gait; 2) the influence of older age on muscle activation variability is muscle- and sex-dependent at mid-stance; 3) the individual activation variabilities of the RF, TA, and GL do not have a substantial role in producing stride time variability.

4.1 Sex-independent effects of older age

In partial agreement with our hypothesis, older age was associated with higher stride time variability and higher muscle activation variability in some, but not all, phases of gait. This mimics the higher stride time variability seen in older age in this study and in previous studies containing large cross-sectional samples [4, 5]. Like in Callisaya et al [4], influences of age on gait speed seem to contribute highly to stride time variability, meaning that a better

understanding of the control of gait speed may help to better understand the production of stride time variability.

The associations between higher age and higher muscle activation variability add to the body of investigations on the neuromuscular features that control gait. Features studied in healthy young adults include both the variability of EMG amplitude shape [24, 25] and the variability of activation modality [26, 27]. These studies highlight the inherent variability that exists in the EMG signal from cycle-to-cycle, but not the source of variability. Although the precise mechanisms are unclear, higher variability with higher age may be partly due to changes in neurophysiology, such as higher neuromuscular noise [28, 29]. Although there are no known studies on how age affects muscle activation variability during gait for comparison to our study, recent studies have analyzed entropy, a non-linear measure of complexity where higher values may indicate higher variability. Kurz & Stergiou [28] found higher entropy in the knee and hip kinematics of older adults than young adults during gait, while Kang & Dingwell [29] found higher entropy in the GL EMG. However, the authors also observed lower entropy of vastus lateralis and biceps femoris EMG [29], contrasting our finding of higher RF muscle activation variability with age in gait. They interpreted their findings to indicate that the fewer MUs of older adults contributed to less complex proximal muscle patterns, and the higher complexity of GL compensated for a loss in proximal muscle pattern complexity [29]. The mechanism driving our finding remains uncertain, but is likely linked to higher variability in MU firing patterns within a single muscle [10–12] and to altered interactions between the MU firing patterns of multiple muscles. These contrasting results and uncertain interpretations point to a need to better understand how entropy and CCV measures of EMG can be combined to explore EMG variability during gait.

As the associations between older age and muscle activation variability were driven mainly by the RF, it seems that the influence of older age on the variability of EMG amplitude is more pronounced in proximal muscles of the lower limb than in distal muscles. This complements previous evidence for muscle- and age-dependent gait muscle patterns, focusing on EMG amplitude [15–17, 30] and EMG entropy [29]. One potential explanation for the muscle-dependence is that the age-related alterations to MU properties are also muscle-dependent [8]. However, the difference in the variability of MU potential shape between young and older adults appears to be similar between distal gait muscles (tibialis anterior) and more proximal gait muscles (vastus medialis and lateralis) [11, 12]. An alternative explanation is that the RF-specific effect on muscle activation variability represents a motor adaptation with aging to maintain gait stability. The RF generally activates during the transition from terminal swing to loading and during terminal stance [26], pointing to its dual purpose in not only propagating forward motion, but also in stabilizing the upper body. Higher variability in RF activation may then be from a change to the central pattern generator of locomotion to avoid overly rigid muscle patterns and reach the optimal state of variability described by Stergiou & Decker [31]. Thus, peripheral (changes in MU physiology) and central (changes in the central pattern generator of locomotion) mechanisms with aging may drive the higher proximal muscle activation variability seen in gait.

4.2 Sex-dependent effect of older age

The association between older age and lower GL CCV in females at mid-stance contrasts with the general effect of older age on EMG CCV, suggesting that there are instances where the variability of muscle activation differs between aging males and aging females. This mirrors our earlier finding where higher GL activation during mid-swing was associated with higher age in females, contrasting the age-independent association with lower TA activation [17]. Together,

these results suggest that aging alters neuromuscular control of the GL during gait in a phase-specific and sex-dependent way.

