The effect of prolonged level and uphill walking on the postural control of older adults.

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**Abstract**

Prolonged walking could alter postural control leading to an increased risk of falls in older adults. The aim of this study was to determine the effect of level and uphill prolonged walking on the postural control of older adults. Sixteen participants (64±5 years) attended 3 visits. Postural control was assessed during quiet standing and the limits of stability immediately pre, post and post 15 minutes rest a period of 30 minutes walking on level and uphill (5.25%) gradients on separate visits. Each 30-minute walk was divided into 3 10-minute blocks, the limits of stability were measured between each block. Postural sway elliptical area (PRE: 1.38±0.22 cm\(^2\), POST: 2.35±0.50 cm\(^2\), p=0.01), medio-lateral (PRE: 1.33±0.03, POST: 1.40±0.03, p=0.01) and anterio-posterior detrended fluctuation analysis alpha exponent (PRE: 1.43±0.02, POST: 1.46±0.02, p=0.04) increased following walking. Medio-lateral alpha exponent decreased between post and post 15 minutes’ rest (POST: 1.40±0.03, POST15: 1.36±0.03, p=0.03). Forward limits of stability decreased between the second walking interval and post 15 minutes’ rest (Interval 2: 28.1±1.6%, POST15: 25.6±1.6%, p=0.01) and left limits of stability increased from pre-post 15 minutes’ rest (PRE: 27.7±1.2%, POST15: 29.4±1.1%, p=0.01). The neuromuscular alterations caused by
prolonged walking decreased the anti-persistence of postural sway and altered the limits of stability in older adults. However, 15 minutes’ rest was insufficient to return postural control to pre-exercise levels.

**Keywords:** Older adults; Postural control; Detrended fluctuation analysis; Walking

**Introduction**

Postural control of older adults is associated with neuromuscular function, falls and functional ability (Ko and Newell, 2016; Kurz et al., 2013; Orr, 2010; Shaffer and Harrison, 2007). An acute reduction in neuromuscular function increases fall risk (Morrison et al., 2016). Fatigue acutely impairs muscle force output, and increases synaptic time delays, motor variability and proprioceptive inaccuracies, resulting in a disturbance to the postural control system (Davidson et al., 2011; Paillard, 2012; Singh and Latash, 2011; Vuillerme and Boisgontier, 2008).

Walking is a common daily activity for older adults that can lead to fatigue (Morrison et al., 2016). Short duration (up to 15 minutes) high intensity walking increases postural sway in older adults during single and double leg stance (Donath et al., 2015a, 2013; Morrison et al., 2016). This suggests the fatiguing exercises acutely altered proprioceptive and vestibular function (Paillard, 2012) and muscle activation patterns (Ortega and Farley, 2015; Paillard, 2012). However, the studies observing postural sway differences (Donath et al., 2015a, 2013; Morrison et al., 2016) have used high intensity exercise over short durations (<15 mins) which is not typical of activity performed by older adults. During longer duration (up to 30 minutes) exercise, younger adults demonstrate an initial decrease in performance of postural control tasks after commencing exercise, however as exercise duration increased,
performance of postural control tasks either remained constant or returned towards baseline (Simoneau et al., 2006; Thomas et al., 2013). However, it is currently unclear if the postural control of older adults can adapt during longer duration exercise, as is seen in younger adults.

In addition to the walking duration, the gradient of the surface could impact the muscle activation and the fatigue and postural control change experienced by the older adult. Older adults have increased muscle activation when walking uphill compared to younger adults (Franz and Kram, 2013; Hortobagyi et al., 2011; Ortega and Farley, 2015). Greater muscle activation could therefore cause uphill walking to have greater effects on postural control than level walking. Furthermore, the neuromuscular alterations associated with fatigue and uphill walking (Franz and Kram, 2013) may alter the postural control dynamics of older adults. More persistent postural sway dynamics, a strategy associated with fewer and less frequent postural adjustments (Borg and Laxåback, 2010), can increase the risk of falls in older adults (Kurz et al., 2013) because the postural control system is less versatile and able to adapt to perturbations or changing environmental conditions (Ko and Newell, 2016; Seigle et al., 2009). However, the effect of walking on the postural sway dynamics of older adults is unknown.

