The underlying mechanisms of improved balance after short- and long-term training in older adults

Leila Alizadehsaravi, Ruud Koster, Wouter Muijres, Huub Maas, Sjoerd M. Bruijn, Jaap H. van Dieën

Department of Human Movement Sciences, Faculty of Behavioural and Movement Sciences, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

Corresponding Author: L.alizadehsaravi@vu.nl

Abstract

With training older adults can improve balance control, but the time course and neural mechanisms underlying these improvements are unclear. We studied changes in balance (robustness and performance), as well as in H-reflex gains, paired reflex depression (PRD) and co-contraction duration (CCI) in ankle muscles after short-term (1 session; STT) and long-term (3 weeks; LTT) balance training in 22 older adults. Mediolateral balance robustness during unipedal stance (time to balance loss in unipedal standing on a robotic platform with decreasing rotational stiffness) improved (33%) after STT, with no further improvement after LTT. Balance performance (mean absolute mediolateral center of mass velocity) improved (18.75%) after STT in perturbed unipedal standing and after LTT (18.18%) in unperturbed unipedal standing. CCI of soleus/tibialis anterior did not change after STT but increased (16%) after LTT. H-reflex gain and PRD excitability did not change with training. Cross-correlations showed that H-reflex gains in unipedal stance were lower and CCI was higher in participants with a more robust balance at the last time-point measurement and, CCI was higher in participants with better balance performance at several time-points. However, changes in robustness and performance were uncorrelated with changes in CCI, H-reflex gain, or PRD. Our results indicate that balance robustness improves over a single session, while balance performance improves more gradually over multiple sessions. Changes in co-contraction and motor neuron excitability of ankle muscles are not exclusive causes of improved balance performance and robustness.

Keywords: Balance training, center of mass velocity, co-contraction, H-reflex, paired reflex depression, motor learning, balance performance, postural balance

Introduction

Balance control is essential to avoid falls during daily-life activities. Impaired balance control due to aging results in falls, injuries and loss of independence in older adults[1]. To resolve this issue, it is important to understand how balance control works and when and how it improves as a result of training. Balancing requires the central nervous system to act rapidly and accurately on an array of sensory inputs[2], consisting of visual, vestibular, and tactile information, as well as proprioceptive sensory feedback[3]. Balance training leads to improved balance performance in older adults[4], observed as a reduction in mediolateral center of mass velocity during unipedal stance[5]. However, the question how balance training induces changes in neuromuscular control remains unanswered. Hence, it is important to investigate the relation between improved balance control in older adults with changes in neural mechanisms at central and/or peripheral nervous system components.

Changes in balance control with training appear to occur at short-time scales, with substantial improvements after a single trial and over a single session[6–8]. Yet most studies have focused on training over several sessions spread over multiple weeks[4]. Since training effects were mostly measured before and after the complete training period only, the difference

between short-term (single session) and long-term effects of balance training in older adults is as yet unclear.

Most studies assess balance control with measures that capture balance *performance*. That is, they asses how good people are at minimizing disturbances from an equilibrium position (often in a non-challenging condition like bipedal stance), for instance by measuring postural sway, in which lower values indicate better performance. There are two problems with this. Firstly, subjects may choose not to minimize their sway, as higher sway values may be unproblematic, and require less energy[9]. Secondly, even if subjects choose to minimize their sway, balance performance does not reflect the maximum capability to avoid balance loss when challenged, i.e. the balance *robustness*. For practical purposes, improved *robustness* may be more important than improved performance. While improved balance *performance* may not necessarily prevent falls, it may indicate improvements of balance control. Hence, we here chose to study effects of training on both balance *performance* and balance *robustness*.

Age-related degenerative processes in the sensory and motor systems induce a shift from reliance on feedback control to reliance on feedforward strategies, such as co-contraction[10]. Antagonistic co-contraction can compensate for impaired sensory feedback[11]. Increasing antagonistic co-contraction when confronted with a challenging balance task is a strategy that is also used by inexperienced young adults[12,13]. Higher co-contraction in older adults with poor balance control compared to young adults with better balance control has been shown previously[13,14]. Balance training can potentially reduce levels of antagonistic co-contraction[15]. In thus it could be expected that balance training will reduce co-contraction in older adults. We note here that many different methods have been used to assess co-contraction in the literature. In the studies mentioned above, the index of co-contraction reflected either the magnitude of antagonistic co-contraction[12] or its magnitude and duration combined[13–15].

Alterations of the H-reflex indicate an adjusted motoneuron output after processing of Ia afferent input at the spinal cord[16]. With age, postural modulation of H-reflexes is reduced[13,17–19] and this may be functionally related to a declined balance performance in older adults[20]. Balance training in young adults has been reported to decrease the soleus (SOL) H-reflex[21–24]. While both young and older adults are capable to down-train the SOL H-reflex[25], it is unclear whether balance training also causes such down-regulation of the H-reflex in older adults. Only a single study investigated H-reflex changes with training in an older population and found no effects of training[26].

In addition to single pulse H-reflex assessment, a double pulse technique, paired reflex depression (PRD), provides insight in the reflex activation history, and as such, the strength of peripherally induced pre-synaptic inhibition[27–29]. The second H-reflex is assumed to be influenced by the synchronous activation of the spindle's afferents during the first H-reflex. Using PRD, the influence of primary spindle afferent feedback and therefore, activation history of the Ia afferents on the motoneuron pool output can be studied [27]. Among middle aged adults (~44 years), subjects with long-term Tai Chi practice showed better balance performance and larger PRD (more H-reflex depression) from supine to standing position compared to the untrained individuals[30]. These authors assumed that a reduced second H-reflex avoids overcorrection and prevents unwanted oscillations. Hence, increased PRD might be expected as a result of balance training.

The aims of the present study were twofold; first, we aimed to assess the functional benefits of short- and long-term balance training in older adults. To do so, we assessed changes in balance robustness (as the duration that participants were able to keep their body balanced while surface stiffness was decreased) and balance performance (measured as the mean absolute value of the center of mass velocity during unipedal balancing). Second, we aimed to explore the associations between the changes in balance robustness and balance performance with co-contraction duration, H-reflex gain and PRD after short-term and long-term training.

