**Retention of gait stability improvements over 1.5 years in older adults: effects of perturbation exposure and triceps surae neuromuscular exercise**

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# Abstract

The plantar flexors play a crucial role in recovery from sudden disturbances to gait. The objective of this study was to investigate whether medium (months) or long-term (years) exercise-induced enhancement of triceps surae (TS) neuromuscular capacities affects older adults’ ability to retain improvements in reactive gait stability during perturbed walking acquired from perturbation training sessions. Thirty-four female adults (65±7y) were recruited to a perturbation training group (n=13) or a group which additionally completed 14 weeks of TS neuromuscular exercise (n=21), 12 of whom continued with the exercise for 1.5 years. The margin of stability (MoS) was analyzed at touchdown of the perturbed step and first recovery step following eight separate unexpected trip perturbations during treadmill walking. TS muscle-tendon unit mechanical properties and motor skill performance were assessed using ultrasonography and dynamometry. Two perturbation training sessions (baseline and after 14w) caused an improvement in the reactive gait stability to the perturbations (increased MoS) in both groups. The perturbation training group retained the reactive gait stability improvements acquired over 14 weeks and over 1.5 years, with a minor decay over time. Despite the improvements in TS capacities in the additional exercise group, no benefits for the reactive gait stability following perturbations were identified. Therefore, older adults’ neuromotor system shows rapid plasticity to repeated unexpected perturbations and an ability to retain these adaptations in reactive gait stability over a long time period, but an additional exercise-related enhancement of TS capacities seems not to further improve these effects.

# Introduction

Falls are a leading cause of injuries among elderly populations (Terroso et al. 2014; MacAulay et al. 2015). Approximately one third of older adults fall at least once a year, with the majority of the falls occurring during locomotion, usually as a result of tripping or slipping (Berg et al. 1997; Talbot et al. 2005). Due to a higher life expectancy and the desire for older adults to remain physically active, developing effective intervention strategies for reducing the risk of falls during locomotion should be one of the major aims of health care.

Balance control during human gait can be characterised using an inverted pendulum model, whereby a loss of stability occurs when the extrapolated center of mass exceeds the boundaries of the base of support (Hof et al. 2005). In order to prevent a fall in such an unstable position, the neuromuscular system must execute postural adjustments to control the velocity and position of the center of mass in relation to the base of support and thereby maintain dynamic stability. During gait, such adjustments can be reactive (feedback-driven) or predictive (feedforward-driven) in nature (for example see: Bhatt et al. 2006b; MacLellan and Patla 2006; Arampatzis et al. 2011; Bierbaum et al. 2011a; Bohm et al. 2015). As falls often occur in the anterior direction during walking, it is not surprising that effectively increasing the base of support in response to a sudden external perturbation is vital for regaining dynamic stability (Maki and McIlroy 2006; Hof 2007).

Given the significance of reactive stepping for maintaining stability, it is promising that older adults show improvement in reactive response to various repeated mechanical perturbations (Bierbaum et al. 2011; Pai et al. 2010; McCrum et al. 2017). As well as acute adaptations, short-term (a few weeks) and long-term (up to 12 months) retention of reactive adaptations acquired during a single slip-perturbation training session have been seen in the same laboratory settings in older adults (Bhatt et al. 2006a, 2012; Pai et al. 2014b). Furthermore, long term benefits of reduced falls incidence (up to 50%) have been observed in older adults up to one year following a single session of repeated gait perturbations, indicating that experiencing unexpected perturbations during gait results in task-specific retention in the locomotor system that can be generalized to daily life situations in older adults (Pai et al. 2014a).

Given that the ability to create a large anterior step is important for maintaining dynamic stability during gait, it is not surprising that the age-related general degradation in the neuromuscular capacities of the lower limbs (Aagaard et al. 2010; Csapo et al. 2014; Karamanidis and Arampatzis 2006; Onambele-Pearson et al. 2006; Stenroth et al. 2012) are often linked to reduced locomotor performance (Beijersbergen et al. 2013; Kulmala et al. 2014) and ineffective reactive recovery actions during perturbed gait (Pijnappels et al. 2005a, 2005b; Epro et al. 2015). In particular, insufficient recovery responses following tripping have been related to age-induced deficits in the ankle plantarflexion moment in the push-off phase of human gait (Pijnappels et al. 2004, 2005a, 2005b), indicating to the importance of the TS for the recovery responses in perturbed gait. Furthermore, reduced lower limb muscle-tendon unit capacities in older adults have been associated with a diminished ability to enlarge the base of support, and thereby, to control stability after sudden release from a forward lean position (Karamanidis et al. 2008). These observations suggest that the TS muscle-tendon unit (MTU) mechanical properties in particular, have a crucial role in the recovery response to unexpected gait disturbances and indicate that improving ankle plantarflexor muscle strength and Achilles tendon stiffness, in addition to repetitive exposure to unexpected perturbations may be beneficial for gait stability in older adults.

Exercise interventions with high magnitudes of mechanical strain on the MTUs are known to counteract the age-related deteriorations in skeletal muscles and tendinous tissues (Reeves et al. 2003; Karamanidis et al. 2014; Epro et al. 2017). However, the reductions in muscle activation due to aging coincide with substantial cortical reorganization (Ward and Frackowiak 2003; Ward et al. 2008) and reduced excitability of efferent corticospinal pathways (Eisen et al. 1991; Sale and Semmler 2005), contributing to the loss of spinal motor neurons and to a reduction in motor performance. Furthermore, specific cortical areas are suggested to play an important role in integration of sensory information and stability control during gait in older adults (Bruijn et al. 2014; la Fougere et al., 2010; Koo et al. 2012), meaning that interventions aiming to modify these cortical areas may be beneficial for older adults. In contrast to conventional muscle strength exercise, specific motor skill training has been shown to increase the excitability (and expand) the cortical areas corresponding to the specific muscles involved in the motor task (Pascual-Leone et al. 1995; Lotze et al. 2003; Tinazzi et al. 2003; Perez et al. 2004; Jensen et al. 2005). To our knowledge, no study has examined whether a TS neuromuscular exercise of the lower limbs incorporating both resistance and specific motor skill training would lead to a more effective dynamic gait stability control during perturbed walking in the elderly.