The aim of the present study was to investigate the effect of level and uphill walking at a moderate intensity on postural control measured during quiet standing and the limits of stability (LOS) and walking muscle activation. Additionally, this study aimed to investigate the effect of exercise duration on the LOS. It was hypothesised that level and uphill walking would increase quiet standing postural sway while decreasing sway anti-persistence the
LOS. It was further hypothesised that uphill walking would have a greater effect on postural control and walking muscle activation than level walking.

Method

Participants

Sixteen older adults (n-female: 6, n-male: 10, age: 64±5 years, height: 1.73±0.14 m, mass: 76.8±12.7 kg, level self-selected walking speed 1.22±0.19 m/s) participated in the study. A priori power calculation revealed a sample size of 16 participants was required based on an effect size of $f=0.56$ for pre-post walking changes in postural control variables (Donath et al., 2013), a desired power of 80% and alpha= 0.05. Participants were excluded if they suffered from neurological conditions, visual impairment, or lower limb injuries. The study received ethical approval from the University ethics committee and all participants were made aware of the nature of the study and their right to withdraw at any time, before providing written informed consent. All aspects of the study were conducted in accordance with the Declaration of Helsinki.

Procedures

Participants attended 3 laboratory visits. During the first visit participants were familiarised with postural control tasks and treadmill walking. The exercise intensity for visits 2 and 3 was also determined during visit 1 as 105% of the average heart rate (HR) during 5 minutes at self-selected walking speed (SSW) on 0% gradient. Pilot data collection established that this intensity was deemed to be moderate intensity (Mazzeo and Tanaka, 2001) and the 5.25% gradient was suitable to complete the 30-minute protocol.
In the second and third visits, participants performed quiet standing, LOS and 1 minute of SSW on 0% gradient immediately before (PRE) and after (POST) 30-minutes of treadmill walking at the predetermined exercise intensity on either 0% or 5.25% gradient (Figure 1). A period of 30-minutes walking was selected as it reflects the duration and intensity of physical activity, to be performed 3-5 days per week, recommended to older adults to maintain a healthy lifestyle (NHS, 2017). The order of 0% and 5.25% conditions was randomised and counterbalanced. Quiet standing and LOS tests were repeated after 15 minutes of rest following completion of POST measurements (POST15).

The 30-minute walk was divided into 3 blocks of 10 minutes. After the first (walking interval 1) and second (walking interval 2) block the LOS test was repeated. Only measurement of the LOS was performed in walking interval 1 and 2 to minimise the time between walking blocks and reduce the time for recovery during the balance tests. Walking speed was increased during the first 10-minute block until the target HR was reached and then speed was fixed for the remainder of the 30-minute walk. Heart rate and rate of perceived exertion (RPE) were recorded in alternate minutes and expired air was collected for the final 2 minutes of each 10-minute block using a Douglas bag for the calculation of oxygen consumption ($VO_2$).

Electromyographic (EMG) activity of the dominant leg Rectus Femoris (RF), Vastus Medialis (VM), Biceps Femoris (BF), Tibialis Anterior (TA), Gastrocnemius Medialis (GM) and Soleus (SOL) was recorded during alternate minutes of each walking block and during PRE and POST SSW. Bipolar Ag/AgCl electrodes were placed with a 2 cm inter-electrode distance.
according to the SENIAM guidelines (Hermens et al., 1999). Prior to placement of electrodes each site was shaved and cleaned with an alcohol wipe. All EMG signals were recorded at 1000 Hz, amplified (gain x1000) and A/D converted using 6 wireless transmitters (BTS FREEEMG 300, BTS Bioengineering, Milan, Italy) and software (EMGAnalyzer, BTS Bioengineering, Milan, Italy). A footswitch was placed under the heel of the dominant leg, and recorded synchronously with EMG signals at 1000 Hz, to allow the detection of heel strike gait events.

Postural control during quiet standing and LOS tests were assessed with participants stood barefoot in a comfortable position on a force plate (Kistler Instruments Ltd, Winterthur, Switzerland) with eyes open. The foot position of each participant was marked on a clear covering placed over the surface of the force plate to ensure the same position was adopted for each trial and visit, as foot placement can alter the calculated postural sway parameters (Chiari et al., 2002). Participants performed 5 trials of 60 seconds quiet standing at PRE, POST, POST15 measurements while the movements of the centre of pressure (COP) were recorded at 48 Hz using Bioware software (Kistler Instruments Ltd, Winterthur, Switzerland).

