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# Development of a rapid stepping test to challenge rapid weight-shifting function in older adults

Margaret M. Ruwitch<sup>1</sup> · Brandi Row Lazzarini<sup>1</sup>

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## Abstract

**Background** The ability to step rapidly, shift weight from side-to-side, and maintain temporal rhythmicity are important functional elements for walking independently and preventing falls in seniors.

**Aims** The purpose of this study was to develop a clinically feasible test of rapid stepping performance that challenges the ability to step rapidly, shift weight from side-to-side, and maintain temporal rhythmicity.

**Methods** Participants were a volunteer sample of healthy, self-ambulating older adults aged 70–98 years. A Repeated Alternating Stair Touch Test was developed, which involved rapidly shifting weight in the medial–lateral direction by tapping each foot alternately onto a step. Performance on the test was assessed using trunk acceleration signals. Associations between the number of steps completed on the Repeated Alternating Stair Touch Test in 20 s and acceleration magnitude, variability, and stepping rhythmicity were assessed using Pearson correlations and linear regression. Repeatability was assessed during a 2-week follow-up period.

**Results** The acceleration magnitude, variability, and stepping rhythmicity variables related moderately with the number of steps on the Repeated Alternating Stair Touch

Test ( $r = 0.534–0.572$ ,  $p < 0.05$ ) and were independent predictors of the number of steps taken ( $R^2$  adj. = 0.624,  $p < 0.001$ ). Repeatability was mixed, though most acceleration variables and number of steps had moderate to high correlations between sessions (intraclass correlations: 0.486–0.828), but a learning effect was evident; performance improved between sessions.

**Conclusion** The Repeated Alternating Stair Touch Test has potential as a simple test of rapid, rhythmic weight-shifting function, but requires modification to improve repeatability.

**Keywords** Acceleration · Aging · Postural balance · Geriatric assessment · Rhythmicity

## Introduction

The ability to avoid a fall upon a destabilization relies upon the ability to step rapidly and effectively [1], which involves the ability to actively shift weight toward the stance side prior to the step or to manage the destabilized medial–lateral (ML) motion of the body following the step [2]. Older adults who are unable to step quickly [3, 4] and rapidly shift weight from side-to-side [5, 6] have an increased incidence of falls. Those at a high risk of falling also display an inability to maintain temporal consistency during walking [7, 8]. The measurement of rapid sideways weight-shifting function and the ability to maintain rhythmicity of movement are currently limited to laboratory settings, or the use of laboratory equipment in clinical settings; the cost and need for technical expertise can limit the applicability of such measurements in the clinic. Therefore, the development of functional tests related to speed of stepping, rapid ML motion, and rhythmicity could

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enhance fall risk prevention efforts by identifying individuals with these related deficits. In order to design a simple, clinically feasible stepping test that challenges ML weight-shifting motion and temporal rhythmicity during repetitive rapid stepping movements, the current study combines elements from previous research that has assessed motion during gait initiation [9–11], single stepping tasks [12–14], and several types of repetitive step tests [15–18] in older patients with stroke [16] and neurological conditions [11], older adults with increased fall risk [15], healthy older adults [9–12, 16–18], and young adults [9–11, 13, 14].

A rapid foot tap onto a step is a more impactful ML weight-shifting challenge than a rapid step forward because of the need to stand on one foot while lifting the other and placing it on the step [13]; increased step time during this task predicted individuals with an elevated fall risk [5]. Controlling ML center of mass motion is more difficult for older adults during rapid stepping compared to natural speed stepping [10], making the speed of the task an important element for a functional test challenging medial–lateral motion. A single rapid foot tap on a step, though, requires equipment like a force platform and a timing device to measure the speed of the motion. Repeated foot placements on a step challenges ML motion while increasing the time duration to one that could be measured with a stopwatch. Hill et al. [16] utilized a repeated step tap test that successfully discriminated between stroke patients and healthy older adults. Participants repeatedly tapped the same foot onto a step and the floor as many times as possible in a short time period, challenging ML weight-shifting to only one side and back to center. Performance on this task was later identified to be related to lower extremity power [18]. A modification that requires participants to alternate tapping each foot on the step would more aggressively challenge ML weight-shifting capacity. The Berg Balance Scale includes a repeated and alternating version of a rapid step-up task, but involves scoring on a scale of zero to four, with highest performance defined as successfully completing eight steps in 20 s [19, 20]. This makes the test subject to a ceiling effect, which seems to be evident also in the adoption of this task in the Balance Evaluation System Test [21]. The current study modifies this version of the stair tap test that requires repeated, alternating ML weight-shifting during rapid stepping by scoring it on a continuous scale to eliminate the risk of a ceiling effect.

