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TITLE:

Evaluating Postural Control and Lower-extremity Muscle Activation in Individuals with Chronic Ankle Instability

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SUMMARY

Individuals with chronic ankle instability (CAI) exhibit postural control deficiency and delayed muscle activation of lower extremities. Computerized dynamic posturography combined with surface electromyography provides insights into the coordination of the visual, somatosensory, and vestibular systems with muscle activation regulation to maintain postural stability in individuals with CAI.

ABSTRACT

Computerized dynamic posturography (CDP) is an objective technique for the evaluation of postural stability under static and dynamic conditions and perturbation. CDP is based on the inverted pendulum model that traces the interrelationship between the center of pressure and the center of gravity. CDP can be used to analyze the proportions of vision, proprioception, and vestibular sensation to maintain postural stability. The following characters define chronic ankle instability (CAI): persistent ankle pain, swelling, the feeling of "giving way," and self-reported disability. Postural stability and fibular muscle activation level in individuals with CAI decreased due to lateral ankle ligament complex injuries. Few studies have used CDP to explore the postural stability of individuals with CAI. Studies that investigate postural stability and related muscle activation by using synchronized CDP with surface electromyography are lacking. This CDP protocol includes a sensory organization test (SOT), a motor control test (MCT), and an adaption test (ADT), as well as tests that measure unilateral stance (US) and limit of stability (LOS). The surface electromyography system is synchronized with CDP to

collect data on lower limb muscle activation during measurement. This protocol presents a novel approach for evaluating the coordination of the visual, somatosensory, and vestibular systems and related muscle activation to maintain postural stability. Moreover, it provides new insights into the neuromuscular control of individuals with CAI when coping with real complex environments.

INTRODUCTION

Computerized dynamic posturography (CDP) is an objective technique for the evaluation of postural stability under static and dynamic conditions and perturbation. CDP is based on the inverted pendulum model that traces the interrelationship between the center of pressure (COP) and the center of gravity (COG). COG is the vertical projection of the center of mass (COM), whereas COM is the point equivalent of the total body mass in the global reference system. COP is the point location of the vertical ground reaction force vector. It represents a weighted average of all the pressures over the surface of the contact area with the ground[1]. Postural stability is the ability to maintain the COM within the base of support in a given sensory environment. It reflects neuromuscular control ability that coordinates the central nervous system with the afferent sensory system (vision, proprioception, and vestibular sensation) and motor command output[2].

Previous evaluation methods for postural control, such as the time for a single-leg stance and the reach distance for Y-balance tests, are results-oriented and cannot be used to objectively evaluate the coordination between sensory systems and motor control[3]. In addition, some studies used portable computerized wobble board, which quantified dynamic balance performances out of laboratory settings[4-6]. CDP differs from the abovementioned test methods, because it can be applied to the analysis of the proportion of vision, proprioception, and vestibular sensation in postural stability maintenance and to the evaluation of the proportion of motor strategy, such as ankle or hip dominant strategy. It has been viewed as a gold standard for postural control measurement[7] because of its accuracy, reliability, and validity[8].

Chronic ankle instability (CAI) is characterized by persistent ankle pain, swelling, and feeling of “giving way”; it is one of the most common sports injuries[9]. CAI originates mostly from lateral ankle sprains, which destroy the integrity and stability of the lateral ankle ligament complex. The proprioception, fibular muscle strength, and normal trajectory of talus are impaired[10,11]. The deficiencies of the weak ankle segment can result in deficient postural control and muscle activation in individuals with CAI[12]. However, few studies have investigated the postural stability of individuals with CAI by using CDP[3,13]. Current measurements could rarely analyze the posture control deficiency of CAI from the perspective of sensory analysis. Therefore, the ability of sensory organization and postural strategy of CAI to maintain postural stability needs further exploration.

Muscle activity is an important component of neuromuscular control that affects the regulation of postural stability[14,15]. However, CDP only monitors the interrelationship between COP and COG through force plates, and its application to the observation of the specific

activation level of lower limb muscles in individuals with CAI is difficult. Currently, few studies have evaluated the postural stability of individuals with CAI through a method that combines CDP with electromyography (EMG).

Therefore, the developed protocol aims to explore postural control and related muscle activity by combining CDP and surface electromyography system (sEMG). This protocol provides a novel approach to investigate neuromuscular control, including sensory organization, postural control, and related muscle activity, for participants with CAI.

PROTOCOL

Prior to tests, the participants signed an informed consent after receiving information about the experimental process. This experiment has been approved by the ethics committee of Shanghai University of Sports.

1. Equipment setup

1.1. Turn on the CDP system, complete self-calibration, and ensure that the instrument operates normally at 100 Hz sampling frequency.

NOTE: Each of the two installed independent force plates measures three forces (Fx, Fy, and Fz) and three moments (Mx, My, and Mz). The x-axis is in the left–right direction and is perpendicular to the sagittal plane. The y-axis is in the forward–backward direction and is perpendicular to the coronal plane. The z-axis is perpendicular to the horizontal plane. The origins are located at the centers of the force plates.

1.2. Double-click **Balance Manager System** | **Clinical Module**, and then click **New Patient** and establish the patient ID. Input an accurate height, weight, and age. Select **Sensory Organization Test, Unilateral Stance, Limits of Stability, Motor Control Test,** and **Adaption Test**.

1.2.1. Fix the participants on the support bar with a safety harness. Correctly align the foot with the force plates and the face with visual surround.

NOTE: Determine the alignment of the participants and the sway amplitude of the dual force plates and visual surround using demographic data. Such data are also used for age-matched normative diagnostic analysis.

1.3. Turn on the surface electromyography (sEMG) system, and double click the **EMG Motion Tools** icon. Specify the trigger signal as **Trigger In (Manual Stop)**, establish the participant ID, and match the measured muscles with the wireless electrode. The muscles of unstable lower limb are vastus medialis (VM), vastus lateralis (VL), biceps femoris (BF), tibialis anterior (TA), peroneal longus (PL), gastrocnemius medialis (GM), and gastrocnemius lateralis (GL).

NOTE: The phrase **Trigger In (Manual Stop)** indicates that CDP triggers the sEMG system to capture EMG data during tests, but the "end" flag requires manual clicking to stop the acquisition.

1.4. Connect sEMG system with CDP system through the synchronization line. Adjust the camera of sEMG system to capture the signal indicator light of the CDP system.

NOTE: The video of the indicator light is collected synchronously with the CDP system and sEMG to cut the corresponding cycle of the EMG in accordance with the CDP tests. “Light on” indicates that the test is in progress, and “light off” indicates that the test is paused/stopped.

2. Participant selection and preparation

2.1. Use the following inclusion criteria for CAI participants: (1) 35 male participants with regular daily activity, excluding professional athletes or sedentary participants; (2) 20–29 years old; (3) history of at least one significant ankle sprain, and the initial sprain must have occurred at least 12 months before enrollment in the study; (4) feelings of “giving away” of the injured ankle joint and/or recurrent sprain and/or “feeling of instability;” and (5) a Cumberland Ankle Instability Tool questionnaire score of less than 24 points[16].

2.1.1. Exclude participants with a history of bilateral sprains, lower limb fracture, operation, nervous and vestibular system diseases, or allergy to taping. Additionally, recruit 35 male participants without CAI, whose demographic data matched with the CAI group, as the control group.

2.2. For preparation, fix the electrode piece on the belly of the measured muscles. Instruct the participants to wear a safety harness and stand barefoot on the force plates to face the visual surround.

2.2.1. Adjust the alignment of the feet on the force plates. Align the malleolus medialis with the horizontal line and lateral edge of foot with the corresponding computer-generated height line (S, M, and T lines). Turn off the screen embedded in the visual surround (**Figure 1**).

NOTE: These guidelines are based on the following heights. “S” means “small” and includes heights ranging from 76 cm to 140 cm. “M” means “medium” and includes heights ranging from 141 cm to 165 cm. “T” means “tall” and includes heights ranging from 166 cm to 203 cm. The screen may produce learning effects, because it can provide real-time visual feedback. Thus, the screen should remain closed during the test, except during the limit of stability (LOS) test[17].

[Place **Figure 1** here]

3. Measurement procedures

3.1. CDP measurement

3.1.1. Sensory organization test

3.1.1.1. Instruct the participants to stand upright and to keep their COG as stable as possible to cope with the interference from vision, somatosensory, and vestibular sensation (singly or combined) (**Table 1**). Complete the measurements of conditions 1–6. Each test lasts for 20 s. Repeat the procedure thrice for each condition.

[Place **Table 1** here]

3.1.2. Unilateral stance

3.1.2.1. Instruct the participants to place their hands on the anterior superior iliac spine with their eyes open/closed. Consider the unstable ankle side as the support leg. Fully extend their knee joint, and bend the knee of their non-supporting leg by approximately 30°. Allow the participants to remain standing stably for 10 s. Repeat the procedure thrice for each visual condition.