Participants performed the LOS task at PRE, between walking blocks 1 and 2 (walking interval 1), between walking blocks 2 and 3 (walking interval 2), POST and POST15. Three trials 30 second trials were performed in the forward, right and left directions at each time point. The backward direction was not included to reduce the risk of falling. Each 30 second trial was divided into 3 phases, in phase 1 participants stood quietly for 10 seconds and then were asked to lean forward, right or left. Phase 2 began at the start of the lean movement and ended when participants reached a lean position they perceived as maximum distance
that they could maintain without falling. The leaning movement was executed at a self-selected speed using an ankle strategy, whilst avoiding bending at the hips and keeping feet flat on the force plate surface. Trials in which participants visibly flexed the hips, or lifted their heels from the force plate surface were repeated. In phase 3 participants were asked to maintain the maximal lean position for the remainder of the 30 second trial. Three trials were performed for each lean direction.

Data Analysis

Walking Muscle Activity

Raw EMG signals were band-pass filtered with a dual-pass 2\textsuperscript{nd} order Butterworth filter with 20 and 450 Hz cut-off frequencies before being full-wave rectified and low-pass filtered with a dual-pass 2\textsuperscript{nd} order Butterworth filter with a 10 Hz cut-off frequency. Low-pass filtered EMG signals were normalised as a percentage of the maximum activity recorded for each muscle during the PRE SSW. The use of a metric EMG value, such as the peak or mean activation, derived from a reference gait condition has been used previously (e.g. Ricamato and Hidler, 2005; Schmitz et al., 2009) and can be more appropriate for normalising gait EMG data than using maximal isometric contractions (Cronin et al., 2015; Yang and Winter, 1984).

Each normalised EMG signal was divided into individual gait cycles using heel-strike events detected using footswitches and interpolated to 1001 data points. For each gait cycle, the mean of the normalised EMG (EMG\textsubscript{Mean}) signal was calculated.
The EMG calculated for each muscle was then averaged for all gait cycles in each individual walking block and PRE and POST SSW. All EMG signals were analysed using custom written Matlab programmes (Mathworks Inc., MA, USA).

**Quiet Standing**

The COP signals were not filtered to avoid removing the natural variability of the signal which would impact the non-linear analyses as the complexity of the signal is removed (Doyle et al., 2004). The postural sway path length (SWAY<sub>PL</sub>) was calculated as the resultant path length of the medio-lateral (ML) and anterio-posterior (AP) COP components. For the calculation of elliptical area (SWAY<sub>EA</sub>) principle component analysis was used to determine the angle of the principle axis, and the minor axis orthogonal to the principle axis. The length of the axes was calculated as 1.96 times the standard deviation along each axis.

Detrended fluctuation analysis (Peng et al., 1995) was performed to calculate the alpha exponent (DFA<sub>α</sub>) separately for the ML and AP COP components. Non-linear analyses provide an indication of the underlying postural control dynamics that cannot be gained from linear measures (Collins et al., 1995). The COP signal was integrated and subsequently divided into non-overlapping boxes of equal length. A linear least squares model was fit to each box and the slope of the model was subtracted to detrend the box. The root mean square fluctuation of the signal was calculated and plotted against the box length on a log-log plot. The process was then repeated with box lengths ranging from 4 to N/4 (Tahayor et al., 2012), where N is the total number of samples. The DFA<sub>α</sub> was then estimated as the slope of a linear least squares model fit to the log-log plot of root mean square fluctuation vs. box length. A DFA<sub>α</sub> value of 1 < DFA<sub>α</sub> < 1.5 represents an anti-persistent signal, one that
tends to anti-correlate with increasing time scales, and a DFA\(_{\alpha}\) value of 1.5 < DFA\(_{\alpha}\) < 2 represents a persistent signal, one that tends to correlate with increasing time scales. Values of 1.5 represent Brownian noise. All quiet standing trials were analysed using custom written Matlab programmes (Mathworks Inc., MA, USA).

**Limits of Stability**

The start and end of each phase during LOS trials was determined as the intersection points of separate linear least squares models fitted to the 3 distinct regions of the COP signal using the Shape Language Modelling Matlab toolbox (Mathworks Inc., MA, USA). The anterior, posterior, left and right boundaries of the base of support (BOS) were determined from the outline of the feet drawn on the force plate as the maximum displacement in each direction respectively. The length of the AP and ML BOS were then calculated as the distance between the anterior and posterior, and left and right boundaries in the respective directions.