Medial–lateral motion of the center of mass of the body is natural during walking as the center of mass is shifted along with the foot placement of the right and left foot. Trunk accelerometry methods have revealed that older adults with increased fall risk exhibit lower acceleration magnitudes in all three planes of motion during walking because they

utilize a cautious locomotion strategy, while exhibiting high variability of step timing when encountering irregular walking surfaces [8]. Given these findings, trunk acceleration was used in the present study to evaluate the magnitude of weight-shifting motion and stepping rhythmicity during a Repeated Alternating Stair Touch Test.

The ultimate goal of this research is to improve existing fall risk assessment methods by developing a clinically feasible test of rapid stepping performance that relates to lab-based measures of function. The current study aims to understand the relationship between a step tap test that requires rapid, alternating ML weight-shifting and measures of acceleration magnitude, variability, and step timing variability, given the relationship of these variables with fall risk. It was hypothesized that:

1. Higher-performing participants who completed a greater number of steps on the step tests would have higher ML acceleration magnitude because they would be faster and more aggressive with their weight-shifting.
2. Higher-performing participants would have lower ML acceleration variability because they would display consistency in their ML motion.
3. Higher-performing participants would be faster and more rhythmic.
4. Performance on the step test would be correlated with performance on standard functional tests, which would indicate an ability to discriminate between participants of varying general mobility functional levels.
5. Men and women would not differ on their performance on the test.
6. The test would be repeatable when conducted again 1–2 weeks following the initial test.

## Methods

### Participants

The study was approved by the university's Institutional Review Board, and informed consent was provided by all participants. Participants from the surrounding community, including two local retirement homes, were recruited via advertising with posted flyers, newspaper, and emails disseminated via the university's lifelong learning group. A priori power analysis using G\*Power identified that a sample size of 34 subjects would enable the detection of statistical significance for any correlation relationships between variables with a small effect size of  $r = 0.4$  ( $\alpha = 0.05$ , power = 0.8), thus, 40 males and females participated in the study. The analyses involving the acceleration signals only included 37 participants, due to an inability to correctly

identify a pattern of steps from three participants' acceleration signals. An exception was the calculation of the root mean square of the acceleration signal; since this calculation does not rely upon step identification, this variable was calculated for the full sample of 40 participants. Participants included were 70 years of age and older, able to walk overground, up stairs, and on a treadmill unassisted (for a partner study), able to walk, sit, and stand repeatedly, and step side-to-side without experiencing any dizziness or lightheadedness. Participants were screened for cognitive function using the Mini-Mental State Examination (MMSE), with a cutoff score of 24 for participation [22, 23], resulting in the exclusion of one participant. Participants were 60 % female, ranged in age from 70 to 98 years (Table 1), self-rated their health as good (19.4 %), very good (52.8 %), or excellent (27.8 %), had a median score of 14 out of 14 points on self-rated activities of daily living, reported taking a median of two medications for chronic conditions (range 2–7 medications), and 11 (42.3 %) participants reported having experienced a fall within the past 12 months.

Participants were interviewed regarding their basic and instrumental activities of daily living (ADL) [24, 25], fall history, health history, and activities-specific balance confidence (ABC) [26]. Before completing the step tests, participants completed the 8-ft Timed Up and Go (TUG) [27] and Short Physical Performance Battery (SPPB) [28] assessments of mobility and balance function.

### Repeated Alternating Step Touch Test

A Repeated Alternating Step Touch Test (RASTT) was evaluated. Participants received verbal instruction during a demonstration were given a short familiarization trial, and then a single RASTT trial was performed.

Eleven participants returned 1–2 weeks later to repeat all of the step tests in order to assess the repeatability of the tests.