We hypothesized that balance robustness and performance would be improved significantly after long-term training, and that such improvements would be accompanied by changes, such as decreased co-contraction duration, lower H-reflex gains and stronger PRD.

Methods

Participants

Twenty-two healthy older adults (11 females, age: 72.6 ± 4.2 years, length: 1.71 ± 0.09 m, weight: 75.6 ± 13.3 kg; mean ± SD) participated in this study. Required sample size was fifteen based on power analysis for an F test of a within factor repeated measure, assuming an effect size of 0.44[4] and correlation among repeated measures of 0.1 ($\beta = 0.8$, G*power 3.1.9.2, Düsseldorf, Germany). To ensure participant safety and data reliability, exclusion criteria included: an inability to stand and walk for 3 minutes without walking aid, cognitive impairments (MMSE < 24), depression (GFS > 5), obesity (BMI > 30), orthopedic, neurological, and cardiovascular disease, use of medication that affects balance, and severe auditory & visual impairments. To prevent ceiling effects in balance robustness and performance and limited training gains, participants practicing sports that explicitly include balance components (e.g. Yoga, Pilates) were excluded as well[31]. To prevent obscuring any training effects, participants were asked to keep their normal activity levels in their daily life throughout the experiment. All participants provided written informed consent prior to participation and the experimental procedures were approved by the ethical review board of the Faculty of Behaviour and Movement Sciences, Vrije Universiteit Amsterdam (VCWE-2018-171).

Experimental procedures

The protocol included an initial measurement session to determine baseline state (Pre), a single-session (short-term) balance training (30 min), a second measurement (Post1), a (long-term) balance training program (9 training sessions, 45 minutes per session), and a third measurement (Post2). The protocol was concluded with a Retention assessment two weeks later. The Pre-measurements, the 30-min training session, and the Post1 measurements were performed on the same day. The measurements consisted of blocks of tests after the familiarization in the following order: *assessment of balance robustness*, *baseline electromyography measurement (EMG, only at Pre and Post2), assessment of H-reflex*, and a series of *unipedal balance performance tests*. During the assessments of the H-reflex and the series of unipedal balance *performance* tests, kinematic and EMG data were recorded. The Retention measurement consisted solely of the assessment of balance robustness (see Figure. 1 for an overview).

Instrumentation and data acquisition

For all unipedal tasks, a custom-made balance platform controlled by a robot (HapticMaster, Motek, Amsterdam, the Netherlands) was used. This platform can rotate 17.5° to either direction in the frontal plane. The rotation of the platform can be controlled by the robot, simulating a tunable stiffness and damping or applying position-control. For safety reasons, the balance platform was equipped with bars in front and on both sides of the participant, and there was ample space to step off the rotating part of the platform (Figure. 2).

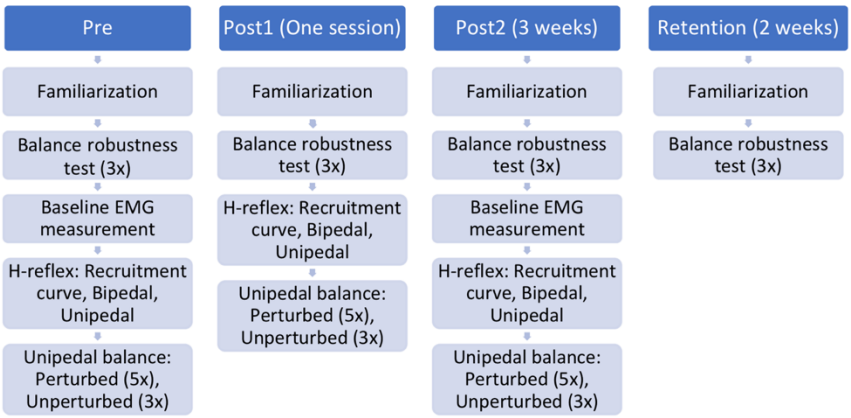

Figure 1. Diagram illustrating the experimental procedures.

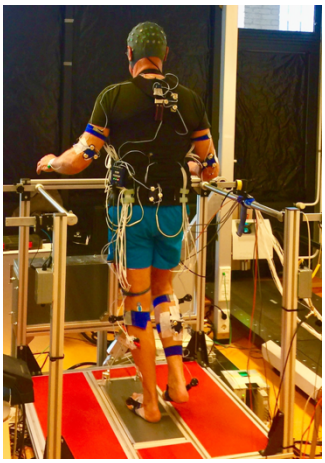

Figure 2. Participant in unipedal stance on the robot-controlled balance platform.

Surface EMG data were collected from three muscles on the preferred stance leg: m. tibialis anterior (TA), m. peroneus longus (PL) and m. soleus (SOL). Bipolar electrodes were placed in accordance with the SENIAM recommendations[32]. The EMG signals were sampled at 2000 Hz and amplified using a 16-channel TMSi Porti system (TMSi, Twente, The Netherlands). The baseline EMG was measured during unipedal stance on a rigid surface. The preferred stance leg was reported by the participant prior to the experiment and confirmed by the experimenter by asking the participant to kick an imaginary soccer ball. The supporting leg was considered the preferred stance leg.

Kinematic data were obtained from 8 active marker clusters containing 3 markers each, placed on the posterior surface of the thorax (1), pelvis (1), arms (2), calves (2), and feet (2). The trajectories of these clusters were tracked by one Optotrak camera array (Northern Digital, Waterloo, Canada). A kinematic model of the participant was formed by relating the cluster positions to anatomical landmarks in an upright position, using a four-marker probe[33].

To elicit the H-reflex in the SOL, the tibial nerve was stimulated using an electrical stimulator (Digitimer, DS7A UK). A large diameter anode, roughly 6 × 9 cm constructed of aluminum foil and conducting gel, was fixed on the patella of the standing leg[34]. The cathode was placed over the tibial nerve in the popliteal fossa of the same leg. The optimal cathode position was determined in each subject by probing the popliteal fossa and delivering 5-10 mA stimulations to find the location that resulted in the largest SOL H-reflex amplitude ~25 ms after stimulation.