3.1.3. LOS

3.1.3.1. Instruct the participants to maintain their COG in the central area. Upon hearing the ring, lean their body and shift their COG quickly into the targeted frame in the screen. Instruct the participants to remain steady for 10 s. Complete the eight directional shifting of their COG (forward, forward-right, right, right-backward, backward, backward-left, left, and left-forward).

NOTE: In the process of COG shifting, the body is kept straight, the heel or toes are not far from the force plates, and the hip joint is not bent.

3.1.4. Motor control test

3.1.4.1. Instruct the participants to respond effectively to restore body stability and to cope with the unexpected slipping of the force plates. Repeat the procedure thrice for each slip condition.

NOTE: The force plates are slipped with small/medium/large amplitude in the anterior/posterior direction. According to the participant's height, the slip amplitude of the force plates is automatically adjusted. Standard procedures must be followed to align the foot

position on the force plates. Random delay exists between trials.

3.1.5. Adaption test

3.1.5.1. Instruct the participants to respond effectively to restore body stability and to cope with five consecutive unexpected rotations at a velocity of 20°/s. Direct the toes upward or downward.

3.2. sEMG measurement and data process

3.2.1. After triggering by CDP system during SOT, US, LOS, MCT and ADT, start the automatic acquisition of lower-limb muscle activity raw data. Manually stop the acquisition during the sEMG system when the light is off. The sample size is 1000 Hz.

3.2.2. Enter the processing window of the sEMG software. Import the C3d file of the EMG raw data and mp4 file of the light video. Cut the trial cycle when the light is on.

3.2.3. In the "processing pipeline" operations, include the following options in the run pipeline: Butterworth filter with low-pass (450 Hz, 2. Order) and high-pass (20 Hz, 2. Order); notch filter at 50 Hz; and root mean square smoothing window of 100 ms.

NOTE: Choose the Butterworth filter with low-pass (450 Hz, 2. Order) and high-pass (20 Hz, 2. Order) to filter out unwanted low and high-frequency components. Set the notch filter at 50 Hz to remove 50 Hz interference from the main power. Use the root mean square smoothing window of 100 ms to smooth the noisy signal.

3.2.4. In the **Generate Events** options, include the following events in the run pipeline. "muscle on" is defined as "all channels go above 5x baseline noise standard deviations for at least 50 ms". "muscle off" is defined as "all channels drop below 5x standard deviations over baseline for at least 50 ms ".

3.2.5. In the **Generate Parameters** options, include the following parameters in the run pipeline: integral electromyography (iEMG); root mean square (RMS); mean power frequency (MPF); medium frequency (MDF); and co-activation ratio.

NOTE: The following are the referenced calculation formulas for the above parameters (Equations 1–5):

$$iEMG = \int_T^{t+T} EMG(t)dt \quad \text{(Equation 1)}$$

$$EMG_{rms} = \sqrt{\frac{1}{T}\int_T^{t+T} EMG^2(t)dt} \quad \text{(Equation 2)}$$

$$MPF = \frac{\int_0^\infty f \times \Phi(f) df}{\int_0^\infty \Phi(f) df} \quad \Phi(f): the\ power\ spectrum\ of\ the\ signal \quad \text{(Equation 3)}$$

$$MDF = \frac{1}{2}\Phi(f) = \int_0^{MDF} \Phi(f) df = \int_{MDF}^0 \Phi(f) df \quad \text{(Equation 4)}$$

$$Co - activation\ ratio = \frac{RMS_{Antagonistic\ muscle}}{RMS_{agonistic\ muscle}} \times 100\% \quad \text{(Equation 5)}$$

3.2.6. Normalize the RMS values of the SOT, US, LOS, MCT and ADT trials with the RMS values of maximal voluntary isometric contraction (MVIC) for each muscle (Equation 6).

$$NEMG = \frac{EMG_{rms}}{MVIC_{rms}} \times 100\%. \quad \text{(Equation 6)}$$

NOTE: MVIC indicates the maximum force contraction of each muscle for participants in the standard posture for 5 s **(Supplementary file 1)**[18].

Representative Results

Representative CDP Results

Sensory organization test

The system evaluates the participant's ability to maintain COG in the predetermined target area, when the environment changes as the peripheral signal input. **Equilibrium score (ES)** is the score under conditions 1–6 that reflects the ability to coordinate the sensory system to maintain postural stability (Equation 6). The **composite score (COMP)** is the weighted average score of all conditions. Great emphasis is given to the challenging conditions of 4, 5, and 6. The composite score is calculated by independently averaging the equilibrium scores for conditions SOT1 and SOT2, by adding these two scores to the equilibrium scores from each three trial of condition SOT 3 through to SOT 6, and by dividing the sum by the total performed trials[19,20]. In the figures, green bars indicate that the participant can coordinate their three sensory systems better and respond more effectively than their age-matched normative counterpoint in the dataset. Red bars indicate that the sensory organization ability of the participant is worse than that of their age-matched normative counterpoint in the dataset (**Figure 2A**).

$$ES = \frac{12.5° - (\vartheta max - \vartheta min)}{12.5°} \times 100. \quad \text{(Equation7)}$$

NOTE: The theoretical maximum anterior–posterior direction displacement of the COG for a healthy adult is 12.5°. θ indicates the sway angle of the COG. The equilibrium score range is 0–100. A score of 0 indicates the loss of balance. Scores close to 100 indicate that the participant has a good balance function.

Sensory Analysis Score: The system coordinates the participation proportion of vision, proprioception, and vestibular sensation under six conditions and deduces the dependence degree on **vision (VIS)**, **proprioception (SOM)**, and **vestibule (VEST)** in the process of

maintaining postural stability (Equations 8–10). The appearance of a red bar indicates that the participant cannot use VIS/SOM/VEST sensory sensation to maintain balance. **Visual preference (PREF)** indicates the ability to ignore wrong visual information in a conflicting visual interference environment (Equation 11). The appearance of a red bar indicates that the participant relies on visual information to maintain balance even with incorrect visual information (Figure 2B).

$$VIS=\frac{Condition4}{Condition1} \quad \text{(Equation 8)}$$

$$SOM=\frac{Condition2}{Condition1} \quad \text{(Equation 9)}$$

$$VEST=\frac{Condition5}{Condition1} \quad \text{(Equation 10)}$$

$$PREF=\frac{Condition3+6}{Condition2+5} \quad \text{(Equation 11)}$$

Strategy Score: The system exports the strategy score (STR) in accordance with the interrelationship of COG and COP during the process of stability maintenance. An STR close to 100 indicates the use of a high proportion of ankle strategy. An STR score close to 0 indicates the use of a high proportion of hip strategy. Marks of conditions 1–6 close to the right side of the quadrant indicate the dominance of the ankle strategy; those close to the left side indicate the dominance of the hip strategy (Figure 2C).

COG Alignment: COG location changes in the form of coordinates under each condition (Figure 2D).

[Place **Figure 2** here]

Unilateral Stance

The **sway velocity of COG** (°/s) during unilateral stance is exported. The appearance of a red bar indicates that the ability to maintain single-stance stability is worse than normal. Left/right difference (%) indicates the comparison of the total swing between the left and right legs (**Figure 3**).

[Place **Figure 3** here]

LOS

LOS is the best voluntary movement measurement in the CDP system. LOS test evaluates the reaction time, movement velocity, perceived ability for LOS, and movement control ability. The following variables are exported:

Reaction Time (RT) (s): The time between the sending of the move signal and the beginning of body movement. The appearance of a red bar indicates delayed reaction time (**Figure 4A**).

Movement Velocity (MVL) (°/s): The average velocity between 5% and 95% from the initial point to the target. The appearance of a red bar indicates that the average velocity of gravity is slower than normal (**Figure 4B**).

Endpoint Excursions (EPE) (%): The COG movement distance from the initial point to the final point. The appearance of a red bar indicates that the movement distance of the COG does not reach the normal range (**Figure 4C**).

Maximum Excursions (MXE) (%): The maximum distance of the COG movement. The appearance of a red bar indicates that the COG's maximum excursion does not reach the normal range (**Figure 4C**).

Directional Control (DCL) (%): The amount of movement toward the intended direction minus the amount of off-axis movement (**Figure 4D**).

[Place **Figure 4** here]

Motor control test: Use this test to evaluate the participant's ability to produce an effective motor response and to restore COG stability to cope with the sudden anterior–posterior displacement of the force plates.

Weight symmetry: This refers to the weight-bearing distribution of both legs. The appearance of a red bar indicates the asymmetrical weight of the left and right legs (**Figure 5A**). The bars show the computer-generated confirmation. If this value is low (≤2), then latency is abnormal. If this value is 0, then the response is missing and needs a retest.

Latency (ms): The response time from the movement of pressure force plates to the movement of the COP. (1) The appearance of a red bar in the unilateral side during forward/backward displacement may be due to unilateral orthopedic injury. (2) The appearance of a red bar in the bilateral sides during forward/backward displacement may indicate the occurrence of damage in the efferent branch of the long circulation pathway. (3) The appearance of a red bar in the bilateral sides during forward and backward displacement may be due to peripheral neuropathy, spinal diseases, multiple sclerosis, and brainstem/cortical pathology (**Figure 5B**).