The distance leaned in each LOS trial was calculated as the absolute distance between the average COP positions in phases 1 and 3. The distance leaned was reported as a percentage relative to the total BOS length (LOS\(_{REL}\)) in the AP direction for forward leaning trials and the ML direction for left and right leaning trials. The root mean square (LOS\(_{RMS}\)) was calculated from the detrended COP signal in phase 3 to indicate the variability of movement in the sustained period of leaning. All LOS variables were averaged across the 3 trials performed at each stage. All LOS trials were analysed using custom written Matlab programmes (Mathworks Inc., MA, USA).
All data were tested for normality using the Shapiro-Wilk test and were normally distributed. Heart rate, RPE, VO₂ and muscle activation of all muscles measured during the 3 walking blocks were analysed using 2x3 repeated measures ANOVAs to determine the effects of gradient (0% vs. 5.25%), time (Block 1 vs. Block 2 vs. Block 3) and interactions. Two-way repeated measures 2x3 ANOVAs were performed to determine the effects of gradient (0% vs. 5.25%), time (PRE vs. POST vs. POST15) and interactions on all quiet standing postural control variables and SSW muscle activation. To determine the effects of time (PRE vs. walking interval 1 vs. walking interval 2 vs. POST vs. POST15), gradient (0% vs. 5.25%) and interactions on all LOS variables 2x5 two-way repeated measures ANOVAs were performed. Post hoc pairwise comparisons with a Bonferroni correction were performed when significant main effects were present. The inter-session reliability (intra-class correlation coefficient: ICC) of quiet standing and LOS variables were also determined from the PRE data of each session. An ICC of 0.75-0.89 and ≥0.90 were considered good and excellent respectively (Portney and Watkins, 2009). Partial eta squared ($\eta^2_p$) was calculated as an estimate of effect size. A $\eta^2_p$ of ≥0.01, ≥0.06 and ≥0.14 represent small, medium and large effects (Cohen, 1988). For all tests the level of significance was set at $p<0.05$. All statistical analysis was performed using SPSS software (v22, IBM UK Ltd., Portsmouth, UK).

**Results**

**Walking blocks**

There were no effects of time, gradient or interactions for the VO₂, RPE, HR (Figure 2) or EMG\textsubscript{Mean} of any muscle between the 3 walking intervals (Table 1).

[Figure 2 here]
Muscle Activation during SSW

The EMG Mean was greater during POST than PRE SSW for RF (F(1,15)=11.65, p<0.01, $\eta_p^2=0.47$) and VM (F(1,15)=10.83, p<0.01, $\eta_p^2=0.46$). The EMG Mean was greater in the uphill than level walking visits (Table 1) for RF (F(1,15)=9.54, p=0.01, $\eta_p^2=0.42$) and BF (F(1,15)=5.74, p=0.03, $\eta_p^2=0.31$). There were no effects of time for BF, TA, GM and SOL, or gradient for VM, TA, GM and SOL. No interactions for any muscle were found.

[Table 1 here]

Quiet standing

There was a significant effect of time for SWAY EA (F(2,30)=7.07, p=0.01, $\eta_p^2=0.32$), AP-DFA $\alpha$ (F(2,30)=2.96, p=0.04, $\eta_p^2=0.17$), and ML-DFA $\alpha$ (F(2,30)=15.61, p=0.01, $\eta_p^2=0.51$). SWAY EA, AP-DFA $\alpha$, and ML-DFA $\alpha$ increased between PRE and POST (p=0.02, p=0.01, p<0.01 respectively), while ML-DFA $\alpha$ also decreased between POST and POST15 (p=0.03). There was no effect of time for SWAY PL. No effects of gradient or interactions for any variable were found (Figure 3).

[Figure 3 here]

Limits of Stability

There was an effect of time for forward LOS REL (F(4,60)=2.98, p=0.03, $\eta_p^2=0.17$), and left LOS REL (F(4,60)=5.92, p<0.01, $\eta_p^2=0.28$), and LOS RMS (F(4,60)=8.56, p<0.01, $\eta_p^2=0.36$). Forward LOS REL decreased between walking interval 2 and POST15 (p=0.01). Left LOS REL and LOS RMS increased between PRE and POST15 (p=0.01, p<0.01 respectively), left LOS RMS also increased between PRE, walking interval 2 and POST (p=0.02, p=0.04 respectively). There were no
effects for gradient, time or interactions for forward LOS$_{RMS}$ or any right LOS variables (Table 2).