Balance robustness

Unipedal balance robustness was assessed using the balance platform. First, participants were familiarized with standing on the platform on their preferred leg for two trials. In the first familiarization trial, the platform imposed ten 8° rotational perturbations at a rate of 16°/s in random direction and returned to horizontal state, every 3 s, to familiarize the subjects with perturbed unipedal balancing. For tests with varying stiffness, the rotational stiffness of the platform was normalized to percentage of mgh (body weight multiplied by center of mass height) of each participant, to factor out differences in participant height and mass. In the second familiarization trial, the platform was set at a stiffness of 100% mgh for 30 s. After familiarization and rest, the participants had to stand on their preferred leg until balance loss occurred, while the stiffness of the platform decreased stepwise every 5 s, asymptotically approximating 0 Nm/rad at the maximum trial duration of 100 s (see EQ. 1, Figure. 3). The time an individual could stay balanced without grabbing the bar or putting down the other foot, was used to assess balance robustness. This was repeated three times, with ample rest (2-5 minutes) in between, and results were averaged.

$$\begin{aligned} &for\ time(T) = [5 * T : 5 * (T+1)];\ [\mathrm{s}] \\ &\mathrm{Stiffness(T)} = \frac{100}{\sqrt{2^T}} * mgh;\ \frac{N.m}{rad} \\ &T = 0,1,2,\ldots,n\ ;\ n \in \mathrm{Z} \end{aligned} \qquad \text{(EQ. 1)}$$

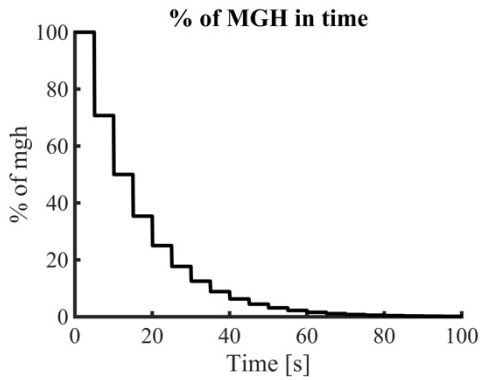

Figure 3. The duration of balancing in [s], and corresponding stiffness as a function of mgh (body mass times gravity times the height of the body center of mass) and time.

Unipedal balance tasks

To measure the duration of co-contraction and balance performance, one unipedal trial on a flat rigid surface as a baseline measurement and 2 unipedal balance tasks on the robot-controlled platform were performed: an unperturbed and a perturbed task. In the unperturbed task, the stiffness of the platform was set at a constant value. To normalize task difficulty to balance robustness, this value was set at 1.3 times the stiffness at which balance loss occurred during the assessment of balance robustness in the Pre-measurement. This task was repeated three times with two minutes rest between trials. In the perturbed task, twelve perturbations were imposed by the platform in the form of mono-phasic sinusoidal rotations either in medial or lateral direction (amplitude of 8°, angular speed of 16°/s). The perturbation direction was randomized and the inter-perturbation duration was randomly selected between 3-5 s. This task was performed five times with two minutes rest in between trials.

H-reflexes and Paired Reflex Depression

Assessment of the H-reflex consisted of three parts: determining the recruitment curve to find Hmax and Mmax, measuring the H-reflex and PRD in bipedal stance, and measuring H-reflex and PRD in unipedal stance, with the intensity of the stimulator set at Hmax. To obtain

the recruitment curve, participants were subjected to low-amplitude (~5 to ~120 mA) electrical stimuli. Participants were instructed to stand still bipedally, with the feet placed at shoulder width, arms besides their body, and to focus on a target in front of them. Subsequently, 1 ms single square pulses with a minimum 4 s inter-stimulus duration were delivered to the tibial nerve at increasing amplitudes to elicit H-reflexes in the SOL and EMG data were recorded. Hmax is the maximum peak-to-peak amplitude of the SOL EMG, between 25 and 50 ms post stimulation, and Mmax is the maximum peak-to-peak amplitude of SOL EMG between 0 and 25 ms post stimulation.

Subsequently, H-reflex and PRD were assessed in two stance conditions[35]. In these conditions, participants were subjected to ten double-pulse stimulations of the tibial nerve. Here, inter-pulse duration was 100 ms, inter-train duration was randomized between 4-8 s, and stimulation intensity was set to the level that previously elicited the Hmax. This stimulation protocol was delivered once in stable bipedal stance and once in unipedal stance on the balance platform, with the stiffness set at 100% *mgh*.

Balance training

In the first session, the participants were trained individually. The nine sessions of the 3-week training program took place in a group setting (6-8 participants). The training program was designed based on previous studies that reported improved balance and reduced fall-risk [36,37]. All training sessions were supervised by a physical therapist who ensured that the sessions remained safe, yet sufficiently challenging for all the participants. The difficulty of the exercise was manipulated by: reducing support (e.g. hand support, two-legged stance, unipedal stance), using unstable objects with varying degrees of freedom and stability, adding motor and cognitive tasks (e.g. catching a ball or passing it in changing directions), and reducing sensory information (e.g. visual fixation or eyes closed). Each session started with a short warm-up. Solely standing balance exercises, focusing on unipedal stance, were included in the training program[38]. Group training sessions were 15 minutes longer than individual training sessions. Extra time was required to switch the devices between the training partners in the exercises with equipment.

Data analysis

Balance robustness

The duration the participant maintained balance, averaged over three trials, served to assess the individual's balance robustness.

Balance performance

The trajectory of the center of mass (CoM) was estimated from a full body kinematic model[39]. Balance performance was expressed as the mean absolute center of mass velocity in the mediolateral direction (vCoM).

Co-contraction index (CCI)

Antagonistic co-contraction is the concurrent activation of antagonistic two muscles. It can be expressed as the duration, magnitude or both duration and magnitude of concurrent activation. Co-contraction was derived from three muscle pairs: SOL/TA, TA/PL and SOL/PL. EMG data were high-pass (35 Hz, bidirectional, 2nd order Butterworth) and notch filtered (50 Hz and its harmonics up to the Nyquist frequency, 1 Hz bandwidth, bidirectional, 1st order Butterworth). Subsequently, the filtered data were rectified using the Hilbert transform and low-pass filtered (40 Hz, bidirectional, 2nd order Butterworth). Finally, we determined the percentage of data points during the perturbed and unperturbed tasks at which both muscles in a pair exceeded the mean muscle activity of baseline unipedal stance. Since for Pre and Post1 time-points the measurements were performed on the same day, the same unipedal trial was used as a reference for these two time-points.