Amplitude scaling: This is the force exerted on the force plate by the leg in response to perturbation. The increase in amplitude scaling (AS) should be bipedally symmetrical and should relate to the amplitudes of force plate slippage (**Figure 5C**).

[Place **Figure 5** here]

Adaption test

The **sway energy score (SES)** is determined based on the velocity and acceleration of the COP

during the first 2 s of perturbation and is exported (**Figure 6**). A red bar that reaches 200 points indicates the loss of balance (fall). (1) If red bars do not reach 200 points in the gray area less than twice in five trials, and other bars remain green, then the variation is normal, and the risk of falling is absent. (2) Red bars that reach 200 points each time in five trials may be due to the following reasons. The COG is backward excessively when the force plates rotate in the toes-up direction and vice versa. The ankle range of motion is limited. The ankle joints or lower limbs are weak. The central nervous system is dysfunctional. (3) The red bars reach 200 points twice in five trials, whereas other bars remain green due to the influence of fear or anxiety. (4) The appearance of a red bar in the gray area five times may be due to weak ankle joints, lower limbs, fear, or anxiety.

[Place **Figure 6** here]

sEMG Results

Taking vastus medialis for example, the raw and processed data of sEMG are shown during SOT, US, MCT, and ADT (**Figure 7** and **Figure 8**). The interval indicated by the red line and tips is the interval where the indicator light of the CDP system is on and is the test stage.

[Place **Figure 7** here]
[Place **Figure 8** here]

The sEMG parameters that correspond to the test stages of SOT, US, LOS, MCT, and ADT are as follows. **iEMG** reflects the muscle energy that is accumulated per unit time. **RMS** reflects the mean power of the EMG signal. **MPF** means the average value of each power in the power spectrum distribution. **MDF** divides the power spectrum into two parts with equal areas. The **coactivation ratio** reflects the coordination between the agonistic and antagonistic muscles of the activation phase in tests.

FIGURE AND TABLE LEGENDS:

Figure 1: Participant preparation for measurement. The participants stand upright barefoot to face the visual surround, wear safety harness, correctly align their feet with the force plates, and fix the wireless EMG electrodes on their legs.

Figure 2: Representative result for participants with CAI during the SOT. (A) Graphic representation of equilibrium and composite scores. **(B)** Graphic representation of sensory analysis results. **(C)** Graphic representation of strategy analysis results. **(D)** Graphic representation of COG alignment results. In the graphical results of SOT, US, LOS, MCT and ADT, the solid green bars represent the results in the normal range. The solid red bars represent the results out of the normal range. The striped bars represent the repeated test. The gray areas represent the abnormal data range.

Figure 3: Sway velocity of COG for participants with CAI during US with eyes open/closed

(°/s).

Figure 4: Representative result for participants with CAI during LOS. (A) Graphic representation of reaction time result(s). **(B)** Graphic representation of movement velocity results (°/s). **(C)** Graphic representation of endpoint and maximum excursion results (%). **(D)** Graphic representation of directional control results (%).

Figure 5: Representative results of participants with CAI during the motor control test. (A) Graphic representation of weight symmetry results. **(B)** Graphic representation of latency results (ms). **(C)** Graphic representation of AS results.

Figure 6: SES of participants with CAI during ADT.

Figure 7: Raw data of sEMG for vastus medialis during SOT, US, MCT, and ADT.

Figure 8: Processed data of sEMG for vastus medialis during SOT, US, MCT, and ADT.

Table 1: Different interference and corresponding anticipated response in sensory organization test. The term "sway-referenced" means that the movement of the force plates and visual surround follows the participant's COG sway.

Supplementary File 1: Introduction for Computerized Dynamic Posturography System

Supplementary Table 1: Application technique on the muscle sites of sEMG electrodes

Supplementary Table 2: Standard Posture for EMG Normalization Method for measured muscles.

DISCUSSION

The presented protocol is used to measure dynamic postural control and related muscle activity in individuals with CAI by synchronizing CDP with sEMG. CDP traces the trajectory of the COP and COG and provides insight into the interaction between sensory information (visual, somatosensory, and vestibular sensation) input and the external environment[8,21,22]. It is an effective tool for the diagnosis of the functional activity limitation caused by sensory or motor system disorders. Muscle activity is collected synchronously during CDP tasks to investigate lower limb coordination. This protocol compensates for the limitations of previous studies under certain circumstances. It allows the comprehensive investigation of the neuromuscular control of CAI through the combination of CDP and related muscle activity.

The following steps in the protocol are critical in investigating postural stability and are associated with the accurate measurement of signals. Pre-experiment results revealed that the completion of the entire test without rest takes 25 min. During this process, participants

concentrate their attention on the adjustment of motor strategies and on the maintenance of balance. Fatigue alters the movement regulation strategy of the central nervous system and interrupts proprioception, muscle response, and dynamic postural control[23,24]. Therefore, a rest time of at least 5 min should be set after each test to avoid cognitive loading and body fatigue[25]. Anthropometric characteristics should be controlled accurately to limit the variability for the accurate evaluation of postural balance[26-28]. Similarly, in this protocol, age, height, weight, and foot position alignment should be controlled accurately, because they determine the location of the COP and affect the analysis of the distribution of weight and force[2]. The safety harness should not be too loose or too tight to protect the safety of the participant without affecting normal movement. After completing the foot alignment, the foot position should not move until the completion of the tests. The participant should not be allowed to grasp the safety harness or lean on the visual surround to seek external support to avoid affecting the accuracy of the result. Randomly sequencing the trials in MCT with different magnitudes helps prevent the participants from predicting the perturbation conditions.

The following limitations need to be considered when implementing measurement. First, only male participants are included to avoid the interference of gender differences in the interpretation of results. Future research needs to explore posture control and muscle activation in female participants with CAI. Second, most CAI injuries are inverted or combined with plantar flexion in the frontal plane, whereas MCT and ADT perturbations involve anterior–posterior slippage in the horizontal plane and flexion–dorsiflexion rotation in the sagittal plane of the force plates. Therefore, future interference models should consider the damage mechanism.

Existing methods are divided into several categories and used to evaluate postural stability, as follows[29]. Clinical scales, such as the Berg Balance Scale, are easy to implement in clinical functional evaluation. However, results are subjective, and the weak segment is difficult to find. The results-oriented measurement of voluntary dynamic control, such as reach distance of Y-balance test, could identify posture control deficiency, but it ignores action quality during the process[30,31]. Changing a certain sensory environment, such as standing with the eyes closed for vision deprivation, standing with one leg to reduce the base of support, or standing on unstable surface (a foam or wobble board), to interfere with the somatosensory system is a low-cost and portable way to differentiate the deficiency of the specific sensory system to achieve dynamic balance control[4,5]. CDP could analyze the dependence proportion of the three sensory systems and could investigate postural strategies by tracing COP and COG. SOT is particularly applied to evaluate the quality of the motor system output (COG dynamic control) by controlling the peripheral environment signal input (sensory weight) in a complete sensory motor loop. US and LOS can evaluate autonomic voluntary motor control ability at the cortical level. MCT and ADT can evaluate automatic posture response at the brainstem and cortical levels through external stimulation. The deficient proprioception, fibular muscle strength, and ligament integrity of individuals with CAI can participate in sensory input and

motor output and can be detected in the weak joint through CDP system measurements. However, the scope of application may be limited by the laboratory setting and complexity.

This explorative protocol measures lower-limb muscle activity during CDP tasks and provides insight into the muscle coordination of an unstable lower limb. Significant differences exist between the CAI and healthy groups due to the deficient stability of lateral ankle ligaments of participants with CAI. Compared with the participants in the healthy group, those in the CAI group may exhibit an anticipated hip strategy and inappropriate use of vision in SOT, greater velocity of COG in US, longer latency and greater amplitudes in MCT, and greater sway energy in ADT. In addition, the muscle activity for peroneal muscles may decrease during CDP tasks. However, making a safe conclusion on the content of this protocol is not possible based on the findings of the current study due to the future application on CAI participants.

This protocol is based on accurate values and a complete sensory motor pathway, which could provide evidence for scientific community. When applied in the clinic, this protocol provides postural strategy in training and specific muscle rehabilitation for the treatment of patients with CAI. Researchers can use this protocol to investigate postural stability and related muscle activity in other situations, as follows: the neuromuscular control assessment of neurologic disorders, such as Parkinson's disease and multiple sclerosis; the postural stability evaluation of supporting aids, such as high heels and lower-limb prosthesis; and the fall risk and muscle activation assessment of special groups, such as the elderly, flatfooted people, and children with cerebral palsy.

The CDP system provides a training mode that can be used to perform balance training, which includes sequence, weight-bearing, and laboratory-customized training for patients during CDP. Researchers can use the research mode of the system to customize the motor mode and duration of the force plates and the visual surround through the sine wave function. Future research on neuromuscular control can use a combination of other instruments, such as motion capture and plantar pressure systems.

DISCLOSURES

The authors have nothing to disclose.