 Reliability of Postural Control Variables

Excellent reliability was found for SWAY$_{EA}$, ML-DFA$_{α}$ and AP-DFA$_{α}$ (ICC: 0.94, 0.92 and 0.91 respectively), however inadequate reliability was found for SWAY$_{PL}$ (ICC: 0.73). Excellent reliability was found for both LOS variables in the forward (LOS$_{REL}$ ICC: 0.93 and LOS$_{RMS}$ ICC: 0.97) right (LOS$_{REL}$ ICC: 0.96 and LOS$_{RMS}$ ICC: 0.99) and left (LOS$_{REL}$ ICC: 0.92 and LOS$_{RMS}$ ICC: 0.95) directions.

Discussion

The present study has demonstrated that prolonged walking alters postural control, and lower limb muscle activation, but that the effect of gradient was minimal. During quiet standing DFA$_{α}$ was increased immediately after walking in both the AP and ML directions and the SWAY$_{EA}$ was also increased. Contrary to the hypothesised effect the LOS was only altered during walking for left LOS$_{RMS}$. However, the forward LOS$_{REL}$ was reduced POST15 compared to walking interval 2 and left LOS$_{REL}$ was increased POST15 compared to PRE. The findings of this study therefore partially support the primary hypothesis that moderate intensity walking would alter postural control. In addition to alterations in postural control the EMG$_{Mean}$ of RF and VM was greater during level SSW following prolonged level and uphill walking.

The current study was the first to use non-linear measures of postural control during quiet standing to examine the effects of moderate intensity walking in older adults. The greater
ML and AP DFA$\alpha$ immediately POST compared to PRE, indicate postural sway was less anti-persistent. An anti-persistent COP signal represents a postural control strategy that relies on rapid corrective impulses (Borg and Laxåback, 2010), becoming more anti-persistent the closer to 1 DFA$\alpha$ is, therefore an increasing DFA$\alpha$ towards 1.5 indicates a postural control strategy less reliant on rapid corrective impulses. A possible explanation could be the increased RF and VM activity found during POST level SSW, indicative of neuromuscular alterations resulting from prolonged walking. It has been suggested previously that fatigue can impair the function of CNS components associated with neuromuscular control and proprioception (Lin et al., 2009; Morrison et al., 2016; Paillard, 2012; Simoneau et al., 2006). An explanation for the less anti-persistent postural sway may therefore be that prolonged walking altered neuromuscular function to the extent that the postural control system constrained the available degrees of freedom (Newell, 1998). However, in opposition to the alterations to quiet standing postural control, neither level or uphill walking had an effect on LOS at the interval measurements, contrary to the hypothesis and previous findings in younger adults (Simoneau et al., 2006; Thomas et al., 2013). A possible explanation is that in the present study there was no change in ankle muscle activation after prolonged walking, similar to the unaltered ankle muscle coordination observed previously following a single high intensity interval training session (Donath et al., 2015b). This could suggest that ankle muscle activation is less prone to fatigue than more proximal muscle and therefore would not impair LOS performance, which is primarily controlled by the ankle muscles.

Previous studies have found increases in SWAY$_{PL}$ (Donath et al., 2013; Stemplewski et al., 2012) and sway velocity (Egerton et al., 2009; Morrison et al., 2016) after exercise, that were not present in the current study. This could be due to the lower exercise intensity used
in the current study that was not sufficient to elicit changes in these SWAY$_{PL}$. However, the increased SWAY$_{EA}$ found in the present study is indicative of a greater sway magnitude and increased fall risk after exercise, in agreement with previous findings (Donath et al., 2013; Morrison et al., 2016).