H-reflexes and Paired Reflex Depression

H-reflex gain and PRD were derived from the high-pass filtered (10 Hz, bidirectional, 2nd order Butterworth) EMG activity of the SOL. The H-reflex gain (EQ. 2) was calculated as the mean, over all pulse trains.

$$Hreflex\ gain\ = \frac{H_1}{bEMG} \qquad \text{(EQ. 2)}$$

where H1 was the maximum peak-to-peak amplitude ~25 ms after the first stimulus of the paired-pulse train and bEMG was the root-mean-square value of the EMG activity over the 100 ms prior to the pulse train. PRD was quantified as the mean relative depression of the second H-reflex relative to the first one (EQ. 3).

$$PRD\% = \frac{(H_2 - H_1)}{H1} * 100 \qquad \text{(EQ. 3)}$$

Statistics

A one-way repeated measures ANOVA was used to test the main effect of time-point (Pre, Post1, Post2, Retention) on balance robustness. Post-hoc comparisons (paired sample t-tests) were performed to investigate the effect of short-term training (Pre vs Post1), long-term training (Pre vs Post2), and retention (Pre vs Retention). In addition, Post1-Post2 and Post2-Retention were compared to obtain insight into the changes over the short- and long-term and in retention.

Two-way repeated-measures ANOVAs were used to identify main effects of time-point (Pre, Post1, Post2) and condition (perturbed/unperturbed or bipedal/unipedal) on vCoM, CCI, H-reflex gain and PRD. When the assumption of sphericity was violated, the Greenhouse-Geisser method was used. Post-hoc analyses (paired samples t-test) were performed to investigate the effect of short-term (Pre vs Post1) and long-term (Pre vs Post2) training when a main effect of Time-point or an interaction of Time-point x Condition was observed. For all post-hoc analyses, Holms' correction for multiple comparisons was applied.

Balance performance and the response to training are heterogeneous in older adults[4]. Therefore, cross-sectional and longitudinal correlation analyses were performed to gain more insight into which (changes in) co-contraction, H-reflexes and PRD were related to (changes in) balance robustness and balance performance. As cross-sectional analyses, the correlations between balance robustness (duration) and CCI (averaged over perturbed and unperturbed trials) for all muscle pairs, H-reflex and PRD were calculated. Moreover, the correlations between balance performance (vCoM) and the CCI for all muscle pairs in perturbed and unperturbed trials, and between balance performance (vCoM) and H-reflex gains and PRD during unipedal and bipedal stance were calculated for the three time-points. For longitudinal analyses, the correlations between changes in the same parameters after short- and long-term training were calculated. In view of outliers, Spearman's correlation (r) coefficients were calculated. In all statistical analyses α=0.05 was used.

Only in balance robustness all participants were included in analysis. For all other analyses twenty-one participants were included because one participant was not able to fully perform the balance performance trials.

Results

Balance robustness

Balance robustness (duration of balancing) increased as a result of balance training ($F_{1.955,41.060} = 10.637,\ p < 0.001$). The mean duration of balancing increased after short-term training ($\mathrm{t} = 3.325,\ p = 0.006$, Figure. 4). While, the duration remained unchanged between Post1-Post2 and Post2-Retention ($\mathrm{t} = -1.257,\ p = 0.427;\ \mathrm{t} = -0.57,\ p =$

0.571, respectively; Figure. 4), long-term training and retention showed higher robustness than Pre time-point ($t = -4.582$, $p < 0.001$; $t = -5.151$, $p < 0.001$, respectively; Figure. 4). Overall, these results indicate a rapid improvement in balance robustness after only one session of training, with no further improvement after the subsequent nine training sessions.

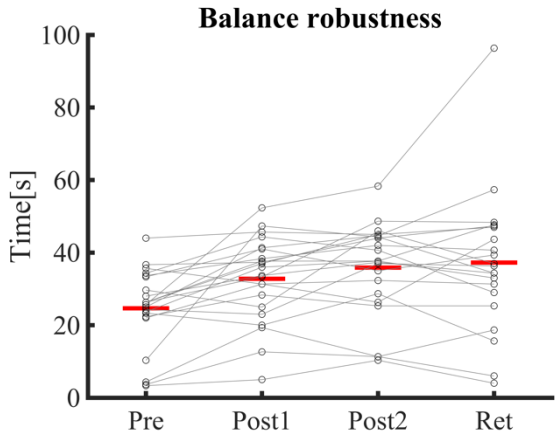

Figure 4. Balance robustness at different time-points, expressed as the duration of maintaining balance under gradually decreasing surface stiffness.

Balance performance

Perturbed and unperturbed

Balance training led to an increase in balance performance (i.e. decreased vCoM, Figure 5, Time-point effect, $F_{1.396,27.921} = 8.106$, $p = 0.004$). Participants showed larger vCoM in perturbed compared to unperturbed standing (Figure 5.a & 5.b, Condition effect, $F_{1,20} = 56.229$, $p < 0.001$). Additionally, there was a significant interaction of time-point and condition on vCoM $(F_{2,40} = 5.264,\ p = 0.009)$. Post-hoc analysis showed that short-term training decreased vCoM in the perturbed condition, but did not change vCoM in the unperturbed condition ($t = 3.178$ $p = 0.021$ and $t = 1.263$, $p = 0.851$, respectively). On the other hand, long-term training, changed vCoM significantly in both perturbed and unperturbed condition ($t = 4.616$, $p < 0.001$; $t = 2.981$, $p = 0.031$; respectively; Figure 5.a & 5b), even though there were no significant changes in vCoM between Post1 and Post2 measurements ($t = 1.439$, $p = 0.783$, and $t = 1.718$, $p = 0.553$, in perturbed and unperturbed conditions respectively; Figure. 5. a & 5. b).