In a recent study (Epro et al. 2017) we were able to increase the TS muscle strength and Achilles tendon stiffness in a group older adults over a time course of 1.5 years, with no changes in the control group. In the current study, we assessed dynamic gait stability during perturbed walking in the same subject groups to determine whether this enhancement of the TS capacities following medium-term (14 weeks) or long-term (1.5 years) exercise would benefit reactive gait stability and if this would have a cumulative effect with retained reactive response improvements made during sessions of perturbations conducted prior to and 14 weeks into the exercise intervention. Based on previous observations, it was hypothesized that dynamic gait stability improvements would be retained by both subject groups, with greater retention at 14 weeks, compared to 1.5 years. The addition of TS neuromuscular exercise was expected to have a cumulative benefit on the reactive response during perturbed walking after 1.5 years. Furthermore, we hypothesized that older adults show a more prolonged transfer (i.e. longer than the typical intervention length of 12-14 weeks) of newly obtained TS capacities to gait due to an overall deterioration of the neuromotor system, represented by only minor or non-existent alterations in gait stability after 14 weeks of exercise.

# Materials and Methods

## *Participants and experimental design*

Thirty-four older female adults [age: 65 ± 7 years; body mass: 65 ± 9 kg; height: 165 ± 6 cm; mean and standard deviation (SD)] from a large-scale knee osteoarthritis study (Kellgren-Lawrence grade of 2-3, but with no pain during locomotion) voluntarily took part in this study. Exclusion criteria were previous Achilles tendon rupture, pain or injury within the last 5 years (e.g. tendinopathy), any neurological or musculoskeletal impairment of the lower limbs (e.g. joint pain during locomotion) or pain during the exercise training which might influence the findings of the current study. The participants were generally healthy and representative for their age group (with an average outcome of the SF-36 general health questionnaire of 73.5%; mean single leg stance time of 39.6 s out of maximal 45 s test duration; average timed up and go test result of 7.2 s). The subjects received medical clearance from their general practitioners prior to all measurements and the training program. All participants provided their written informed consent after being informed about the procedures and possible risks of the study. Thirteen participants (age: 66 ± 8 years; body mass: 64 ± 9 kg; height: 165 ± 5 cm; mean and SD) were assigned to the dual-session perturbations training (GAIT) group and the remaining twenty-one participants (age: 65 ± 7 years; body mass: 66 ± 10 kg; height: 165 ± 8 cm; mean and SD) formed an exercise group (GAIT+NM), which, in addition to the perturbations, completed a TS neuromuscular exercise intervention (resistance and motor skill training). The study was approved by the responsible ethics committee of the German Sport University Cologne (ethical approval number 026/2013) and met all requirements for human experimentation in agreement with the Declaration of Helsinki.

In order to examine whether experimentally enhanced TS capacities would further improve the ability to recover balance during perturbed walking in older adults, in addition to improvements made through perturbation experience, a longitudinal exercise intervention was implemented to increase TS muscle strength and Achilles tendon stiffness, and to improve TS specific motor skills over a time period of 1.5 years. Before (Pre), after 14 weeks (Post 14 weeks) and after 1.5 years (Post 1.5y) of TS neuromuscular exercise intervention, each participant was analyzed on two separate days in order to acquire the following main outcome measures: (i) dynamic stability (specifically the margin of stability) during perturbed and unperturbed walking and (ii) the TS neuromechanical properties (TS muscle strength, Achilles tendon stiffness and TS specific motor skill performance).

## *Analysis of dynamic stability following unexpected gait perturbation*

Dynamic stability was examined by applying a trip perturbation during treadmill walking via manually controlled custom-built pneumatic brake-and-release system (Fig. 1), similar to the magnetic system described in previous studies (Süptitz et al. 2013; McCrum et al. 2014). A resistance (creating approximately 55 N) was applied to and removed from the lower right limb during the swing phase via an ankle strap and Teflon cable (Fig. 1). The protocol began with subjects walking at 1.4 m·s-1 on a motor-driven treadmill (pulsar 4.0, h/p/cosmos; Nussdorf-Traunstein, Germany) while wearing the ankle strap, and a safety harness connected to an overhead frame (Fig. 1). After four minutes of walking (Karamanidis et al. 2003), a baseline measurement (non-perturbed walking over 20 stride cycles) was recorded, from which six consecutive strides were used to determine the baseline (Base) of the parameters of interest. Following this baseline measurement, the gait perturbation was applied (Fig. 2). The subjects were not informed about the onset or removal of the resistance, but were aware that at some point during walking their gait was going to be perturbed. The unexpected perturbation was repeated eight times (trials), separated by washout periods of unperturbed walking (varied lengths of about two to three minutes) with the first and eighth trial of the perturbation session at baseline (T1Pre, T8Pre), after 14 weeks (T1Post14w, T8Post14w) and after 1.5 years (T1Post1.5y, T8Post1.5y) being considered for further analysis. We considered only the first and eighth trials simply because these are the most relevant, as they represent the initial and post-perturbation exposure performance of the subjects on each test day (perturbation training). The aim of the study was foremost to describe and examine the retention effects following perturbation training rather than the trial to trial adaptation within the training sessions, and therefore the focus was solely on first and last perturbation trials.

**Insert Figure 1**

In order to examine the dynamic stability during treadmill walking, a motion capture system (120 Hz; Nexus 1.8.2; Vicon Motion Systems, Oxford, UK) was used to trace five retro reflective markers (radius 16 mm) placed at the greater trochanter of the right and left leg, seventh cervical vertebra (C7), left and right forefoot. The 3D coordinates of the markers were smoothed using a fourth order digital Butterworth filter with a cut-off frequency of 20 Hz. Two 2D-accelerometers (1080 Hz; ADXL250; Analog Devices, Norwood, MA, USA) placed on the right and left tibia were used to determine each foot touchdown (TD) based on the tibia peak accelerations (Süptitz et al., 2012). The margin of stability (MoS), a valid method to analyse biomechanical stability during human gait (Bruijn et al. 2013), was calculated in the anteroposterior direction at each foot TD using the extrapolated center of mass approach (Hof et al. 2005), as the difference between the extrapolated center of mass and the anterior boundary of the base of support (anteroposterior position of the toe projection to the ground), adapted for the simplified kinematic model based on Süptitz et al. (2013):

where PTroch is the average anteroposterior component of the projection of the trochanter markers to the ground, vTroch is the average horizontal velocity of the trochanter markers, vC7 is the horizontal velocity of the C7 marker, vBoS the average anteroposterior velocity of the foot markers during stance (approximately equal to the velocity of the treadmill belt), g is the gravitational acceleration, and L is the pendulum length (the average distance between the trochanter and the ankle joint center of rotation of the left and right leg in the sagittal plane). This reduced kinematic model has been previously validated (Süptitz et al. 2013) as appropriate to assess dynamic stability parameters (XCoM and MoS) during unperturbed and perturbed treadmill walking (perturbed and recovery steps) with the same perturbation task and walking velocity as the current study with the same age group of the current study, showing significant correlations (on average r = .90; p < 0.05) with a full body kinematic model across trials.