The present findings suggest that 15 minutes rest is insufficient to return postural control to pre-exercise levels after 30 minutes walking in older adults, in agreement with findings following eccentric fatiguing exercises (Papa et al., 2015). The SWAY$_{EA}$ and AP DFA$_{α}$ did not return to baseline values and forward LOS$_{REL}$ decreased compared to walking interval 2. However, the ML DFA$_{α}$ did return to baseline values and left LOS$_{REL}$ increased after 15 minutes’ rest, but this was associated with a greater LOS$_{RMS}$ indicating less control of the leaned positions. In young adults, 5 and 10 minutes rest were sufficient to recover postural control to pre-exercise levels following Triceps Surae and repeated sit-to-stand fatiguing exercises (Bryanton and Bilodeau, 2016; Noda and Demura, 2007). It is possible that the age-related decline in neuromuscular function results in reduced capacity to recover postural control after exercise.

There are some limitations of the present study that must be taken into consideration. Due to the population studied, conclusions are limited to healthy, active older adults. Frail older adults or those with additional comorbidities would likely be more susceptible to fatigue caused by moderate intensity level and uphill walking leading to a greater extent of impairment to postural control. It should also be considered that the process of measuring the LOS between each 10-minute period of walking took approximately 5 minutes and likely resulted in a level of recovery between periods. Consequently, the overall intensity of
exercise may not have been maintained across the 30-minute period of walking, potentially
impacting on muscle activation and limiting the cumulative effects of continuous walking.

In conclusion, both level and uphill walking at a moderate intensity altered quiet standing
and limits of stability postural control measures but there was no effect of exercise duration
on LOS in older adults. Postural control was altered immediately post exercise becoming
less anti-persistent to meet the demands of maintaining balance following 30 minutes of
walking. Secondly, when exercise intensity is matched, walking uphill does not result in
additional effects on postural control. Furthermore, the results of this study indicate that 15
minutes of rest is insufficient to completely restore postural control measures to pre-
exercise levels after 30 minutes walking.

Conflicts of Interest
None. This study received no external funding.

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Thomas, K.S., VanLunen, B.L., Morrison, S., 2013. Changes in postural sway as a function of


Figure captions

Figure 1. Overall study design.

SSW: Self-selected speed walking, PC: postural control assessments including quiet standing and limits of stability, LOS: limits of stability test

Figure 2. Mean and standard deviation values of VO₂, heart rate and rate of perceived exertion in walking block 1, 2 and 3.
Figure 3. Mean and standard deviation values of quiet standing postural control variables for level and uphill walking at PRE, POST and POST15 measurements.

* indicates POST is greater than PRE, ** indicates that POST15 is less than POST.
Table 1. Mean and standard deviation values for mean EMG activation of all muscles at all measurement points.

<table>
<thead>
<tr>
<th></th>
<th>PRE</th>
<th>Block 1</th>
<th>Block 2</th>
<th>Block 3</th>
<th>POST</th>
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<td></td>
<td>Level</td>
<td>Uphill</td>
<td>Level</td>
<td>Uphill</td>
<td>Level</td>
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<tr>
<td>Mean</td>
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<td>35.6±9.0</td>
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<tr>
<td>Activation (%)</td>
<td>VM</td>
<td>24.0±7.8</td>
<td>30.8±8.0</td>
<td>30.8±11.1</td>
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<tr>
<td></td>
<td>BF</td>
<td>28.7±9.6</td>
<td>34.8±11.4</td>
<td>34.4±13.6</td>
<td>49.9±16.4</td>
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<td>TA</td>
<td>28.1±9.6</td>
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<td>GM</td>
<td>31.2±10.5</td>
<td>30.4±6.5</td>
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<tr>
<td></td>
<td>SOL</td>
<td>29.4±11.7</td>
<td>32.0±7.5</td>
<td>34.6±12.3</td>
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Table 2. Mean and standard deviation values for all limits of stability variables at all measurement points

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<th>PRE</th>
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<th>Walking Interval 2</th>
<th>POST</th>
<th>POST15</th>
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<td>Forward</td>
<td>LOS&lt;sub&gt;REL&lt;/sub&gt;</td>
<td>25.7±5.5</td>
<td>26.2±4.9</td>
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<td></td>
<td>LOS&lt;sub&gt;RMS&lt;/sub&gt;</td>
<td>4.4±1.9</td>
<td>4.6±1.6</td>
<td>4.7±1.9</td>
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<td>28.0±5.6</td>
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<tr>
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<td>LOS&lt;sub&gt;RMS&lt;/sub&gt;</td>
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* indicates values are greater than PRE, † indicates values are lower than walking interval 2.