This test, adapted from Berg et al. [19, 20] and Hill et al. [16], was designed to require rapid ML weight-shifting. Participants stood unassisted with their toes behind a line of tape placed on the floor 15 cm in front of a 15-cm tall exercise step, which was placed on the floor on a nonslip mat against the wall [13, 16, 19, 20]. A piece of tape was placed in the center of the step in order to enforce tapping the foot near the midline of the body, aiming to elicit maximum ML weight-shifting.

Participants were asked to wear comfortable, closed-toed walking shoes. To perform the RASTT, they were instructed to start with both feet behind the tape mark on the floor, then tap each foot alternately onto the piece of tape in the middle of the exercise step as many times as safely possible within 20 s. Speed was emphasized over accuracy, so the participants were not required to line their foot up behind the tape mark on the floor after each tap. The number of times each foot returned to the floor was recorded as the number of steps. It took <5 min to explain, demonstrate, practice, and test one RASTT trial.

### Instrumentation

A triaxial accelerometer (G-Link<sup>®</sup> LXRS<sup>®</sup> Wireless Accelerometer Node, LORD Microstrain Sensing Systems, Williston, VT, USA) was leveled with a bubble level in the ML direction and taped to the participant's skin overtop of the fourth lumbar vertebra. Acceleration data were recorded using LORD Microstrain Node Commander 2.7.0 software.

**Table 1** Participant characteristics and functional test scores

Characteristic or test	Total sample (n = 40) Mean (SD)	Females (n = 24)	Males (n = 16)
Age (years)	80.4 (6.5)	81.2 (7.1)	79.3 (5.6)
Weight (kg)	71.9 (16.7)	61.5 (8.5)*	86.8 (14.2)*
Height (cm)	167.5 (10.1)	161.1 (7.4)*	176.2 (6.2)*
BMI (kg/m <sup>2</sup> )	25.4 (4.0)	23.5 (2.7)*	27.9 (4.1)*
RASTT number of steps	21.0 (5.0)	21.7 (5.8)	20.1 (3.4)
Grip strength (kg) <sup>a</sup>	28.6 (16.3–52.6)	25.2 (16.3–36.3)*	41.7 (18.1–52.6)*
SPPB (points out of 12) <sup>a</sup>	11 (6–12)	11 (6–12)	11 (9–12)
TUG time (s) <sup>a</sup>	8.71 (5.75–16.13)	8.91 (6.19–16.13)	8.42 (5.75–14.13)
ABC (%) <sup>a</sup>	96.7 (74.4–100)	95.4 (77.4–100)	96.9 (86.9–100)
MMSE (points out of 30) <sup>a</sup>	28 (24–30)	28.5 (26–30)	27 (24–30)

BMI body mass index, SPPB Short Physical Performance Battery, TUG Timed Up and Go, ABC activities-specific balance confidence, RASTT Repeated Alternating Stair Touch Test

\* Statistically significant sex difference (*t* test *p* < 0.05)

<sup>a</sup> Values are displayed as median (range) due to being nonnormally distributed

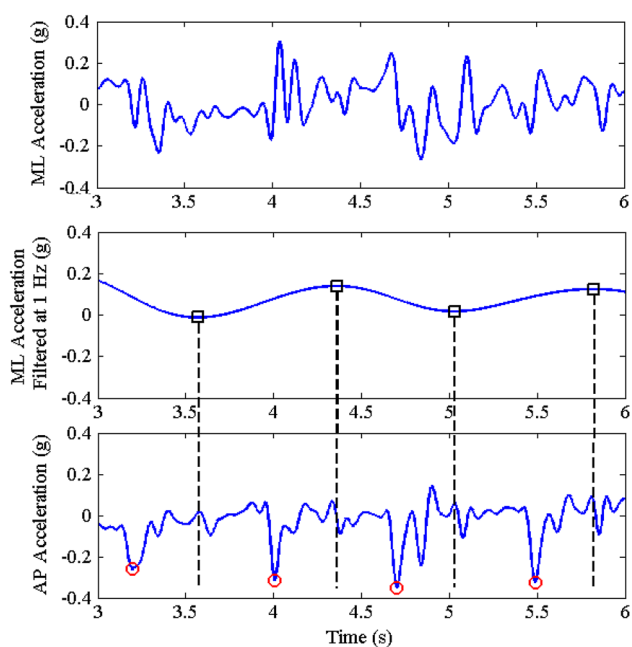


## Signal processing

Signals were processed using custom written Matlab code. Raw acceleration signals were filtered using a fourth order Butterworth filter with a 15-Hz cutoff frequency. For all three planes of motion, the mean acceleration of the trial was subtracted from the signal in order to remove the acceleration due to gravity represented in the signal from any instrument positioning errors. The adjusted trial mean should be zero since the participant did not travel during the trial [29].