**Insert Figure 2**

## *Analysis of triceps surae neuromechanical properties*

The mechanical properties of the TS MTU of the preferred leg for step initiation were assessed using ultrasonography and dynamometry synchronously with the participants seated, ankle and knee joints fixed at 90° (thigh and foot perpendicular to the shank) and the foot placed on a custom-made strain gauge dynamometer (1000 Hz; please also see Epro et al. 2017). Inverse dynamics were used to calculate the resultant joint moments acting about the ankle joint, accounting for the gravitational moments using a passive measurement. Following a standardized warm-up (Maganaris et al. 1998), all participants performed six separate maximal voluntary isometric ankle plantarflexion contractions (MVC) with visual feedback and verbal encouragement from the researchers. The first three MVCs (“as strong as possible”) were foremost performed to assess the maximal ankle plantarflexion moment for each participant, whereas the second three were performed as MVC ramp contractions in order to calculate the Achilles tendon force-length relationship. Standardized ramp contractions (3 seconds until maximum plantarflexion moment) ensure that the excursion of the tendon is relatively slow and thereby improve post data processing of the ultrasound image sequences. All six MVC plantarflexion contractions were used to assess the individual TS muscle strength (maximal plantarflexion moment). Achilles tendon force was determined by dividing the resultant ankle joint moment by the individual tendon moment arm. The Achilles tendon moment arm was individually determined using the tendon excursion method (An et al. 1983, 1984; Maganaris et al. 1998) for each participant at the Pre measurement between 85 and 105 degrees ankle joint angles (Epro et al. 2017). The displacement of the myotendinous junction of the m. gastrocnemius medialis during the controlled ramp contractions was analysed using a securely positioned linear array ultrasound probe (29 Hz; MyLabTMFive, Esaote; Genoa, Italy) and by manually digitizing the displacement of the myotendinous junction using video analysis software (Simi Motion 5.0, SIMI Reality Motion System GmbH, Unterschleißheim, Germany). During each contraction, the effect of inevitable ankle joint angular rotation on the obtained tendon elongation was taken into account using a potentiometer positioned under the heel to calculate changes in the ankle joint angle through the inverse tangent of the ratio of the heel lift to the distance between the ankle joint axis (Ackermans et al. 2016; Epro et al. 2017). The Achilles tendon stiffness (mean value of three MVC ramp contractions) was determined as slope of the calculated tendon force and resultant tendon elongation relationship between 50% and 100% of maximum tendon force using linear regression.

In order to assess TS motor skill performance, the participants had to trace different pre-defined functions displayed on a computer screen by controlling a moving circular cursor with precise bilateral ankle plantarflexions, limited to a maximum intensity of 30% MVC. Each function was presented two times with two different cursor movements: time over the function with 80s of continuous tracing (slow) and with 20s of continuous tracing (fast). The measurement was carried out using a custom-made training device described in more detail in the section *“Triceps surae neuromuscular exercise intervention”*. The TS motor skill performance was determined by calculating the mean deviation from the presented function, which was then normalized to the individuals’ bilateral maximal plantarflexion moment (relative mean deviation) at each measurement time point (Pre, Post 14w and Post 1.5y). In order to avoid possible errors through differences in subjects’ attention between the measurement time points, the first and last 5% of the traced data were excluded from the mean and relative mean deviation calculations. For the Pre, Post 14w and Post 1.5y measurement time points four separate functions were used to determine the motor skill performance: two pre-defined functions, which occurred only in these measurement sessions (transfer functions: FunctTR1 and FunctTR2; Fig. 3) and two functions, which were practiced in each training session (constant functions: FunctCT1 and FunctCT2; Fig. 3).

**Insert Figure 3**

## *Triceps surae neuromuscular exercise intervention*

The GAIT+NM group underwent a supervised resistance exercise intervention (approximately 50 minutes each session) three times per week for 14 weeks (except for week 1 and 2, where training was performed twice), after which the exercise was continued twice a week for 1.5 years. Each training session consisted of five sets of four repetitions of isometric plantarflexion contractions for both legs individually and then together (90% of MVC; 3s loading, 3s relaxation), which has shown to increase TS muscle strength, as well as tendon stiffness, in both younger and older adults (Arampatzis et al. 2007; Karamanidis et al. 2014; Epro et al. 2017). The exercise was carried out using eight custom-made training devices each equipped with two custom-made strain gauge type dynamometers (1000 Hz; ankle and knee angle fixed at 90°; Fig. 3) to determine the plantarflexion moments for both legs separately, and a visual feedback system developed with LabVIEW (2013 SP1; National Instruments, Austin, TX, USA). The resultant moments at the ankle joint were determined as described in the previous section (*“Analysis of triceps surae neuromechanical properties”*).

The exercise intervention also incorporated a TS specific motor skill training program intended to provoke greater excitability of the motor cortex areas representing specific muscles using the same devices as for the isometric contractions, which was based on previous work of the research groups of Perez and Jensen (Perez et al. 2004; Jensen et al. 2005). The TS specific motor skill training was carried out by using bilateral ankle plantarflexion contractions with a low intensity (30-45% of MVC), where the participants had to trace four different pre-defined functions displayed on a computer screen with two different cursor movement (20s and 80s of continuous tracing) as described above in the section “*Analysis of triceps surae neuromechanical properties”*. In comparison to the measurement sessions, the two transfer functions were replaced with two further functions, which were varied with each training session accordingly so that all subjects traced the same functions in a given training session (varied functions; Fig. 3). Each training session consisted of two repetitions of four functions, once with both velocities (80s or 20s) with approximately one minute of rest between each set.

For each subject the intensity for every individual training session (90% of MVC for the TS MTU resistance training and 30-45% of MVC for the specific motor skill training) was determined according to the maximal resultant ankle plantarflexion moment (both unilateral and bilateral) performed at the start of each training session, conducted after a standardized warm-up of 2–3 minutes of submaximal isometric contractions. Thereby the training load was adjusted individually at every session and was gradually increased according to the progression made in TS muscle strength over the 1.5 years of exercise intervention. Furthermore, to ensure maximal attention to the tasks, all training sessions were supervised.