Bipedal and unipedal (H-reflex trials)

In one participant at time-point Pre, during the bipedal H-reflex measurement, a marker on the left arm was not visible. Therefore, for this participant the arms were excluded in calculating CoM trajectories. There was a significant effect of Time-point on balance performance ($F_{1.163,23.267} = 5.233$, $p = 0.027$). Participants showed larger vCoM in bipedal compared to unipedal standing (Figure 5.c & 5.d, Condition effect, $F_{1,20} = 63.924$, $p < 0.001$). There was a significant interaction of Time-point x Condition on vCoM $(F_{1.249,24.974} = 6.237,\ p = 0.014)$. Post-hoc analysis showed that vCoM decreased only in the unipedal condition, after the short- and long-term training ($t = 4.101$, $p = 0.001$, $t = 4.147$, $p = 0.001$ respectively), even though there were no significant changes between Post1-Post2 time-points ($t = 0.046$, $p = 1$).

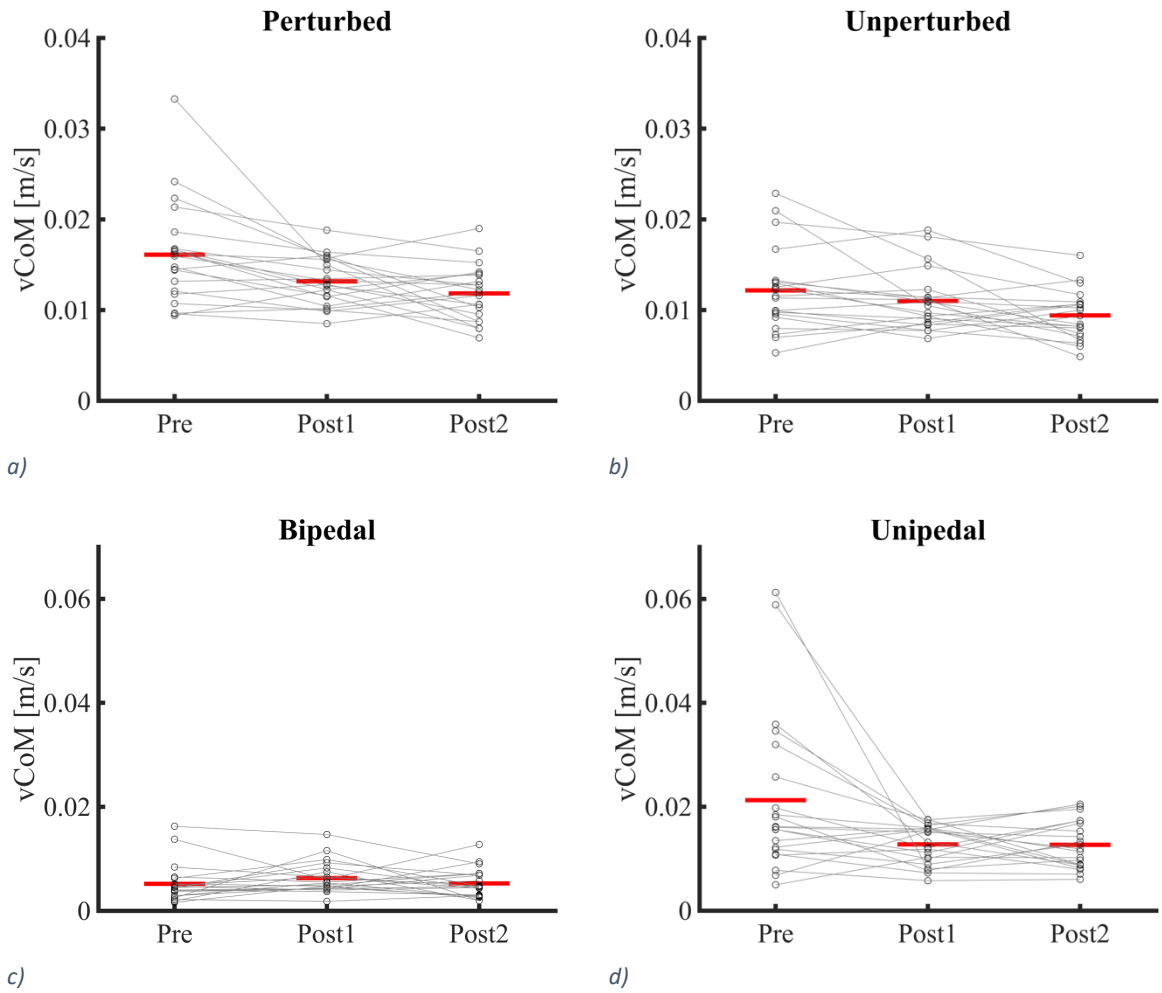

Figure 5. The mean absolute center of mass velocity in mediolateral direction at all three measured time-points a) in the perturbed condition b) in the unperturbed condition c) in H-reflex bipedal stance condition d) in H-reflex unipedal stance condition. Circles and connecting lines represent individual results. The red lines indicate averages across subjects.

Duration of Co-contraction

The CCI of the SOL/TA muscle pair was affected by time-point (Table. 1). Post-hoc comparison showed that the CCI was not changed after the short-term but had increased after long-term training ($t = 1.623, p = 0.112$; $t = -2.372, p = 0.045$ respectively; Figure 6 a). No effects of Time-point and Condition, nor an interaction were observed for the other muscle pairs (Table. 1). Overall our results showed no changes in SOL/TA CCI after short-term training but an increased SOL/TA CCI after long-term training.

Table 1. Results of repeated-measures ANOVA of the duration of co-contraction of three muscle pairs, in perturbed and unperturbed standing at three Time-points of Pre, Post1 and Post2. Bold numbers indicate a significant effect.