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REFERENCES

1 Winter, D. A. Human balance and posture control during standing and walking. *Gait & Posture*. **3**, 193-214 (1995).

2 Vanicek, N., King, S. A., Gohil, R., Chetter, I. C., Coughlin, P. A. Computerized dynamic posturography for postural control assessment in patients with intermittent claudication. *Journal of Visualized Experiments*. (82), e51077 (2013).

3 Yin, L., Wang, L. Acute Effect of Kinesiology Taping on Postural Stability in Individuals With Unilateral Chronic Ankle Instability. *Frontiers in Physiology*. **11**, 192 (2020).
4 Fusco, A. et al. Dynamic Balance Evaluation: Reliability and Validity of a Computerized Wobble Board. *Journal of Strength and Conditioning Research*. **34** (6), 1709-1715 (2020).
5 Fusco, A. et al. Wobble board balance assessment in subjects with chronic ankle instability. *Gait & Posture*. **68**, 352-356 (2019).
6 Silva Pde, B., Oliveira, A. S., Mrachacz-Kersting, N., Laessoe, U., Kersting, U. G. Strategies for equilibrium maintenance during single leg standing on a wobble board. *Gait & Posture*. **44**, 149-154 (2016).
7 Domènech-Vadillo, E. et al. Normative data for static balance testing in healthy individuals using open source computerized posturography. *European Archives of Oto-Rhino-Laryngology*. **276** (1), 41-48 (2019).
8 Harro, C. C., Garascia, C. Reliability and validity of computerized force platform measures of balance function in healthy older adults. *Journal of Geriatric Physical Therapy* (2001). **42** (3), E57-e66 (2019).
9 Doherty, C. et al. The incidence and prevalence of ankle sprain injury: a systematic review and meta-analysis of prospective epidemiological studies. *Sports Medicine*. **44** (1), 123-140 (2014).
10 Hertel, J. Sensorimotor deficits with ankle sprains and chronic ankle instability. *Clinics in Sports Medicine*. **27** (3), 353-370, vii (2008).
11 Munn, J., Sullivan, S. J., Schneiders, A. G. Evidence of sensorimotor deficits in functional ankle instability: a systematic review with meta-analysis. *Journal of Science and Medicine in Sport*. **13** (1), 2-12 (2010).
12 Arnold, B. L., De La Motte, S., Linens, S., Ross, S. E. Ankle instability is associated with balance impairments: a meta-analysis. *Medicine & Science in Sports & Exercise*. **41** (5), 1048-1062 (2009).
13 de-la-Torre-Domingo, C., Alguacil-Diego, I. M., Molina-Rueda, F., Lopez-Roman, A., Fernandez-Carnero, J. Effect of kinesiology tape on measurements of balance in subjects with chronic ankle instability: a randomized controlled trial. *Archives of Physical Medicine and Rehabilitation*. **96** (12), 2169-2175 (2015).
14 Jaber, H. et al. Neuromuscular control of ankle and hip during performance of the star excursion balance test in subjects with and without chronic ankle instability. *PLoS One*. **13** (8), e0201479 (2018).
15 Simpson, J. D., Stewart, E. M., Macias, D. M., Chander, H., Knight, A. C. Individuals with chronic ankle instability exhibit dynamic postural stability deficits and altered unilateral landing biomechanics: A systematic review. *Phys Ther Sport*. **37**, 210-219 (2019).
16 Gribble, P. A. et al. Selection criteria for patients with chronic ankle instability in controlled research: a position statement of the International Ankle Consortium. *Br J Sports Medicine*. **48** (13), 1014-1018 (2014).
17 Wrisley, D. M. et al. Learning effects of repetitive administrations of the sensory organization test in healthy young adults. *Archives of Physical Medicine and Rehabilitation*. **88** (8), 1049-1054 (2007).

18 Tabard-Fougère, A. et al. EMG normalization method based on grade 3 of manual muscle testing: Within- and between-day reliability of normalization tasks and application to gait analysis. *Gait & Posture*. **60**, 6-12 (2018).
19 Shim, D. B., Song, M. H., Park, H. J. Typical sensory organization test findings and clinical implication in acute vestibular neuritis. *Auris Nasus Larynx*. **45** (5), 916-921 (2018).
20 Nam, G. S., Jung, C. M., Kim, J. H., Son, E. J. Relationship of vertigo and postural instability in patients with vestibular schwannoma. *Clinical and Experimental Otorhinolaryngology*. **11** (2), 102-108 (2018).
21 Faraldo-Garcia, A., Santos-Perez, S., Crujeiras, R., Soto-Varela, A. Postural changes associated with ageing on the sensory organization test and the limits of stability in healthy subjects. *Auris Nasus Larynx*. **43** (2), 149-154 (2016).
22 Gofrit, S.G. et al. The association between video-nystagmography and sensory organization test of computerized dynamic posturography in patients with vestibular symptoms. *European Archives of Oto-Rhino-Laryngology*. **276** (12), 3513-3517 (2019).
23 Gribble, P. A., Hertel, J., Denegar, C. R., Buckley, W. E. The effects of fatigue and chronic ankle instability on dynamic postural control. *Journal of Athletic Training*. **39** (4), 321-329 (2004).
24 Gribble, P. A., Hertel, J., Denegar, C. R. Chronic ankle instability and fatigue create proximal joint alterations during performance of the Star Excursion Balance Test. *International Journal of Sports Medicine*. **28** (3), 236-242 (2007).
25 Le Clair, K., Riach, C. Postural stability measures: what to measure and for how long. *Clinical Biomechanics*. **11** (3), 176-178 (1996).
26 Fusco, A. et al. Y balance test: Are we doing it right? *Journal of Science and Medicine in Sport*. **23** (2), 194-199 (2020).
27 Riemann, B., Davies, G. Limb, sex, and anthropometric factors influencing normative data for the Biodex Balance System SD athlete single leg stability test. *Athletic Training & Sports Health Care*. **5**, 224-232 (2013).
28 Chiari, L., Rocchi, L., Cappello, A. Stabilometric parameters are affected by anthropometry and foot placement. *Clinical Biomechanics*. **17** (9-10), 666-677 (2002).
29 Chaudhry, H., Bukiet, B., Ji, Z., Findley, T. Measurement of balance in computer posturography: Comparison of methods--A brief review. *Journal of Bodywork and Movement Therapies*. **15** (1), 82-91 (2011).
30 Hertel, J., Braham, R. A., Hale, S. A., Olmsted-Kramer, L. C. Simplifying the Star Excursion Balance Test Analyses of Subjects With and Without Chronic Ankle Instability. *Journal of Orthopaedic & Sports Physical Therapy*. **36** (3) (2006).
31 Gribble, P. A., Hertel, J., Plisky, P. Using the Star Excursion Balance Test to assess dynamic postural-control deficits and outcomes in lower extremity injury: a literature and systematic review. *Journal of Athletic Training*. **47** (3), 339-357 (2012).

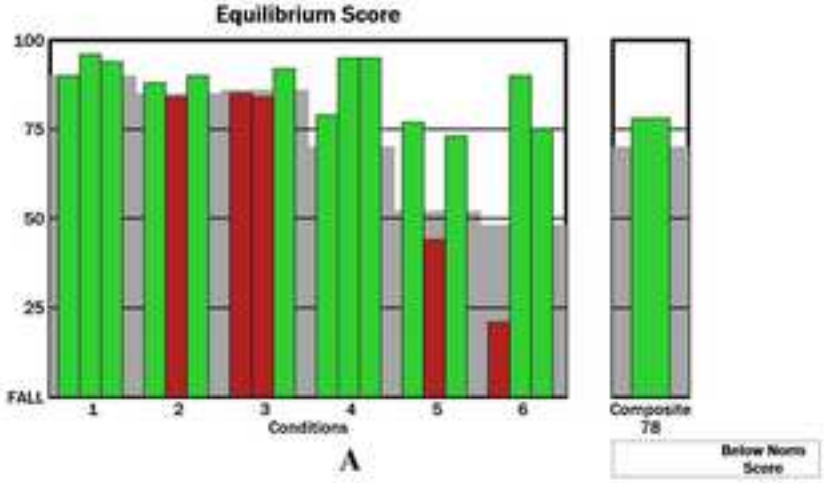
Figure 1. Participant preparation for measurement.

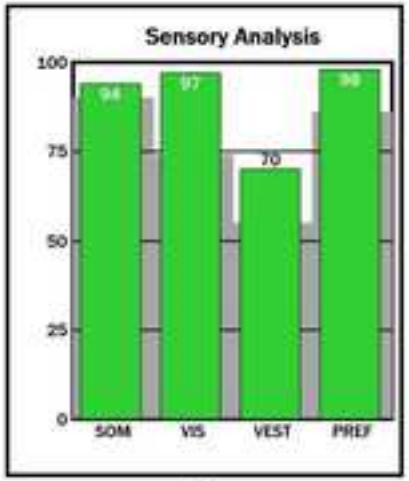
Click here to access/download;Figure;Figure 1.jpg

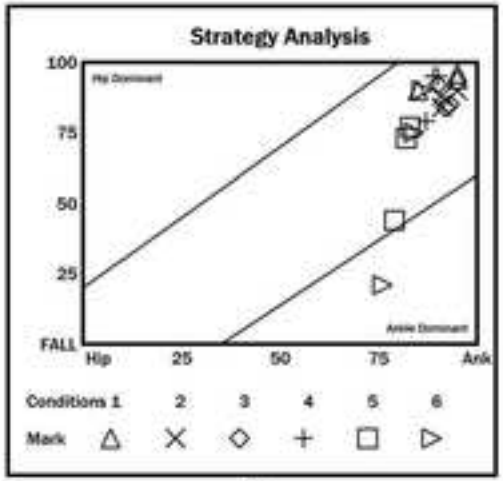
Figure 2. Representative result for participants with CAI during the SOT.