## *Statistics*

The GAIT+NM group (n=21) was decreased after 14 weeks of intervention to a subsample of 12 older adults, who continued the exercise for the whole 1.5 years. The GAIT group (n=13) took part at all three measurement time points (Pre, Post 14 weeks and Post 1.5 years). Accordingly, the statistical analyses with the GAIT+NM group were performed once with the 12 subjects completing the long-term intervention (all three measurement time points) and once with the 21 subjects finishing the medium term intervention (first two measurement time points). For better understanding, these subjects are marked as GAIT+NM14w and GAIT+NM1.5y respectively in the text and figures in the following sections. Independent samples T-tests were used to identify possible differences in age, body mass and body height between the formed subject groups. A two-way repeated measures analysis of variance (ANOVA) with the subject group (GAIT, GAIT+NM14w and GAIT+NM1.5y) and time point (Pre, Post 14 weeks and Post 1.5 years) as factors was implemented to identify potential exercise-related effects on TS muscle strength, Achilles tendon stiffness and motor skill performance (relative mean deviations) in the four pre-defined functions (FunctCT1, FunctCT2, FunctTR1 and FunctTR2). Two-way repeated measures ANOVAs with the subject group and trial (T1Pre, T8Pre, T1Post14w, T8Post14w, T1Post1.5y and T8Post1.5y) as factors was used to determine possible acute adaptive improvements and retention of MoS during the recovery response to the unexpected trip perturbation task separately for the perturbed step (TD Pert) and first recovery step after the perturbation (TD Reco1L). For the unperturbed gait (TD Base; assessed always prior T1 as an average of 12 consecutive steps of unperturbed walking with the ankle strap), a further two-way repeated measures ANOVA was implemented with subject group and time point (Pre, Post 14 weeks and Post 1.5 years) as factors. Significant main effects and interactions were further analysed using the Bonferroni post-hoc comparison. The significance level was set at α=0.05, with all results in the text, tables and figures presented as mean and SD. All statistical analyses were conducted using the software Statistica (Release 10.0; Statsoft Inc., Tulsa, OK, USA).

# Results

## *Changes in triceps surae neuromechanical properties*

For the 14 weeks of exercise intervention with 21 older female adults (GAIT+NM14w group), the implemented two-way ANOVA revealed a statistically significant subject group × time point interaction for the maximal ankle plantarflexion moment (F (1, 32) = 17.67, p < 0.05) and Achilles tendon stiffness (F (1, 32) = 6.26, p < 0.05). The Bonferroni post-hoc analysis revealed significant changes in the maximal ankle plantarflexion moment (Pre: 116.4 ± 25.4 Nm vs. Post 14 weeks: 145.8 ± 35.3 Nm; p < 0.001) and Achilles tendon stiffness (Pre: 513.3 ± 183.6 N·mm-1 vs. Post 14 weeks: 614.1 ± 190.1 N·mm-1; p < 0.05) for the GAIT+NM14w group Post 14 weeks in comparison to Pre measurement.

Considering the TS specific motor skill performance, the two way repeated measures ANOVA revealed a statistically significant (10.79 ≤ F (1, 32) ≤ 33.36, p < 0.05) subject group × time point interaction for each analyzed function. *Post hoc* tests found no statistical differences in the relative mean deviation between the GAIT and GAIT+NM14w group at the Pre measurement, regardless of the cursor movement. For the GAIT+NM14w group, we found significantly (p < 0.05) lower relative mean deviations for both velocities (slow: 80s and fast: 20s) after the time period of 14 weeks in comparison to the Pre measurements for all four analyzed functions (slow: on average over all functions Pre 4.1 ± 2.1 % vs. Post 14 weeks 1.2 ± 0.4 %; fast: Pre 4.5 ± 1.8 % vs. Post 14 weeks 1.8 ± 0.5 %; Table 1 and Fig. 4).

Following the 1.5 year exercise intervention with the 12 older female adults (GAIT+NM1.5y group), a subject group × time interaction was revealed for the maximal ankle plantarflexion moment (F (2, 46) = 3.43, p < 0.05) and Achilles tendon stiffness (F (2, 46) = 4.67, p < 0.05). The *post hoc* test revealed a significantly (p < 0.001) higher maximal ankle plantarflexion moment (Pre: 116.3 ± 30.8 Nm vs. Post 14 weeks: 141.5 ± 30.2 Nm vs. Post 1.5 years: 145.9 ± 30.2 Nm) and significantly (p < 0.001) increased Achilles tendon stiffness (Pre: 488.4 ± 136.9 N·mm-1 vs. Post 14 weeks: 598.2 ± 141.2 N·mm-1 vs. Post 1.5 years: 637.1 ± 183.2 N·mm-1) after post 14 weeks and post 1.5 years exercise when compared with baseline values. Furthermore, a subject group × time interaction was revealed for each analyzed function from the TS specific motor skill training (6.70 ≤ F (2, 46) ≤ 28.27, p < 0.05). The following *post hoc* tests detected an improvement compared to baseline in Post 14 weeks and Post 1.5 years measurements in the TS specific motor skill performance, were the GAIT+NM1.5y group showed significantly (p < 0.05) lower relative mean deviation values in comparison to baseline in all four analyzed functions and velocities (slow: on average over all functions Pre 4.1 ± 2.0 % vs. Post 14 weeks 1.1 ± 0.4 % vs. Post 1.5 years 1.0 ± 0.3 %; fast: Pre 4.5 ± 1.8 % vs. Post 14 weeks 1.7 ± 0.4 % vs. Post 1.5 years 1.6 ± 0.4 %; Table 1 and Fig. 4). However, the *post hoc* comparisons did not show any differences in the TS muscle strength, Achilles tendon stiffness and TS specific motor skill performance values between the Post 14 weeks and Post 1.5 years measurement time points, which indicates a maintenance of, rather than further improvements in the analyzed parameters.