Paradigm	Muscles	Time-point			Condition			Time-point*Condition		
		df	F	p	df	F	p	df	F	p
CCI	SOL TA	**1.512, 30.242**	**8.073**	**0.003**	1,20	0.416	0.526	2,40	0.298	0.726
	TA PL	1.148, 22.952	1.285	0.275	1,20	0.005	0.944	1.567, 31.336	1.018	0.370
	SOL PL	1.08883,37.665	0.522	0.492	1,20	0.616	0.441	2,40	0.762	0.473

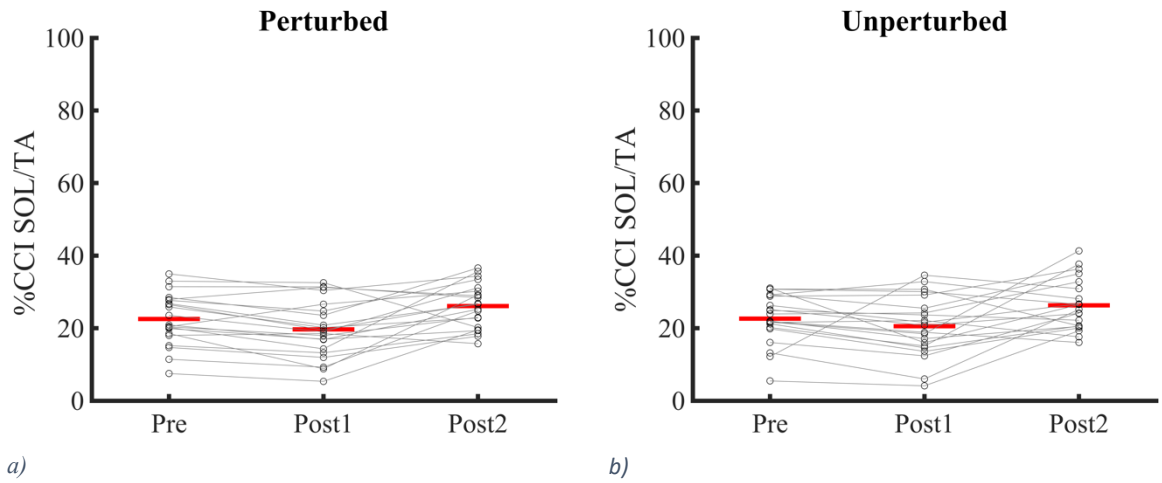

Figure 6. Co-contraction index at three time-points in a) perturbed and b) unperturbed standing, for the muscle pairs SOL/TA. Circles and connecting lines represent individual results. The red lines indicate averages across subjects.

Reflexes

There was no effect of Time-point, nor an interaction effect of Time-point x Condition on H-reflex gains $\left(F_{1.567,31.344} = 0.467,\ p = 0.585 \text{ and } F_{2,40} = 1.859,\ p = 0.169, \text{respectively; Figure 7}\right)$. H-reflex gains were significantly higher in bipedal compared to unipedal stance ($F_{1,20} = 26.549,\ p < 0.001$). Similarly, there was no effect of Time-point, nor an interaction effect of Time-point x Condition, on PRD $\left(F_{2,40} = 1.043\ p = 0.360 \text{ and } F_{2,40} = 0.204,\ p = 0.802, \text{respectively; Figure 8}\right)$, but PRD was stronger in bipedal compared to unipedal stance ($F_{1,20} = 39.613,\ p < 0.001$). Overall our results did not show any changes in the reflexes as a result of training.

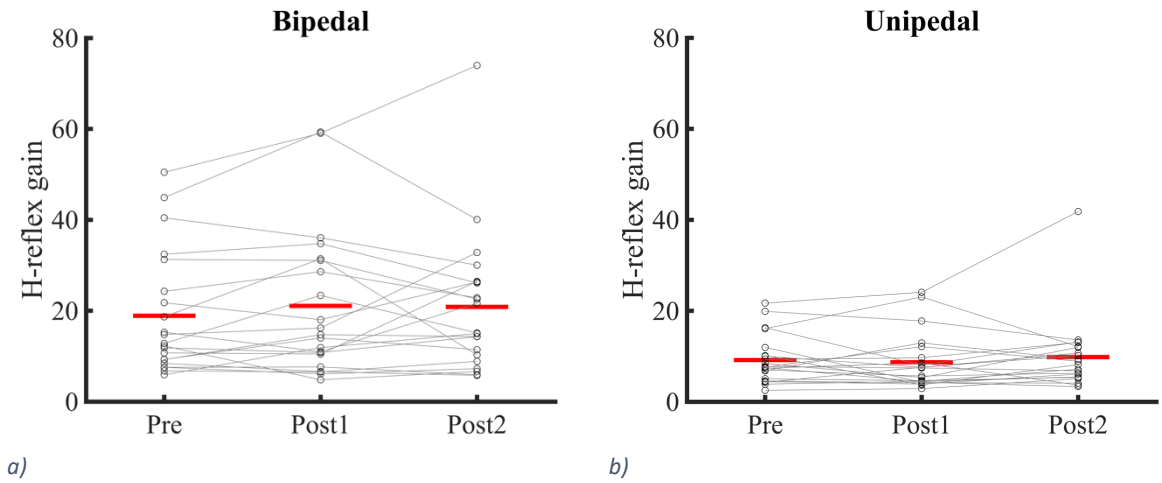

Figure 7. H-reflex gains at three time-points a) shows the reflex gain for the bipedal condition b) shows the reflex gain for the unipedal condition. Circles and connecting lines represent individual results. The red lines indicate averages across subjects.

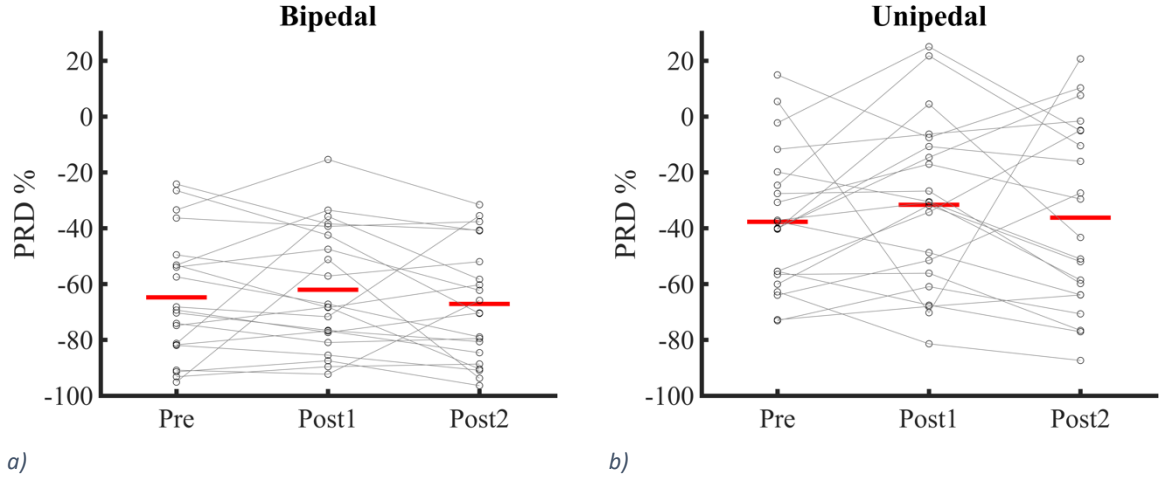

Figure 8. Paired reflex depression at three time-points. The paired reflex depression is displayed for a) the bipedal condition, and b) unipedal condition. Circles and connecting lines represent individual results. The red lines indicate averages across subjects.