Click here to access/download;Figure;Figure 2.jpg

Conditions	EQUILIBRIUM			STRATEGY			COG Alignment					
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1		Trial 2		Trial 3	
1	90	96	94	85	95	95	-0.7	-0.8	-0.8	-0.3	0.3	-0.8
2	88	84	90	91	91	95	-0.5	0.0	-0.3	-0.5	-0.4	-0.4
3	85	84	92	93	92	90	-0.7	-0.7	-0.6	-0.7	-0.6	-0.3
4	79	95	95	87	90	89	-0.8	-1.0	-0.8	-0.6	-0.7	-0.7
5	77	44	73	83	79	82	-0.5	-0.5	-0.5	-0.7	-0.5	0.1
6	21	90	75	76	86	84	-0.7	-0.5	0.2	-0.8	-0.2	-0.4







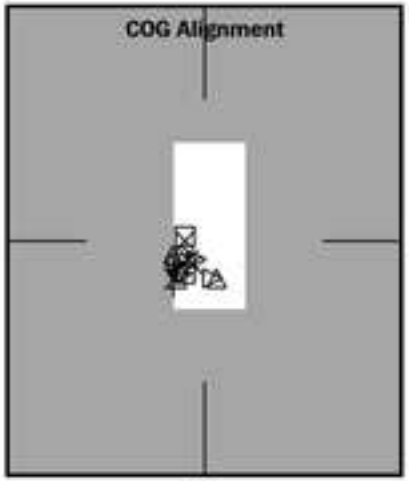


Figure 3. Sway velocity of COG for participants with CAI during US with eyes open/closed (°/s).

Click here to access/download;Figure;Figure 3.jpg

SWAY VELOCITY(deg/sec)/LOB(sec)

Conditions	Trial 1	Trial 2	Trial 3
Left-EO	1.4 /10.0	1.2 /10.0	1.4 /10.0
Left-EC	0.7 /10.0	1.2 /10.0	1.0 /10.0
Right-EO	0.8 /10.0	0.9 /10.0	0.9 /10.0
Right-EC	1.3 /10.0	1.1 /10.0	0.8 /10.0

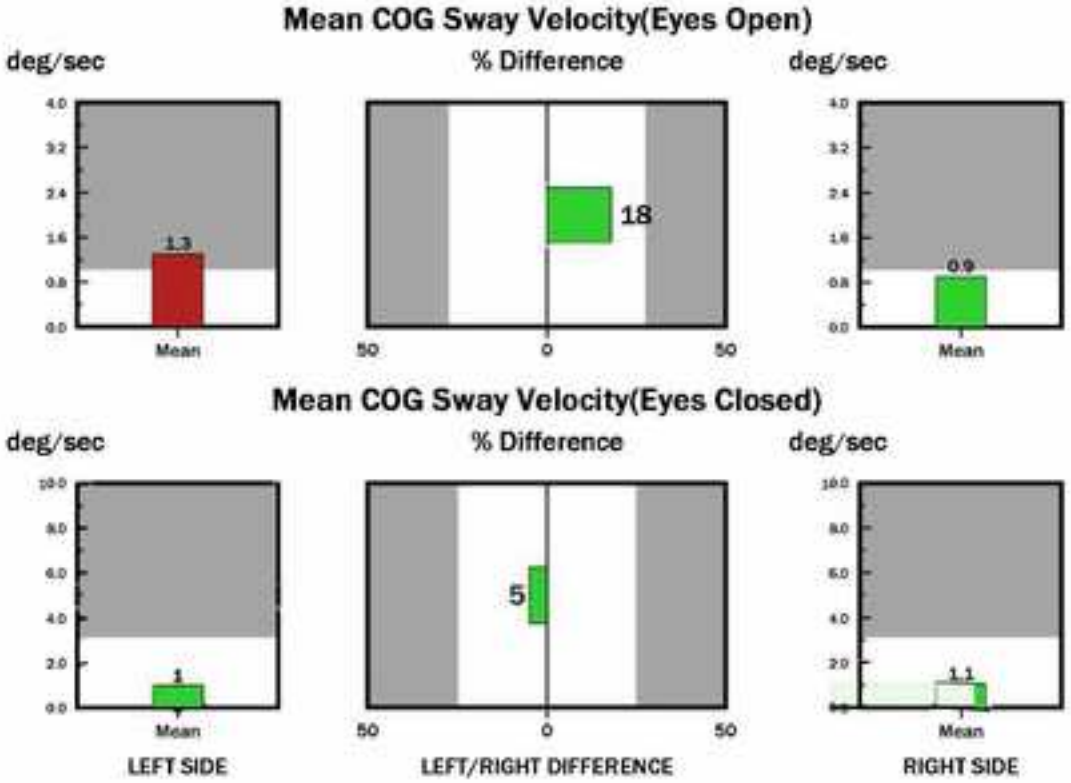


Figure 4. Representative result for participants with CAI during LOS.

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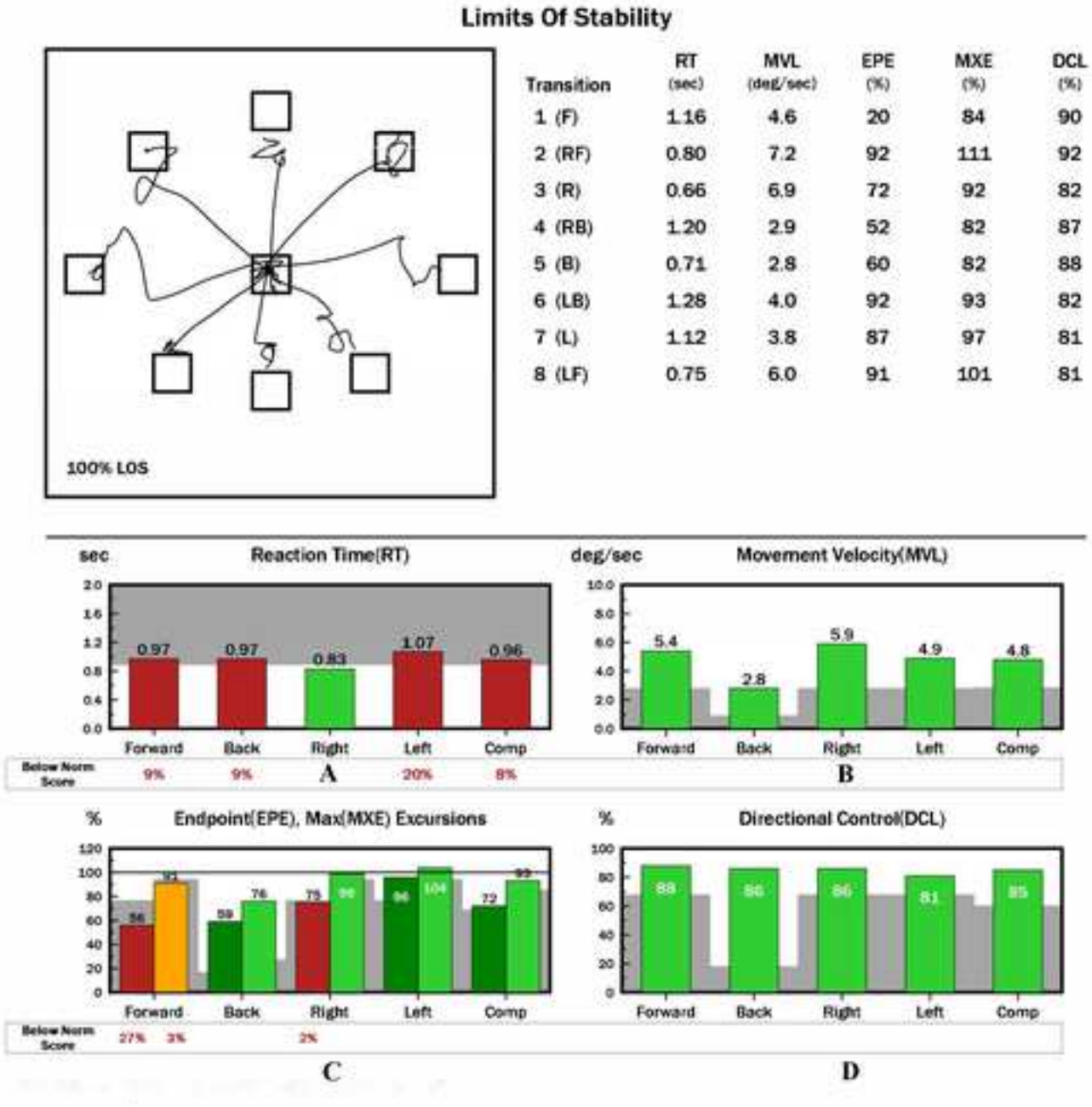
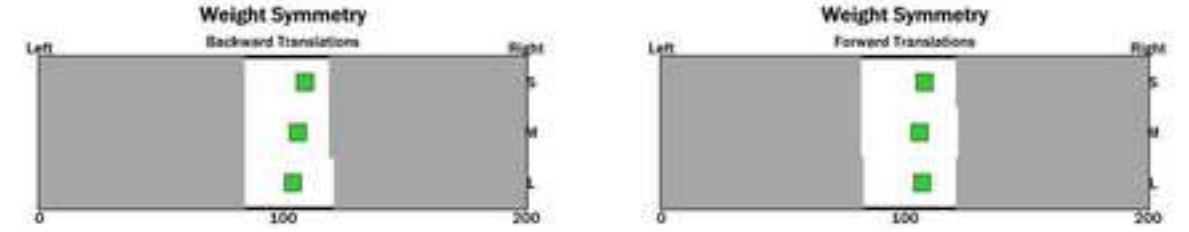