**Insert Figure 4**

The GAIT group, who experienced only the two single sessions of perturbations, did not demonstrate any statistically significant differences in maximal ankle plantarflexion moment (Pre: 117.5 ± 28.6 Nm vs. Post 14 weeks: 124.3 ± 29.5 Nm vs. Post 1.5 years: 126.9 ± 28.9 Nm) and Achilles tendon stiffness (Pre: 499.3 ± 121.0 N·mm-1 vs. Post 14 weeks: 463.3 ± 148.2 N·mm-1 vs. Post 1.5 years: 466.1 ± 130.8 N·mm-1) between all three measurement time points. In the baseline (Pre) measurement no statistical differences in maximal ankle plantarflexion moment and Achilles tendon stiffness were found between the subject groups (GAIT, GAIT+NM14w and GAIT+NM1.5y). Similarly, the GAIT group showed no differences over the time points in the TS specific motor skill performance (relative mean deviation) in either cursor movement for the analyzed functions (slow: on average over all functions Pre 3.0 ± 1.0 % vs. Post 14 weeks 2.6 ± 1.0 % vs. Post 1.5 years 2.5 ± 0.8 %; fast: Pre 3.5 ± 0.9 % vs. Post 14 weeks 3.2 ± 0.9 % vs. Post 1.5 years 3.1 ± 0.7 %; Table 1 and Fig. 4). Further, in the baseline (Pre) measurement, no statistical differences in relative mean deviation for the functions FunctCT1, FunctCT2 and FunctTR1 were found between the subject groups. However, the GAIT group showed significantly (p < 0.05) lower relative mean deviation in FunctTR2 at Pre measurement in comparison to the GAIT+NM14w and GAIT+NM1.5y group. In the measurement time points after 14 weeks and after 1.5 years, GAIT+NM14w and GAIT+NM1.5y groups showed significantly (p < 0.05) lower relative mean deviation when compared to the GAIT group. There were no significant differences in subjects’ age, body mass and body height (see “*Participants and experiment design*”) between the GAIT, GAIT+NM14w and GAIT+NM1.5y group.

## *Changes in dynamic stability* *following unexpected gait perturbations*

Regarding the dynamic stability during treadmill walking, the two-way repeated measures ANOVAs revealed no significant subject group (GAIT, GAIT+NM14w and GAIT+NM1.5y) or time point (Pre, Post 14 weeks and Post 1.5 years) effects for the MoS values during the non-perturbed baseline walking (average value over 12 consecutive steps; Fig. 5).

**Figure 5**

Considering the first 14 weeks of exercise, the analysis of MoS at TD of the perturbed and first recovery steps, revealed a statistically significant trial effect for both analyzed steps (F (3, 96) = 12.01, p < 0.05 and F (3, 96) = 5.21, p < 0.05 for TD Pert and TD Reco1L respectively) with no significant trial × subject group interactions. The following *post hoc* test revealed significantly (p < 0.05) higher MoS values at TD Pert and TD Reco1L in the T8Pre, T1Post14w and T8Post14w trials in comparison to T1Pre (Fig. 6 and Fig. 7). However, a significantly (p < 0.05) lower MoS at TD Pert could be determined in the T1Post14w when compared to the T8Pre (Fig. 6). After the second perturbation training session, the MoS at TD Pert was significantly (p < 0.05) higher in the T8Post14w in comparison to the T1Post14w trial. No significant differences in the MoS at TD Reco1L between the T8Pre, T1Post14w and T8Post14w trials were identified.

When considering the subject group undergoing the 1.5 year of exercise with the GAIT and GAIT+NM1.5y groups, the two-way ANOVA revealed a statistically significant trial effect for both, MoS at TD Pert (F (5, 115) = 15.11, p < 0.05) and TD Reco1L (F (5, 115) = 5.63, p < 0.05), with no significant trial × subject group interactions. The *post hoc* tests showed significantly (p < 0.05) higher MoS values at TD Pert and TD Reco1L for T8Pre, T1Post14w, T1Post1.5y and T8Post1.5y trials in comparison to T1Pre (Fig. 6 and Fig. 7). However, a significantly (p < 0.05) lower MoS at TD Pert was identified in T1Post14w in comparison to T8Pre (Fig. 6). After the second perturbation training session, the MoS at TD Pert in the T8Post14w showed a significant increase in comparison to the T1Post14w trial (Fig. 6). Following 1.5 years the MoS at TD Pert was significantly (p < 0.05) reduced in T1Post1.5y compared to T8Post14w and to T8Pre (Fig. 6). Further, the third perturbation training showed a significant (p < 0.05) increment of the MoS at TD Pert in the T8Post1.5y in comparison to the T1Post1.5y trial. No significant differences in the MoS at TD Reco1L were found between the T1Post14w, T8Post14w, T1Post1.5y and T8Post1.5y.

**Figure 6**

**Figure 7**

# Discussion

The current study aimed to determine whether an enhancement of the TS neuromuscular capacities following medium-term (14 weeks) or long-term (1.5 years) exercise intervention would benefit reactive dynamic gait stability and if this would have a cumulative effect with retained reactive response improvements made during sessions of perturbations conducted prior to and 14 weeks into the exercise intervention in older adults. Our hypothesis, that dynamic stability improvements would be retained by older adults over both 14 weeks and 1.5 years after experiencing perturbation training sessions was confirmed, as all groups retained adaptations made in the perturbation sessions. However, the addition of TS neuromuscular exercise was not cumulative with the effects of retention in reactive response during perturbed walking in the intervention group after both the medium-term (14 weeks) and, against our hypothesis, the long-term (1.5 years) exercise time period.

## *Adaptation of reactive dynamic stability during perturbed walking*

The first trial of the initial session of trip perturbations (T1Pre) revealed comparable MoS values at TD Base, TD Pert and TD Reco1L in all three subject groups, thereby indicating that the applied perturbation and task demand was similar with respect to dynamic stability (Fig. 6 and Fig. 7). The current results suggest that older female adults acutely adapted their reactive response following the unexpected perturbation in all perturbation sessions (Fig. 6 and Fig. 7). When combining these results with earlier studies using various perturbations to gait (Bhatt et al. 2006b; Bierbaum et al. 2011; Pai et al. 2010), it can be concluded, that older adults seem to be capable of adapting their reactive recovery behavior in response to repeated exposure to gait perturbations in the anterior-posterior direction. Repetitive exposure to sudden perturbations drives foremost the central nervous system to adapt and cope with unexpected environmental changes. Perturbation training can induce involuntary prediction errors, which cannot be readjusted solely by volitional corrective motor output (Keck et al. 1998; Tseng et al. 2007). Experiencing these kinds of errors is crucial for the central nervous system to reorganize the existing internal representation of the environment (Malone et al. 2012; Shadmehr et al. 2010), providing the necessary basis for learning a motor task, in this case to modify both the predictive and reactive control of gait stability. The reactive control is shown to be mainly influenced by feedback mechanisms, such as spinal reflexes and supraspinal automatic postural responses potentially involving the brainstem, the cerebellum and the cerebral cortex (Jacobs and Horak 2007). Even though aging is associated with several neurodegenerative changes (Dorfman and Bosley 1979; Haug and Eggers 1991; Edström et al. 2007; Park and Reuter-Lorenz 2009), this acquisition of fall resisting skills can still happen rapidly in older adults after a novel perturbation (Pai et al. 2003; Pai et al. 2010). Such locomotor adaptation may depend both on the spinal and supraspinal structures and their plasticity (Wolpaw 2010; Jacobs and Horak 2007). However, the degree to which such central nervous system plasticity may influence the rapid adaptation in the reactive recovery behavior during perturbed walking in older adults (Bhatt et al. 2006b; Bierbaum et al. 2011; Pai et al. 2010; Patel and Bhatt 2015) needs further investigation.