Step frequency, the number of steps the participant completed per second, was calculated in Matlab and used as a cutoff frequency to severely filter the ML signal [30]. The severely filtered ML signal was used to identify the basic ML weight shifts due to the step cycle rhythm. A step was defined as each foot returning to the ground after tapping the step and was identified from the AP acceleration signal as the negative peak that occurred between each weight shift cycle in the severely filtered ML signal. A stride was identified as every other step, corresponding to the same foot returning to the ground, as during walking, and this completes one full cycle of the overall ML motion (Fig. 1).

ML per-stride peak-to-peak acceleration (P-P ACC) was calculated as the difference between the maximum and minimum acceleration amplitude in each stride. Mean ML



**Fig. 1** ML and AP acceleration signals demonstrating the process of identifying steps for the RASTT. *Squares* represent the peaks in the severely filtered (at 1 Hz, the step frequency for this trial) ML acceleration signal. These peaks correspond with the peak ML position during which the stepping foot tapped the top of the step. The *circles* represent each foot returning to the floor, which were identified by the AP peaks occurring between each of the severely filtered ML peaks

P-P ACC and ML root mean square (RMS) are both measures of the average acceleration magnitude during the trial. The standard deviation (SD) of the ML P-P ACC represents stride-to-stride acceleration variability. Mean step time, the average time between each of the identified steps in the trial, and step time SD, the amount of variation in step time throughout the trial, were also calculated, representing the speed and rhythmicity of stepping, respectively.

## Statistical analysis

All statistical analysis was conducted using Systat 13 software. The normality of each variable was assessed using the Shapiro–Wilk and Anderson–Darling tests and a visual inspection of histogram plots of the data. Nonnormally distributed data were log transformed for use with parametric statistical tests, affecting all RASTT variables except mean step time and number of steps. All functional test results were also nonnormally distributed; visual analysis of histograms revealed that most participants' values clustered near higher performance scores with a tail directed toward lower functioning scores. SPPB, MMSE, and ABC scores were not normal even after a log transformation: thus, correlations involving these variables were conducted using the Spearman's tests.

Associations between the number of steps completed and outcome measures of acceleration magnitude and variability, stepping speed, and stepping rhythmicity were analyzed using Pearson correlations. Additional associations between participant characteristics, functional test scores, and performance on the step tests were analyzed using Pearson or Spearman correlations. Forward stepwise linear regression was used to determine which outcome measures best predicted number of RASTT steps completed. Only variables that were significantly correlated with number of steps were included in the stepwise regression analyses. Mean step time was not included as a possible predictor, given that it (time between steps) was essentially another way of representing the outcome measure (number of steps in 20 s). Sex differences in performance were assessed using independent *t* tests. Differences between participants who reported a fall in the past year and those who did not were assessed using independent *t* tests. Repeatability was assessed using intraclass correlations and dependent *t* tests to test for between-session differences.

## Results

All participants were able to complete the RASTT without any adverse events. Participants' RASTT scores ranged between 9 and 31 steps. The number of RASTT steps was

**Table 2** Step timing and acceleration variables were significantly correlated with the number of steps completed on the RASTT ( $n = 37$ )

	Median (range)	Correlation with RASTT number of steps (Pearson's $r$ )
Mean step time (s) <sup>a</sup>	0.949 (0.199)	-0.959*
SD step time (s)	0.072 (0.032-0.365)	-0.572*
ML RMS (g)	0.145 (0.054-0.332)	0.534*
Mean ML P-P ACC (g)	0.594 (0.378-1.416)	0.536*
SD ML P-P ACC (g)	0.108 (0.039-0.470)	0.560*