Figure 5. Representative results of participants with CAI during motor control test.

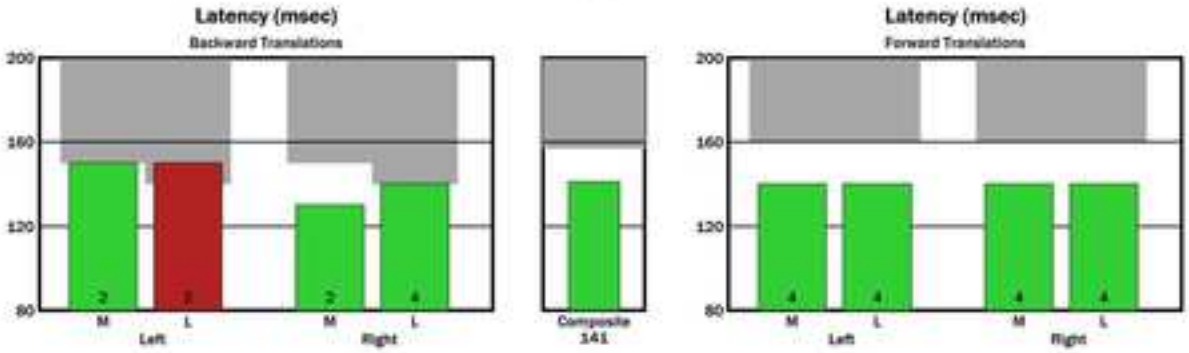
Click here to access/download;Figure;Figure 5.jpg

Translation	WEIGHT SYMMETRY	Latency (msec) Left	Latency (msec) Right	Amplitude Scaling Left	Amplitude Scaling Right	STRENGTH SYMMETRY
Small B	109	150 2	150 3	3	7	140
Medium B	106	150 2	130 2	4	7	127
Large B	104	150 2	140 4	8	12	120
Small F	108	140 2	140 1	5	7	116
Medium F	106	140 4	140 4	7	9	112
Large F	107	140 4	140 4	9	14	121

Composite = 141



A



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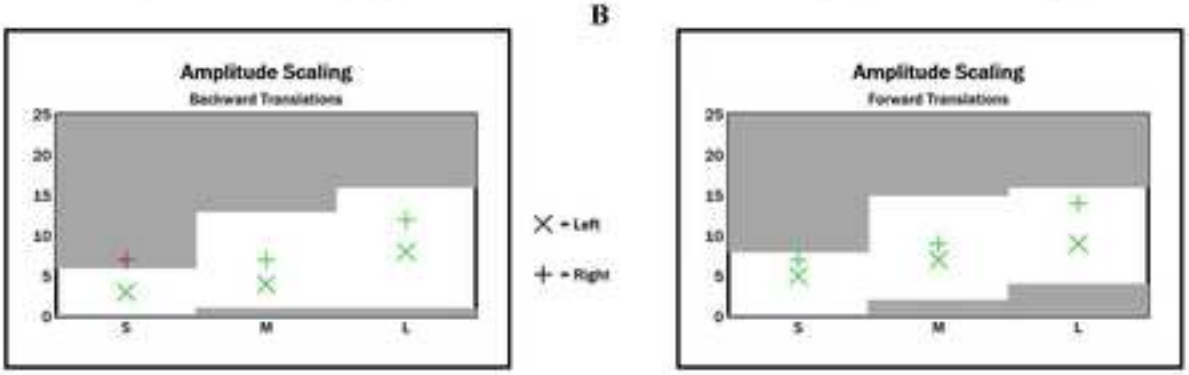
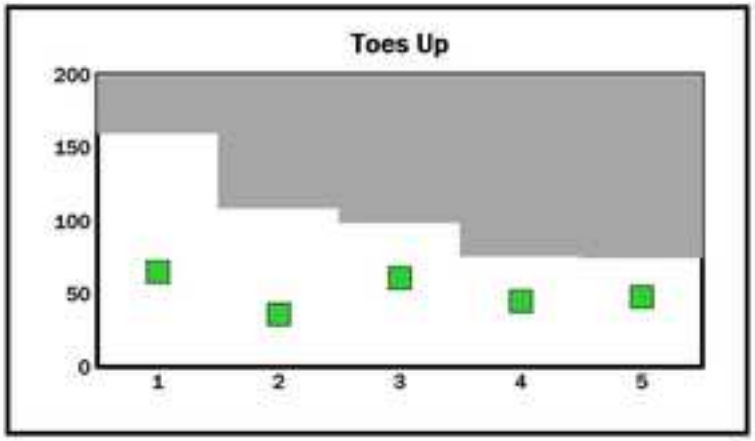


C

Figure 6. SES of participants with CAI during AT.

Click here to access/download;Figure;Figure 6.jpg

	Score				
Conditions	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Toes Up	65	36	61	45	48
Toes Down	50	24	33	38	31



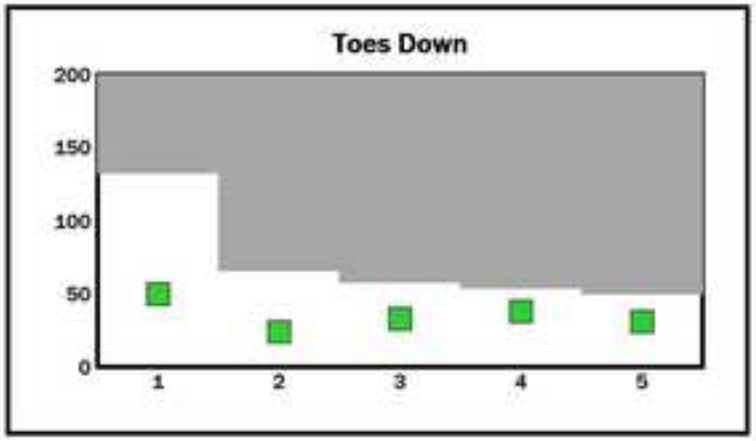


Figure 7. Raw data of sEMG for vastus medialis during SOT, US, MCT and ADT.