Associations of balance robustness with co-contraction and reflexes

All correlation results are shown in Tables 2 and 3. For co-contraction, the average of the perturbed and unperturbed SOL/TA CCI was positively correlated with balance robustness at time-point Post2 ($r = 0.564, p = 0.007$). No correlations were observed between changes after short- or long-term training. For reflexes, H-reflex gains in unipedal stance were negatively correlated with balance robustness (duration) at time-point Post2 ($r = -0.585, p = 0.005$). No correlations were observed between changes after short- or long-term training.

Table. 2 Results of the correlational analysis between co-contraction (averaged over perturbed and unperturbed trials), reflexes in bipedal and unipedal with balance robustness (duration) at each Time-point. Bold numbers indicate a significant effect.

	Pre Balance robustness		Post1 Balance robustness		Post2 Balance robustness	
	r	p	r	p	r	p
CCI TAPL	-0.183	0.425	0.326	0.148	0.037	0.873
CCI SOLTA	-0.015	0.948	0.115	0.617	**0.564**	**0.007**
CCI SOLPL	-0.277	0.221	-0.114	0.621	-0.229	0.317
H-reflex gain Bi	0.193	0.398	-0.183	0.426	0.050	0.827
H-reflex gain Uni	0.183	0.425	-0.044	0.849	**-0.585**	**0.005**
PRD Bi	-0.363	0.105	0.119	0.605	0.114	0.621
PRD Uni	-0.384	0.086	-0.063	0.783	0.113	0.625

Table. 3 Results of the correlational analysis between the changes of co-contraction (averaged over perturbed and unperturbed trials), changes of reflexes in bipedal and unipedal with changes of balance robustness (duration) after short- and long-term training

	Short-term Δbalance robustness		Long-term Δbalance robustness	
	r	p	r	p
ΔCCI TAPL	0.076	0.743	-0.380	0.089
ΔCCI SOLTA	0.302	0.182	-0.013	0.957
ΔCCI SOLPL	0.085	0.713	-0.392	0.079
ΔH-reflex gain Bi	-0.162	0.481	0.015	0.948
ΔH-reflex gain Uni	0.296	0.191	0.102	0.657
ΔPRD Bi	-0.352	0.116	0.053	0.819
ΔPRD Uni	-0.005	0.982	0.100	0.665

Associations of balance performance with co-contraction and reflexes

All correlation results are shown in Tables 4 and 5. For co-contraction duration, at time-point Post1, TA/PL CCI was negatively correlated with vCoM in perturbed standing ($r = -0.448, p = 0.043$), at time-point Post2, SOL/TA CCI was negatively correlated with vCoM in perturbed standing ($r = -0.471, p = 0.032$), and at time-point Pre, TA/PL CCI was negatively correlated with vCoM in unperturbed standing ($r = -0.453, p = 0.040$). Negative correlations indicate that higher duration of co-contraction was associated with better performance (lower sway velocity). No correlations were observed between changes after short- or long-term training (Table. 5).

For reflexes, at time-point Post1, PRD was positively correlated with vCoM in bipedal stance ($r = 0.583, p = 0.006$), indicating that stronger PRD was associated with better performance. No correlations were observed between changes after short- or long-term training (Table. 5).

Table. 4 Results of the correlational analysis between co-contraction with vCoM in perturbed and unperturbed, and between reflexes in bipedal and unipedal with vCoM in bipedal and unipedal stance at each Time-points of Pre, Post1 and Post2. Bold numbers indicate a significant effect.

	Perturbed					
	Pre vCoM		Post1 vCoM		Post2 vCoM	
	r	p	r	p	r	p
CCI TAPL	-0.310	0.170	**-0.448**	**0.043**	0.040	0.863
CCI SOLTA	-0.411	0.064	-0.276	0.223	**-0.471**	**0.032**
CCI SOLPL	0.292	0.198	-0.053	0.819	0.154	0.501
	Unperturbed					
	r	p	r	p	r	p
CCI TAPL	**-0.453**	**0.040**	-0.274	0.228	0.168	0.462
CCI SOLTA	-0.268	0.237	-0.285	0.208	-0.189	0.408
CCI SOLPL	0.277	0.221	0.194	0.395	0.288	0.204
	Bipedal					
H-reflex gain Bi	0.066	0.775	-0.137	0.550	0.310	0.170
PRD Bi	-0.375	0.094	**0.583**	**0.006**	-0.275	0.226
	Unipedal					
H-reflex gain Uni	0.284	0.210	-0.009	0.970	0.185	0.418
PRD Uni	-0.045	0.845	0.305	0.178	-0.088	0.702

Table. 5 Results of the correlational analysis between changes of co-contraction with changes of vCoM in perturbed and unperturbed, and changes of reflexes in bipedal and unipedal with changes of vCoM in bipedal and unipedal stance after short- and long-term training

	Short-term ΔvCoM		*Long-term ΔvCoM*	
	r	*p*	*r*	*p*
	Perturbed			
ΔCCI TAPL	*0.032*	*0.890*	*-0.263*	*0.247*
ΔCCI SOLTA	*0.087*	*0.707*	*0.041*	*0.858*
ΔCCI SOLPL	*0.172*	*0.452*	*0.077*	*0.736*
	Unperturbed			
ΔCCI TAPL	*-0.002*	*0.993*	*0.022*	*0.925*
ΔCCI SOLTA	*0.306*	*0.176*	*0.085*	*0.711*
ΔCCI SOLPL	*0.374*	*0.095*	*0.203*	*0.373*
	Bipedal			
ΔH-reflex gain Bi	*-0.367*	*0.101*	*0.347*	*0.124*
ΔPRD Bi	*-0.115*	*0.616*	*-0.386*	*0.085*
	Unipedal			
ΔH-reflex gain Uni	-0.414	*0.063*	*0.234*	*0.306*
ΔPRD Uni	0.144	*0.531*	*-0.039*	*0.868*

Discussion

We investigated the functional benefits and neural mechanisms associated with functional benefits of short- and long-term balance training in older adults. We found that only one session of balance training increased older adults' balance robustness. Extra training sessions did not

further improve but maintained the acquired robustness. In addition, balance performance in perturbed unipedal balancing was improved after only one training session, again with no further improvement over subsequent training sessions. Performance in unperturbed unipedal balancing, significantly improved over the long-term training period, in line with previous studies[4].