ML RMS medial-lateral acceleration root mean square, Mean ML P-P ACC mean medial-lateral acceleration peak-to-peak magnitude, SD ML P-P ACC standard deviation of the medial lateral peak-to-peak acceleration magnitude, SD step time standard deviation of the step time

\* Statistically significant ( $p < 0.05$ )

<sup>a</sup> Displayed as mean (SD)

**Table 3** Regression coefficients for the variables that significantly predicted the number of steps completed on the Repeated Alternating Stair Touch Test in a forward stepwise multiple regression analysis

Independent variable	B coefficient	Standard error	$\beta$ standardized coefficient	Tolerance	$t$	$p$ value
Constant	21.871	3.382			6.468	0.000
Log SD step time	-4.614	0.900	-0.526	0.993	-5.129	0.000
Log SD ML peak-to-peak acceleration	2.685	0.924	0.363	0.668	2.904	0.007
Log ML RMS	3.068	1.356	0.283	0.667	2.262	0.030

The model was significant [ $F(3,33) = 20.92$ ,  $R_{adj}^2 = 0.624$ ,  $p < 0.001$ , standard error of the estimate = 2.746]

highly negatively correlated with mean step time ( $r = -0.959$ ,  $p < 0.001$ ), moderately positively correlated with all measures of acceleration magnitude (ML RMS:  $r = 0.534$ ,  $p = 0.019$ ; and mean ML P-P ACC:  $r = 0.536$ ,  $p = 0.018$ ) and variability (SD ML P-P ACC,  $r = 0.560$ ,  $p = 0.009$ ) and moderately negatively correlated with SD step time ( $r = -0.572$ ,  $p = 0.006$ ; Table 2). There was a trend toward age being mildly negatively correlated with number of steps, revealing that older participants completed fewer steps ( $r = -0.313$ ,  $p = 0.059$ ). Sex comparisons revealed that men in the sample were taller, heavier, had larger BMI, and stronger handgrip strength than the women, but that they did not differ on the number of RASTT steps (Table 1). The majority of participants were high functioning; most had not fallen within the past year and scored high on questionnaires and tests of functional mobility (Table 1). There was not a significant difference between participants who reported a fall in the past year ( $n = 12$ ) and those who did not for any of the functional tests, including the number of RASTT steps [20.6 (SD 4.8) vs. 21.2 (SD 5.1) steps, respectively;  $t(38) = 0.365$ ,  $p = 0.717$ ].

Forward stepwise linear regression revealed SD step time, SD ML P-P ACC and ML RMS, to be the best predictors of the number of steps completed [ $F(3,33) = 20.92$ ,

$R_{adj}^2 = 0.624$ ,  $p < 0.001$ , standard error of the estimate: 2.746, Table 3]. The tolerance values were high (Table 3), indicating that the model does not appear to be affected by multicollinearity. Higher-performing participants had less step timing variability (Fig. 2), greater acceleration magnitude (Fig. 3), and greater RASTT acceleration variability.

Repeatability was good for number of steps, mean step time, and mean ML P-P ACC, moderate for ML RMS, and SD step time, and mild and nonsignificant for SD MLP-P ACC (Table 4). Number of steps, mean step time, and SD step time significantly improved between sessions (Table 4).

RASTT performance was moderately related to tests of balance and mobility function; RASTT number of steps was moderately correlated with TUG (Fig. 4) and SPPB scores, but was not related to grip strength, ABC scores, or MMSE scores (Table 5).

## Discussion

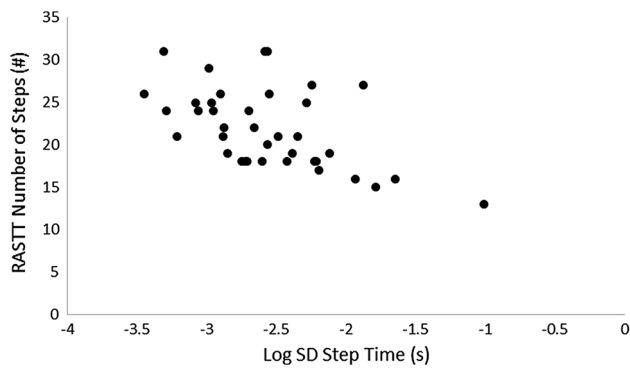
The unique potential of the RASTT to contribute to functional assessment of older adults is that it specifically challenges medial-lateral weight-shifting and rapid,

rhythmic, alternating stepping ability while utilizing continuous scale scoring. Higher-performing participants on the RASTT exhibited larger ML acceleration magnitudes, indicating that they were more aggressive with their ML weight-shifting and were faster and more rhythmic with their stepping. These results are consistent with previous studies associating faster gait and stepping-in-place speed