Click here to access/download;Figure;Figure7.jpg

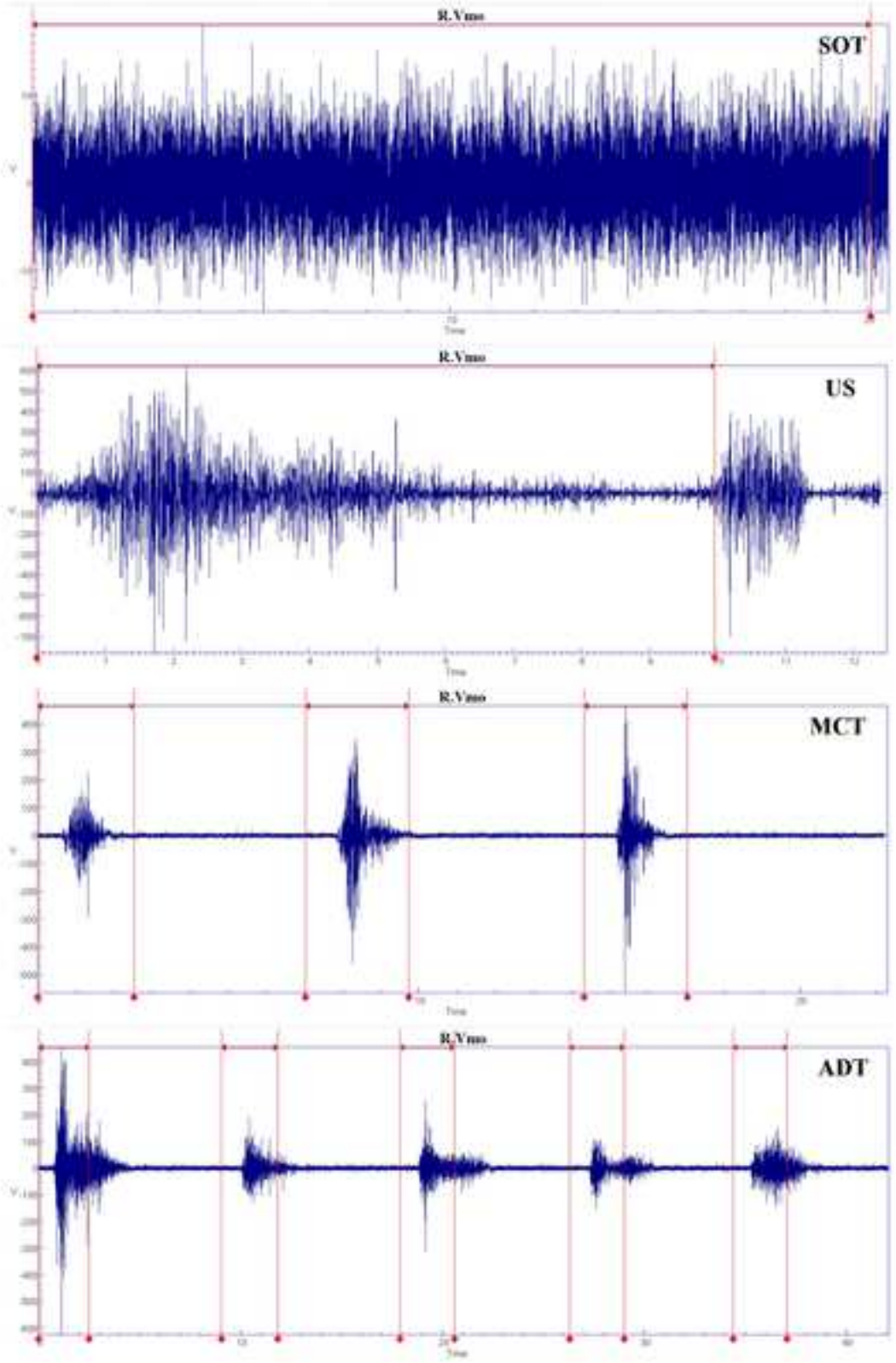


Figure 8. Processed data of sEMG for vastus medialis during SOT, US, MCT and ADT.

Click here to access/download;Figure;Figure 8.jpg

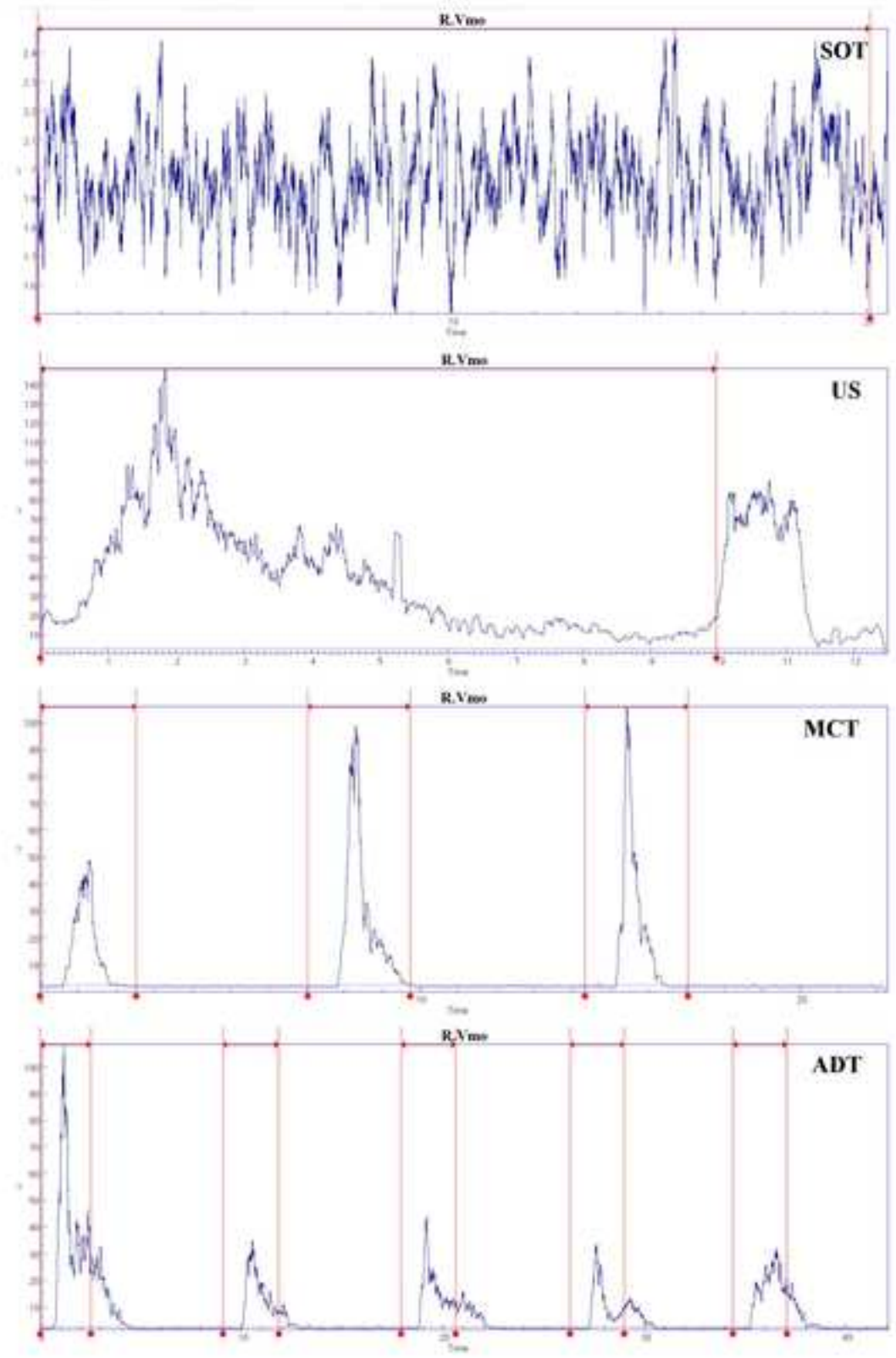


Table 1. Different interference and corresponding anticipated response in sensory organization test.

Condition	Eyes	Force plates	Visual surround	Interference	Anticipated Response
1	Open	Fix	Fix		Somatosensory
2	Close	Fix	Fix	Vision	Somatosensory
3	Open	Fix	Sway-reference	Vision	Somatosensory
4	Open	Sway-reference	Fix	Somatosensory	Vision, vestibular
5	Close	Sway-reference	Fix	Somatosensory, vision	Vestibular
6	Open	Sway-reference	Sway-reference	Somatosensory, vision	Vestibular

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
NeuroCom Balance Manager SMART EquiTest	Natus Medical Incorporated，USA		Its major components include: NeuroCom Balance Manager Software Suite, dynamic dual force plate (rotate & translate), moveable visual surround with 15” LCD display (it could provide a real time display of the subject’s center of gravity shown as a cursor during the task) and illumination, overhead support bar with patient harness, computer and other parts.
wireless Myon 320 sEMG system	Myon AG		The system consists of 16 parallel channels of transmitter signals, receiver, "EMG motion Tools" and "ProEMG" software,computer and other parts.

Editorial comments:

1. Please employ professional copy-editing services as the language in the manuscript is not publication grade. For examples, lines 41-42 is not a sentence: Postural stability and fibular muscle activation level in individuals with CAI decreasing due to the injuries of the lateral ankle ligament complex.

Answer: Thanks for the recommendation, we have sent this manuscript to professional editing company for editing service.

2. The supplemental files and tables have been moved to separate files. Please include a short title and legend for each in the manuscript.

Answer: We have included the short title and legend for each supplemental file. (line623-626)

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

Dear authors, You have revised most of the points raised in my original review and you have improved the clarity of this study but some concerns of mine still remain. To be more specific, you have well defined the aims scopes of this newly introduced protocol (lines 56-59, 68-76, 83-87, 90-96). There is also an addition of control group to ascertain whether there is reduction in stability (lines 154-156). There is a good definition but not a satisfying justification of inclusion/exclusion criteria (lines 147-154, 460-463). For instance you have not explained the Cumberland Ankle Instability Tool or why the initial sprain occurrence is 1 year or more before the recruitment of each subject? Could you please provide the rationale behind your inclusion criteria? For instance, have you complied with the recent selection criteria presented by Gribble et al? [Gribble PA, Delahunt E, Bleakley CM, et al. Selection criteria for patients with chronic ankle instability in controlled research: a position statement of the International Ankle Consortium. J Athl Train. 2014;49(1):121-127. doi:10.4085/1062-6050-49.1.14]

Answer: Thanks for the comments, the inclusion criteria complied with “Selection Criteria for Patients With Chronic Ankle Instability in Controlled Research: A Position Statement of the International Ankle Consortium”. We have added this reference (line154).