In terms of challenge, the perturbed balance performance test and also the unipedal test during H-reflex stimulation can be considered intermediate to the unperturbed balance test and the test for robustness. We suggest that robustness and perturbed performance outcomes are mainly limited by the ability to deal with near balance loss, while the unperturbed balance test reflects the ability to minimize sway in a situation where balance loss is not likely to occur. The fast changes in the ability to recover balance would be in line with results on perturbation training[7,8]. Overall, this suggests that balance training can increase robustness rapidly, while long-term training refines balance performance and maintains the acquired balance robustness and performance. Given the functional relevance of balance robustness, this finding would put into question the predominant use of balance performance in conditions with a low challenge as outcome measures of training. We note here that balance performance during bipedal standing was not affected by training.

Contrary to our hypothesis, co-contraction was not decreased after balance training; short-term training did not change the co-contraction duration and long-term training even led to an increased co-contraction duration of SOL/TA. Moreover, cross-sectional correlation analysis showed higher co-contraction duration was correlated with a higher balance robustness and performance. Co-contraction may be an adaptation and training could reduce the need for it - older adults show more co-contraction than young adults[14]. But, training also could increase the use of this adaptation. Co-contraction of antagonistic muscles has been shown to increase joint stiffness and serve a zero-delay corrective response to unexpected disturbances in challenging motor tasks[40]. In addition, co-contraction may reduce electromechanical delays by pre-tensioning tendons and as such improved feedback control[41] and co-contraction may improve feedback response by allowing dual control of agonist and antagonistic muscles[42]. Therefore, older adults may increase co-contraction to enhance balance control. However, longitudinal analysis did not show any correlation between the changes in co-contraction duration and changes in balance robustness or performance. Therefore, it seems that increased co-contraction duration is not the mechanism underlying improved balance after short- or long-term training. Possibly, training causes some individuals to use co-contraction more, whereas it reduces the need for co-contractions in others.

Also, in contrast with our hypothesis, neither short-, nor long-term training affected H-reflex gains or PRD. In line with previous studies[13,43,44], H-reflex gains decreased when going from bipedal to unipedal stance. This has been suggested to help in dealing with the higher postural demand of unipedal stance[45], where monosynaptic stretch reflexes may fail to contribute to maintenance of balance. However, we found stronger PRD in bipedal than unipedal stance. It has been suggested that the inhibitory effect of the first H-reflex stimulus is less when more background afferent discharge is present, which could explain the difference between unipedal and bipedal stance[27]. Alternatively, PRD may be affected by descending pathways projecting onto spinal interneurons, resulting in a larger second H-reflex (less depression) in unipedal compared to bipedal stance[46]. Functionally this decreased depression could act to facilitate responses to external perturbation, but this would be at odds with the decreased gain of the first H-reflex. Cross-sectional analyses showed that, in unipedal stance, smaller H-reflex gains were correlated with higher balance robustness, and stronger PRD in bipedal stance correlated with better balance performance. Longitudinal correlational analyses did not show any significant correlation between the neuromuscular mechanisms and the performance or the robustness. All in all, these data support that lower excitability in response

to type 1a afference and stronger suppression of responses to such input is beneficial for balance control, in line with outcomes of studies in middle-aged adults[30], but changes in H-reflex sensitivity or depression do not appear to account for the effect of training.

Limitations

Since multiple randomized controlled trials have shown the efficacy of balance training in older adults, the present study was done without a control group[4]. This implies however, that we cannot exclude that some of our findings were due to repeated testing, which in itself could be seen as a form of training. The finding that balance robustness did not drop two weeks after the last training session and hence five weeks after initial testing indicates that the improvement was a result of learning. Second, our hypothesis that co-contraction duration will decrease after the balance training in older adults, was based on the findings from our previous study, where we found higher co-contraction duration in older adults compared to younger adults. Hence, we used the method presented in the current study comparable with our first study, which takes the duration of co-contraction as a percentage of when muscles are active, as determined from a reference activation. This EMG baseline measurements itself could be influenced by training, and higher co-contraction duration after the long-term training resulted in our study could be simply due to lower baseline measurement at Post2 Time-point. Also, co-contraction could be an adaptation mechanism and training could reduce or increase it, considering the impairment and the task. Third, for reflex measurements it is generally recommended to elicit H-reflex between 15-40% of Mmax [47,48], while we elicited H-reflex at Hmax, in line with our previous study. However, for 20 out of 22 participants Hmax was less than 40% of Mmax (see supplementary materials). Lastly, we calculated a large amount of correlations, and did not apply a correction for multiple testing while doing so. Hence, our results should be considered as explorative, and future, confirmative studies should be undertaken to confirm our findings.

Perspective

Previous studies showed improved balance performance as a result of balance training in both young and older adults[4,6,49]. In young adults improved balance control has been shown to be accompanied with decreased H-reflexes[24] and decreased co-contraction[15]. In older adults, the mechanisms underlying improvements in balance performance and robustness after the training remain unclear. Our results indicate that short-term training improves balance robustness, while long-term training led to a better balance performance with no further improvement in but potentially contributing to retention of balance robustness. The underlying neural mechanisms studied here (i.e., co-contraction duration, H-reflex gain and peripherally induced inhibition measured with PRD) were not exclusive mechanisms underlying short- or long-term balance improvement.

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