A loss in GL activation variability at mid-stance likely indicates a lack of flexibility in the motor patterns able to be selected by older females. This seems to align with previous evidence [32–34]. Barrett et al [32] reported lower variability of ankle dorsiflexion/plantar flexion in females in their sample of young adults and Rathleff et al [33] reported lower variability of the navicular height during stance in females in their sample of middle aged adults. Our results seem to suggest that this sex difference in motor variability at the ankle is maintained in older age. Interestingly, the lack of flexibility of ankle motor patterns in aging females may help explain sex- and age-based gait instability. In a recent study, van Kooten et al [34] measured the gait instability of 114 healthy older females and males aged 55-84 years old. After controlling for gait speed, they found higher instability with older age during loading and higher instability in females than in males during mid-stance and terminal stance, indicating higher instability with older age and in females. Further, they showed that age- and sex-based instability only occurred during the stance phase, aligning with our age and sex effects on GL activation variability. Although the interaction effect of older age and sex on gait instability remains to be determined, these studies suggest that instability during single-leg support in aging females may originate from a lack of flexibility in the available neuromuscular and kinematic patterns.

4.3 Associations between the variability of muscle activation and variability of gait motor output

In contrast with our hypothesis, associations between muscle activation variability and stride time variability were not stronger in females than males. In fact, the correlations we found were mostly in the RF and poor in strength, suggesting that the variability in RF EMG, and not

TA or GL EMG, minimally affects the variability in gait motor output. This was somewhat surprising since older adults in our sample had both higher RF activation variability and higher stride time variability. We speculate that the variabilities of individual muscle activations may sum to produce variability in gait motion; that is to say, activation variabilities may sum to produce variability in each joint motion, and the variabilities of each joint motion may sum to produce stride time variability. Our RF-specific correlations, taken with the theorized shift to greater hip control during gait in healthy older adults [35], suggests that the hip is a particular joint of interest. In fact, Schloemer et al [36] observed higher activity of hip extensors (gluteus maximus and gluteus medius) and lower activity of hip flexors (iliacus and psoas) in older adults than young adults during gait. The authors assessed the contribution of these muscles to center of mass support, finding that they had a greater role in older adults than in young adults, but not in compensation for decreases in the contribution at other muscles of the knee and ankle joints. These results were interpreted as evidence for an age-related change in neuromuscular control at the hip [36]; however, the cycle-to-cycle variability patterns of these muscles remain to be evaluated. Clearly, further investigation is needed to understand how variability in lower limb muscle activation produces variability in joint motion, particularly at the hip, to help better identify the neuromuscular origin of stride time variability.

4.4 Limitations

Interpretations of our results are limited by the sample, definitions of gait subphases, and muscles analyzed. Our sample consisted of a large number of males and females for an experimental study, allowing us to model age as a continuous variable. However, as described in our previous study [17], these adults were concentrated in the 20-29 and 60+ years ranges and may underrepresent adults aged 30-59 years. Also, we defined phases of gait by subdividing the

gait-normalized EMG signal, in line with prior studies [15, 17]. While an accepted practice for analyzing EMG during gait, this procedure does not factor in the inter-individual differences that may exist in gait timing; these inter-individual differences may be reduced by synchronizing EMG with signals on gait progression (for example, information on foot-floor contact using foot switches or pressure mats). Finally, the RF, TA, and GL were a focus of this investigation since previous studies have reported various influences of age on their activation patterns [15–17] and we were limited to three EMG sensors on each leg; effects on the activation variability of other muscles could differ, such as those that produce motion in other planes (non-sagittal). Thus, detailed investigations of the knee and hip musculature that produce non-sagittal plane motion may help better understand how age influences muscle activation variability and how stride time variability is produced.

5. Conclusions

Older age was associated with higher RF muscle activation variability during gait. Sex-dependencies at mid-stance, however, point towards lower flexibility in muscle activity to the ankle during single-leg support for aging females, which may contribute to gait instability. The variability of RF muscle activation was weakly related to stride time variability; further investigation is needed to determine how the EMG patterns of multiple muscles interact to produce variability in the motor output of individual joints during gait.

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Author Contributions

C.A.B. and J.N.C. were responsible for study conception, while M.P., G.P., F.A., and M.P. were responsible for the study organization and execution. C.A.B. and J.N.C. designed and executed the statistical analyses. C.A.B. wrote the first draft of this manuscript, which was reviewed and critiqued by M.P. and J.N.C. All authors read and approved the final manuscript.

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Highlights

- higher variability in RF EMG amplitude with older age during gait
- in females, lower variability in GL EMG amplitude with older age at mid-stance
- significant but weak relationships between EMG variability and stride time variability