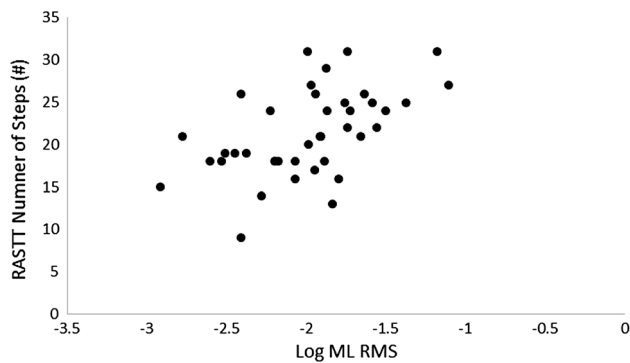
[3], increased acceleration magnitudes during walking [8], and decreased step time variability with higher-functioning and lower fall risk participants [31–33]. The addition of the RASTT to functional assessments of seniors would enable practitioners to understand function on this group of variables without expensive equipment. It is currently unknown, however, if the RASTT can predict falls in seniors, as the current sample was high functioning. The RASTT is being developed to serve as a screening tool to identify individuals who are at a higher risk of experiencing a fall prior to the onset of severe mobility limitations. Its use may be limited to individuals who are not already experiencing severe mobility disability—those who can stand and shift weight from side-to-side while placing the feet on a step without the use of an assistive device.

Contrary to our hypothesis, higher-performing participants on the RASTT exhibited greater acceleration variability; they did not maintain consistency in acceleration magnitudes from step to step. This finding was also observed with frail older participants who exhibited lower ML trunk acceleration variability during walking than healthy seniors [32]. A loss of variability in physiological systems, including the ability to vary movement while walking and balancing, is associated with a decreased ability to adapt to environmental stresses [34]; thus, an increase in variability of motion can indicate a more adaptable system. Rapid stepping increases the incidence of overshooting the ML center of mass position in older adults [10]. Therefore, higher-functioning participants in the current study may have overshoot their intended ML center of mass position during the RASTT more frequently because they were moving faster, causing them to exhibit greater ML acceleration variability. Presumably, the ability to correct the incongruity between the intended and actual center of mass position brought on by rapid stepping [10] is only possible for higher-functioning participants.

RASTT number of steps was also positively correlated with age, but age did not independently predict RASTT performance in the forward stepwise regression analysis



**Fig. 2** Higher-performing participants who completed more steps on the Repeated Alternating Stair Touch Test (RASTT) had lower values of the log of the standard deviation (SD) of step time



**Fig. 3** Higher-performing participants who completed more steps on the Repeated Alternating Stair Touch Test (RASTT) had higher values of the log of the medial–lateral (ML) root mean square (RMS)

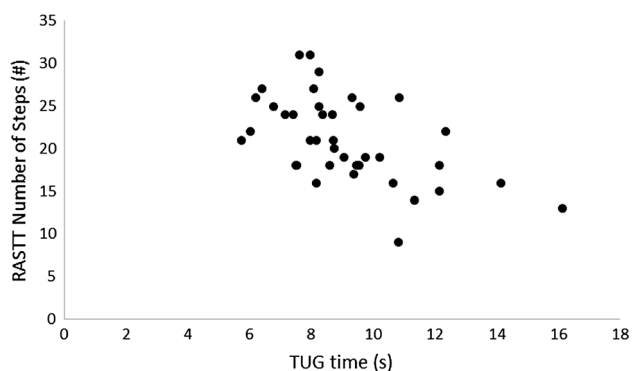
**Table 4** Repeatability results for Repeated Alternating Stair Touch Test ( $n = 11$ )