## *Retention of reactive gait stability improvements following perturbation exposure*

For the older population, it is important to determine how long these improvements may be retained. Previous slip-perturbation studies illustrate that the improvements in feedforward control seem to be fully retainable for longer time periods (up to 1 year) after just few perturbation trials, however the retention effect for the reactive response seems to be limited (Bhatt and Pai 2005). Nonetheless, when using more frequent exposure to perturbations in a single training session, the long-term retention of the acquired skills becomes meaningful in the reactive response over a few months (Bhatt et al. 2006a, 2012; Pai et al. 2014b). Furthermore, the inclusion of an additional session (consisting of only one “booster” perturbation trial) can augment the retention effects seen months after performing a single session of perturbation training and can thereby, slow down the decay of newly obtained motor skills controlling gait stability over time (Bhatt et al. 2012). The current study found noticeable retention of the dynamic stability improvements acquired in the perturbation sessions 14 weeks and 1.5 years post-perturbation exposure. However, the improvements in MoS at TD Pert showed a minor but significant decay compared to the last perturbation trial of the first training session (T8Pre), whereas the MoS at TD Reco1L did not show any significant drop. These findings are in accordance with previous studies conducted with slip-perturbations, where older adults have also demonstrated partial retention after few months of the acute rapid adaptation in various stability outcome measures compared with the novel slip with an evident performance decay in comparison to the last training slip (Bhatt et al. 2006a, 2012). After the longer non-exposure period at 1.5 years, the GAIT group demonstrated long-term retention of the improvements made in reactive response. However, interestingly after 1.5 years, contrary to TD Pert, the MoS at TD Reco1L showed again no significant decay in the T1Post1.5y when compared to the T8Post14w. This indicates that the first recovery step may not be characterized as a pure reactive response, as it seems to be dependent of the performance made in perturbed step. This means that the identified superior retention of the MoS at TD Reco1L seems to be a combined effect of the improved perturbed step and the retention itself, as the MoS at TD Pert showed a significant decay in T1Post14w and T1Post1.5y after the non-exposure periods.

Accordingly, it can be concluded, that older adults experiencing two sessions of unexpected trip perturbations are able to retain the acquired improvements in dynamic stability over a time period of 1.5 years. Whether the retention effects can be generalized for daily life situations in elderly adults cannot be answered based on these results obtained in a standardized laboratory-settings. However, in combination with earlier studies it seems to be plausible to suggest this possibility (Bhatt and Pai 2009; Pai et al. 2014a), as long term benefits of reduced falls incidence (up to 50%) have been observed in older adults up to one year following single sessions of repeated with slip-perturbations (Pai et al. 2014a) and inter-task transfer from trained slip-perturbation paradigms to other perturbation settings has been shown (Bhatt & Pai 2009; Yang et al. 2013; Lee et al. 2016).

## *Effects of triceps surae neuromuscular exercise on the retention of reactive gait stability improvements*

The current study included a physical exercise intervention in order to improve TS capacities, as a rapid generation of high plantarflexion moments is proven to be an important factor for successful balance recovery after various perturbations (Pijnappels et al. 2004, 2005a, 2005b; Karamanidis et al. 2008). Furthermore, earlier studies (Grabiner et al. 2005; Karamanidis et al. 2008) and our recent findings (Epro et al. 2015) suggest that the TS muscle strength contributes approximately 30-40% to the ability to increase the base of support (Reco1L step in the current study) and thereby recover balance during perturbed gait or forward falls. Accordingly, it was expected that the GAIT+NM group (perturbation + TS neuromuscular training) would show higher improvements in the dynamic stability in comparison to the GAIT group (only perturbation training). However, despite a significant enhancement of the TS neuromuscular capacities over 14 weeks of exercise (higher TS muscle strength, increased Achilles tendon stiffness and improved motor skill performance), both GAIT+NM groups (GAIT+NM14w and GAIT+NM1.5y) did not display any further meaningful improvements in the reactive response following unexpected perturbations to gait. This would support our suggestion that transfer of the newly obtained TScapacities into gait modifications may be prolonged in older adults, and thereby explain the non-existent effects on gait stability 14 weeks into the exercise. However, the observation that our subgroup of 12 older adults who continued the TS exercise intervention over 1.5 years also showed no further gait stability improvements shows that the effect of exposure to gait perturbations seems to provide a much larger stimulus for improvement of dynamic stability control in older adults than experimentally improved TS capacities. Therefore, an additional effect from the improved TS capacities might not be required, at least for the Reco1L step, because stability was already sufficiently controlled at the onset of perturbation due to the perturbation exposure, thereby negating or reducing the requirement for large recovery steps and hence increased TS muscular output.

Interestingly, some of our previous observations identified that stronger, in comparison to weaker older adults demonstrate a more effective reactive response following unexpected gait perturbation (Epro et al. 2015). It is important to highlight, however, that the TS muscle strength differed by 42% between these two groups, which is considerably greater than the 25% increase achieved by the GAIT+NM group of the current study after 1.5 years of exercise. Accordingly, one might imply that the exercise-induced improvements in the lower extremity MTUs in the current and previous exercise interventions (e.g. Gillespie et al. 2012; Sherrington et al. 2008) may not have resulted in sufficient increases in strength, and that a certain threshold in strength has to be achieved before notable modifications in the stability performance occur. In order to handle perturbations a certain strength level is necessary, but maximum TS muscle strength appears not to be the most critical determinant. For instance, rather than the individual maximal joint moments, but the ability to generate joint moments in a proper temporal framework has shown to be the main reason for an improved dynamic stability in simulated forward falls after an intervention (Arampatzis et al. 2011). In the current study, we additionally attempted to stimulate the neural factors (motor skill training) to provoke a greater excitability of the cortical representation of specific muscles and both GAIT+NM groups (GAIT+NM14w and GAIT+NM1.5y) were able to significantly improve their motor skill performance after 14 weeks of combined TS neuromuscular exercise intervention, which was not the case in the GAIT group. However, similar to the TS muscle strength and Achilles tendon stiffness, a maintenance of, rather than further improvements in motor skill performance were detected after continuing the exercise intervention up until 1.5 years. Accordingly, even a potentially improved excitability of the cortical representation did not aid the older adults in utilizing the improved TS MTU capacities in the reactive recovery response during perturbed walking. Therefore, we suggest that in order to improve the ability to cope with disturbances to gait, older adults may benefit more from exposure to unexpected gait perturbations than from interventions targeting the leg-extensor neuromuscular capacities. This kind of perturbation-training may have significant clinical and practical implications for in falls prevention in older adults. Perturbation training could have multiple benefits for healthcare systems, due to the potential to provide such training at low cost, in combination with the short training time that appears to be needed, as well as the long-term benefits for older adults (Gerards et al. 2017). The above suggestion is supported by earlier slip-perturbation studies, which have shown a long-term (up to 12 months) retention of the improvements in the reactive recovery response obtained in a single perturbation session as well as a reduced annual daily life fall risk up to 50% (Bhatt et al. 2006, 2012; Pai et al. 2014a; Pai et al. 2014b). However, whether the current exercise paradigm using tripping while walking on a treadmill is transferable to other types of perturbations (e.g. slip-perturbations) or to daily life environments and activities (and if it could lead to a reduced incidence of falls) in elderly, needs further investigation.

## *Limitations*

One might argue that the participants may have anticipated the onset of the perturbation and thereby predictively modified their gait. However, no clear predictive adjustments were observed in either group or time point (no significant differences in MoS of the step prior to the perturbation compared to baseline). Despite this, the detected improvements in the recovery behaviour may not be fully attributable to reactive, feedback-driven motor adjustments, because prior experience of the perturbation task may have led to a heightened awareness and concentration and thereby a faster and more effective response compared to a novel situation (Pater et al. 2015)[42]. While this potential drawback might affect our identified degree of retention effects post 14 weeks and post 1.5 years within subject groups, this drawback may be less relevant for our conclusion that an additional TS MTU exercise intervention does not further enhance the retention of improvements made in reactive response in perturbed gait over a prolonged time period. Concerning the motor skill training, it could be argued that our single exercise duration was lower than used in studies by Perez et al. (2004) and Jensen et al. (2015), potentially not providing a sufficient stimulus to provoke plastic changes in the central nervous system in the older adults. However, the participants of the current study performed an exercise intervention 2-3 times a week up to 1.5 years with a total training duration over 1100 minutes for the motor skill training. Therefore, we are quite confident that the total exercise duration was sufficient to provoke neural adaptation in the examined older adults. Furthermore, a limitation of the current study is the relatively low number of subjects in each group (n=13 for GAIT group; n=21 GAIT+NM14w group and n=12 GAIT+NM1.5y) which reduces the potential for determining statistical differences between the exercise interventions (GAIT vs GAIT+NM), reflected also by a low statistical power (0.32 for the non-significant group x trial interaction for the MoS at TD Pert). Therefore we cannot exclude that a higher number of subjects might have led to a significant result regarding effectiveness of the combined perturbation and TS neuromuscular exercise intervention in comparison to only perturbation exposure. However this drawback does not affect our observation that the aged neuromotor system shows plasticity in response to repeated exposure to unexpected perturbations and an ability to retain these acquisitions in reactive recovery responses over a prolonged time period of 1.5 years.

## *Conclusions*

In conclusion, the current findings provide evidence that older adults are able to partly retain the reactive response improvements in fall**-**resting skills acquired during perturbation training sessions over a prolonged period of 1.5 years. An additional exercise-induced enhancement of the TS neuromuscular capacities over 14 weeks or 1.5 years seems not to lead to further meaningful benefits for the reactive recovery response to control the center of mass state following a gait perturbation in elderly adults. Thus, the central nervous system of older adults shows rapid plasticity to repeated exposure to unexpected perturbations and an ability to retain these acquisitions in reactive recovery responses over a prolonged time period (1.5 years), but it seems, that an additional exercise-related enhancement of TS neuromuscular capacities is not necessarily transferred to the recovery behavior during unexpected perturbations to gait in older adults. Accordingly, perturbation training alone seems to optimize task performance to a necessary level of stability. In order to improve the ability to cope with disturbances to gait, older adults may benefit more from a brief exposure to unexpected gait perturbations than from interventions targeting the neuromuscular capacities of the lower limbs.

# Author contributions

G.E., G-P.B. and K.K. conception and design of research; G.E., A.M., M.L., and K.K. performed experiments; G.E., C.M. and K.K. analyzed data; G.E., C.M., G-P.B. and K.K interpreted results of experiments; G.E. and K.K. prepared figures; G.E. and K.K. drafted manuscript; G.E., C.M., A.M., M.L., G-P.B. and K.K. edited and revised manuscript; G.E., C.M., A.M., M.L., G-P.B. and K.K. approved final version of manuscript.

# Conflict of Interest Statement

The authors declare no conflicts of interest.