	Session 1 mean (SD)	Session 2 mean (SD)	Intraclass correlation
Number of steps	21.0 (4.3)	23.2 (4.4)*	0.763
Mean step time (s)	0.971 (0.191)	0.874 (0.184)*	0.793
SD step time (s)	0.086 (0.035)	0.067 (0.033)*	0.609
ML RMS (g)	0.134 (0.047)	0.144 (0.064)	0.618
Mean ML P-P ACC (g)	0.557 (0.184)	0.607 (0.256)	0.828
SD ML P-P ACC (g)	0.107 (0.064)	0.137 (0.074)	0.486

*ML RMS* medial–lateral acceleration root mean square, *Mean ML P-P ACC* mean medial–lateral acceleration peak-to-peak magnitude, *SD ML P-P ACC* standard deviation of the medial–lateral peak-to-peak acceleration magnitude, *SD step time* standard deviation of the step time, *IQR* interquartile range

\* Statistically significant improvement between sessions (paired  $t$  test,  $p < 0.05$ )





**Fig. 4** Higher-performing participants who completed more steps on the Repeated Alternating Stair Touch Test (RASTT) had shorter (faster) times for the completion of the 8-foot Timed Up and Go (TUG) test

**Table 5** Relationship between Repeated Alternating Stair Touch Test (RASTT) and demographic and functional test scores ( $n = 40$ )

	Pearson's correlation with RASTT number of steps
Age (years)	-0.313 <sup>a</sup>
Height (cm)	-0.128
Body mass (kg)	-0.211
BMI (kg/m <sup>2</sup> )	-0.197
TUG time (s)	-0.542**
SPPB (points) <sup>b</sup>	0.512**
MMSE (points) <sup>b</sup>	0.290 <sup>a</sup>
ABC (%) <sup>b</sup>	0.213
Grip strength (kg)	-0.077

BMI body mass index, TUG Timed Up and Go, SPPB Short Physical Performance Battery, MMSE Mini-Mental State Exam, ABC activities-specific balance confidence

\*\* Statistically significant Pearson's  $r$  ( $p < 0.01$ )

<sup>a</sup> Trend toward significance,  $0.05 < p > 0.07$

<sup>b</sup> Spearman's correlation

when the acceleration and temporal variables were taken into account. There was a notable amount of variance (37.5 %) in RASTT performance that could not be explained by the variables measured in this study, however. Given the association between RASTT, speed of movement, and rhythmicity, other unmeasured variables that also relate to these aspects of function could underlie the relationships identified in this study, or could account for additional variance in RASTT performance. These include lower extremity muscle power [18, 35], body composition [36], vision [37], balance and sensorimotor function [38], vestibular function [39, 40], osteoarthritis [41, 42], cognitive function [43, 44], and psychological factors, such as a fear of falling [45].

Repeatability for the RASTT was moderate, but effects of learning were apparent. Before it can be useful as a functional assessment, the RASTT will require modifications to improve repeatability, such as additional familiarization time and perhaps altering the time period of the test, the step height, and the distance between the participant and the step. Additionally, the RASTT's responsiveness to changes in function will need to be assessed, along with its relationship to fall rates, before its utility as a clinical assessment can be fully understood.

The RASTT related fairly well to tests of general mobility and balance function. It is expected that the scores would not be strongly related because RASTT, TUG, and SPPB functional tests challenge different domains of function; the step test was designed specifically to challenge ML weight-shifting function while stepping quickly, whereas the functional tests challenge lower extremity function, mobility, and the ability to maintain static balance.

**Study limitations**

The results of this study may be limited by the participant pool. Participants who volunteered for the study were generally high functioning, the majority were community-dwelling, many had not fallen, and only two reported multiple falls within the last year. Even still the range of RASTT scores was broad.

**Conclusion**

Participants who completed more RASTT steps in 20 s were able to shift their weight aggressively in the ML direction and tolerate more variability in their ML accelerations from step to step, while maintaining rapid and rhythmic stepping. RASTT would be a low cost, unique addition to functional assessments of older adults; however, modifications to the RASTT will first be needed to improve its repeatability. In addition, the RASTT's responsiveness to change and its ability to identify individuals at a higher risk of falling have not yet been evaluated.

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**Compliance with ethical standards**

**Conflict of interest** The authors have no conflicts of interest regarding this study.

**Statement of human and animal rights** All procedures performed involving human participants in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

**Informed consent** All participants gave written informed consent.

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