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# Tables

**Table 1**: Relative mean deviation in the motor skill performance with the slow (upper values) and fast (lower values) cursor movement (80s and 20s of continuous tracing respectively) in all analyzed functions (exercise functions: FunctCT1 and FunctCT2; transfer functions: FunctTR1 and FunctTR2) at Pre, Post 14 weeks and Post 1.5 years measurement time points for the GAIT group (n = 13), GAIT+NM14w group (n = 21) and GAIT+NM1.5y group (n = 12).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **GAIT** | | | **GAIT+NM14w** | | **GAIT+NM 1.5y** | | |
| **Pre** | **Post 14 weeks** | **Post 1.5 years** | **Pre** | **Post 14 weeks** | **Pre** | **Post 14 weeks** | **Post 1.5 years** |
| FunctCT1 (%) | 3.5 ± 1.2 | 2.8 ± 1.3 | 2.4 ± 0.6 | 4.0 ± 1.8 | 1.2 ± 0.4\*# | 3.8 ± 1.6 | 1.1 ± 0.3\*# | 1.0 ± 0.2\*# |
| 3.9 ± 0.9 | 3.4 ± 1.1 | 3.5 ± 0.7 | 4.4 ± 1.7 | 1.7 ± 0.4\*# | 4.3 ± 1.6 | 1.7 ± 0.4\*# | 1.6 ± 0.5\*# |
| FunctCT2 (%) | 2.8 ± 0.6 | 2.5 ± 0.7 | 2.5 ± 0.6 | 4.0 ± 1.9 | 1.2 ± 0.5\*# | 3.8 ± 1.7 | 1.1 ± 0.3\*# | 1.0 ± 0.4\*# |
| 3.4 ± 0.9 | 3.0 ± 0.7 | 3.0 ± 0.6 | 4.2 ± 1.8 | 1.8 ± 0.6\*# | 4.2 ± 1.7 | 1.6 ± 0.4\*# | 1.5 ± 0.3\*# |
| FunctTR1 (%) | 2.3 ± 0.9 | 1.9 ± 0.7 | 1.9 ± 0.6 | 3.1 ± 1.8 | 0.9 ± 0.3\*# | 3.1 ± 1.6 | 0.8 ± 0.2\*# | 0.8 ± 0.2\*# |
| 3.0 ± 0.8 | 2.7 ± 0.7 | 2.7 ± 0.6 | 3.9 ± 1.6 | 1.6 ± 0.4\*# | 3.9 ± 1.6 | 1.6 ± 0.4\*# | 1.5 ± 0.3\*# |
| FunctTR2 (%) | 3.5 ± 0.9 | 3.1 ± 1.0 | 3.2 ± 0.9 | 5.5 ± 2.5# | 1.4 ± 0.4\*# | 5.6 ± 2.3# | 1.4 ± 0.4\*# | 1.2 ± 0.3\*# |
| 3.8 ± 0.8 | 3.6 ± 1.1 | 3.4 ± 0.8 | 5.3 ± 1.9# | 2.0 ± 0.5\*# | 5.7 ± 1.8# | 1.9 ± 0.4\*# | 2.0 ± 0.4\*# |

\*p < 0.05 compared with Pre.

#p < 0.05 compared with respective function of the GAIT group.

# Figure legends

**Figure 1**:Custom-made pneumatic perturbation device, which allows to apply and remove external resistance (generating approximately 55 N, within about 20 ms) to the lower limb during walking via an ankle strap and a Teflon cable. The system’s control board is synchronized with the PC and motion capturing system via a TTL signal, which changes when the perturbation in turned on/off.

Figure 2: A typical recovery behavior following the unexpected trip perturbation in one of the participants The pull from the pneumatic brake-and-release system is activated (ONSET Pert) during the support phase of the right leg and deactivated (OFFSET Pert) in the following support phase of the same leg, thereby causing the subject to experience the pull only during the swing phase. This causes a reduction in the base of support at foot touchdown of the perturbed right leg (TD Pert), a higher velocity and more anterior position of the center of mass in comparison to the step before the perturbation (TD PRE Pert), which leads to a decreased margin of stability. In order to maintain dynamic stability, the subject has to increase the base of support in the following recovery step (TD Reco1L).

**Figure 3:** Schematic diagram of the resistance and motor skill training. A: Subjects were seated on the training device with their lower leg secured, with knee and ankle joints fixed at 90° (shank perpendicular to thigh). B: Ankle plantarflexion MVC moment was measured with 2 custom-made strain gauge-type dynamometers. The foot was positioned on the dynamometer by aligning the axis of rotation of the ankle joint with the plate’s centre of rotation (ankle plantarflexion MVC moment equivalent to the moment measured by the dynamometer). Therefore, the resultant moment was calculated as the product of F(resultant force) and r (distance between the plate’s centre of rotation and the strain gauge). C: feedback for the resistance training (5 times of 4 plantarflexion contractions with 90% of MVC). D: motor skill training paradigm. Each training session consisted of four separate functions: two pre-defined functions (constant functions) and two regularly varied functions. E: transfer functions: in the measurement sessions the varied functions were replaced with two pre-defined transfer functions only occurring in the measurement sessions. The relative mean deviation (mean deviation normalized to maximal plantarflexion moment) was calculated for the exercised (each training session) and transfer (pre and post measurement) functions.

**Figure 4:** Average trajectory of the plantarflexion moment and respective mean relative deviations in one of the constant functions (FunctCT1) with fast cursor movement (20s continuous tracing) at baseline (Pre), after 14 weeks (Post 14 weeks) and after 1.5 years of motor skill training in the perturbation training group (GAIT) and the combined perturbation and TS neuromuscular exercise intervention group (GAIT+NM1.5y). The black line represents the optimal trace (target function), the red and green lines represent the average actual traced relative plantarflexion moment performed by the subjects in this measurement time point for the GAIT and GAIT+NM1.5y group. Gray areas represent the excluded data during the first and last 5% of the traced data (to avoid possible errors in mean and relative deviation through differences in subjects’ attention between the measurement time points).

\*: Statistically significant difference to Pre for the GAIT+NM1.5y group (p < 0.05).

**Figure 5:** Margin of stability (MoS) at touchdown during unperturbed walking (TD Base: average of 12 consecutive steps) in the perturbation training group (GAIT) and the combined perturbation and TS neuromuscular exercise intervention groups (GAIT+NM14w and GAIT+NM1.5y) at baseline (Pre), post 14 weeks (Post 14w) and post 1.5 years (Post 1.5y) measurement time points. All values are expressed as means with SD (error bars).

**Figure 6:** Margin of stability (MoS) at touchdown of the perturbed leg (TD Pert) in the perturbation training group (GAIT) and the combined perturbation and TS neuromuscular exercise intervention groups (GAIT+NM14w and GAIT+NM1.5y) at baseline (Pre), post 14 weeks (Post 14w) and post 1.5 years (Post 1.5y) measurement time points. All values are expressed as means with SD (error bars). a: Statistically significant difference to T1Pre (p < 0.05); b: Statistically significant difference to T8Pre (p < 0.05); c: Statistically significant difference to T1Post14w (p < 0.05); d: Statistically significant difference to T8Post14w (p < 0.05); e: Statistically significant difference to T1Post1.5y (p < 0.05).

**Figure 7:** Margin of stability (MoS) at touchdown of the first recovery step (TD Reco1L) following the perturbation in the perturbation training group (GAIT) and the combined perturbation and TS neuromuscular exercise intervention groups (GAIT+NM14w and GAIT+NM1.5y) at baseline (Pre), post 14 weeks (Post 14w) and post 1.5 years (Post 1.5y) measurement time points. All values are expressed as means with SD (error bars). a: Statistically significant difference to T1Pre (p < 0.05).