About the probable flaws or limitations I am pleased (lines 460-510). Regarding the equipment setup and the yellow highlighted lines I would strongly recommend you consider a presentation in an appendix or a supplemental file only. Also, I strongly recommend setup section be presented in supplemental file for the sake of saving the journal's space. These lines interfere with the text flow and cause confusion as they locate between purpose and representative computerised dynamic posturography results and scores. I have to add one more comment about the structure of your paper; it is inappropriate to write „notes", you have to express them in a continuous text form. The math equations were abridged to the minimum and most necessary (lines 244-

250).

Answer: Thanks for the detailed suggestions, Regarding the equipment setup, the yellow highlighted lines and "NOTES" parts, please allow us to explain the reason.

(1) The equipment setup part was arranged in the main text in accordance with the requirements of the JoVE guideline: *The protocol text should provide a detailed description to enable the accurate replication of the presented method (including setup, materials, actions, conditions, etc.) by both experts and researchers new to the field.*

(2) The yellow lines part complies with the JoVE too: *For a protocol section that exceeds 3 pages, highlight in yellow up to 2.75 pages (no less than 1 page) of protocol text (including headers and spacing) to be featured in the video. Our scriptwriters will derive the video script directly from the highlighted text.*

(3) As for the "NOTE" part, it also complies with the typesetting requirements of the JoVE: *Short notes ("NOTE:") can be used sparingly to describe nonactionable items.*

Last but not least, I would strongly recommend you to compose a paragraph in introduction where the role of this paper as a new protocol introducer will be expressed.

Answer: Thanks for the recommendation, we have added related statement in introduction. (line97-99)

The current article version is lacking result and comparison reporting. Instead, you just present the protocol of a future study. Given this methodological features is not clearly mentioned in the title or the text, I believe this represents a major flaw. You should also elaborate on this generalisation issue in the discussion section. Safe conclusion on the content of this innovative study cannot be made based on the findings of the current study due to future application on subjects.

Answer: Thanks for the comments, we have revised the title as "***Method to Evaluate Postural Control and Lower-extremity Muscle Activation in Individuals with Chronic Ankle Instability"*** in order to highlight this methodological features. *(line2-3).*

In addition, we added related descriptions for this generalisation issue in discussion. (line 501-502)

Reviewer #2:

Manuscript Summary: The authors accepted the comments made in the previous review and implemented the necessary corrections, in particular the choice of the authors of introducing an internal control group for the evaluation of the results in addition to the normative data provided by the equipment.

Minor Concerns: It is not clear the type of study the authors depict: in case of an explorative study this could be sufficient at the aim, but they should explain what results they expect to see. In case of a confirmative study they need to establish also a more definite endpoint and a power calculation. The possible availability of a cohort of historical data on healthy subjects could help in drawing up a primary endpoint. Due to the way the equipment works, researchers should expect to see significant differences during unilateral stance test and the LOS test. CAI instability reduces control of foot inversion and eversion, involving muscle activity in the frontal more than other planes. The unilateral stance test should show the most interesting results when comparing compensative muscle activation patterns with healthy controls. The SOT should show an anticipated hip strategy and inappropriate use of vision even when unreliable. ADT should show an increase in latencies.
Answer: Thanks for the careful comments, this protocol is explorative, we have added related descriptions for possible results in discussion. (line497-501).

Reviewer #3:
Manuscript Summary:
Thank you for providing a revised version for assessment. Overall, the manuscript has greatly improved from the original version. I have no further suggestions.

Major Concerns:
Not applicable.

Minor Concerns:
Not applicable

Thank you for your approve.

Reviewer #4:
No more comments from my part. I thank the authors for addressing all my previous comments and suggestions.

Thank you for your approve

Introduction of Computerized Dynamic Posturography System

The computerized dynamic posturography system (see table of materials) is the gold standard in balance assessment with Computerized Dynamic Posturography. Its major components include: computerized dynamic posturography software suite (see table of materials), dynamic dual force plate (rotate & translate), moveable visual surround with 15" LCD display (it could provide a real time display of the subject's center of gravity shown as a cursor during the task) and illumination, overhead support bar with patient harness, computer and other parts. During dual force plates, there are five force transducers to measures three Forces (Fx, Fy, Fz) and Moments (Mx, My, Mz). It could evaluate the postural stability which based on the inverted pendulum model by tracing the interrelationship between the center of pressure and center of gravity. This system comprised of clinical module and research module. Clinical module consists of objective balance assessment (sensory organization test (SOT), unilateral stance (US), limits of stability (LOS), motor control test (MCT), adaption test (ADT), rhythmic weight shift (RWS), weight bearing squat (WBS))and dynamic training protocols (sequence training, weight bearing and custom training).

1. Double click "Balance Manager system" icon, double-click "clinical module", click "new patient" and establish patient ID, input accurate height, weight age and other information, select "sensory organization test, unilateral stance, limits of stability, motor control test and adaption test", fix participants on support bar with safety harness, align foot correctly with force plates and face with visual surround.
2. SOT: Inform participants: "This test includes 6 different combinations of sensory conditions. In each test, I will remind you to open or close your eyes in advance. You may experience the sway of visual surround or force plates, or both/none. Each sensory condition lasts for 20s and repeats for three times. During test, you need to keep your center of gravity stable as much as possible without moving your feet, your hands naturally hang on both sides of your body, do not touch the visual surround or safety harness. If the balance is lost, this test fails." When participants are ready, click "Start" until each test ends automatically. SOT evaluates the ability of sensory organization and strategy to maintain postural stability through six different combinations of visual, somatosensory and vestibular interference. The movement of dual force plates and visual surround is sway-referenced, which means their movement amplitude and direction will follow the participant's COG. For example, if participant's COG moves forward one degree, the force plate will tilt toes-down one degree.
3. US: Inform participants: "This test require to place your hands on the anterior superior iliac spine with eyes open/closed, take this ankle side as the support leg, fully extend your knee joint, bend the knee of your non-supporting leg by approximately 30°, and remain standing stably for 10 s. Do not touch the visual surround or safety harness. If the balance is lost, this test fails". When participants are ready, click "Start" until each test ends automatically.
4. LOS: Turn on the display and inform participants: "Please look at the cursor in the screen, it represents your center of gravity, first, keep your COG in the central frame, when hearing ring, lean your body and shift quickly your COG into the one of eight targeted frames in the screen and remain steady for 10 seconds. In the process of shifting the COG, your body should be straight, the heel or toes should not away from the force plates, and the hip joint should not bend. When the cursor keeps stable in the central frame, click "Start" until each test ends automatically. Complete eight directional shift in random sequence.

5. MCT: Inform participants: “you will experience the unexcepted slide of the force plates, you need to restore balance through postural coordination. This test includes anterior/posterior slide with small, medium and large amplitude. Each condition repeats three times.” When participants are ready, select randomly direction and amplitude and click “Start” until each test ends automatically.
6. ADT: Inform participants: “you will experience the unexcepted rotation with toes up or down of the force plates, you need to restore balance through postural coordination. Each direction repeats five times.” When participants are ready, select randomly direction and click “Start” until each test ends automatically.
NOTE: there is a display embedded in the visual surround, A frame and cursor will be displayed on the screen. The cursor represents the position of the participant's COG. Generally speaking, when the participant's feet are aligned correctly and stand symmetrically, the cursor would be in the center of the frame. Before test, the screen is turned on, participant would know roughly the position of their COG. In the formal test, in order to simulate reality more realistically and avoid real-time feedback, we turn off the screen before the test in SOT, US, MCT and ADT. During LOS, open the screen due to the display needs to provide orientation.

Application technique on the muscle sites of sEMG electrodes

Muscles	Starting posture	Location of electrode placement
Vastus medialis	Sitting on a table with the knees in slight flexion and the upper body slightly bend backward.	The electrodes need to be placed at lower 80% on the line between the anterior spina iliaca superior and the joint space in front of the anterior border of the medial ligament.
Vastus lateralis	Sitting on a table with the knees in slight flexion and the upper body slightly bend backward.	The electrodes need to be placed at lower 2/3 on the line from the anterior spina iliaca superior to the lateral side of the patella.
Biceps femoris	Lying on the belly with the face down with the thigh down on the table and the knees flexed (to less than 90 degrees) with the thigh	The electrodes need to be placed at 50% on the line between the ischial tuberosity and the lateral epicondyle of the tibia.
Tibialis anterior	Supine or sitting	The electrodes need to be placed at upper 1/3 on the line between the tip of the fibula and the tip of the medial malleolus
Gastrocnemius medialis,	Lying on the belly with the face down, the knee extended and the foot projecting over the end of the table.	The electrodes need to be placed on the most prominent bulge of the muscle
Gastrocnemius lateralis	Lying on the belly with the face down, the knee extended and the foot projecting over the end of the table.	The electrodes need to be placed at upper 1/3 of the line between the head of the fibula and the heel.
Peroneal longus	Sitting with extremity medially rotated.	The electrodes need to be placed at upper 25% on the line between the tip of the head of the fibula to the tip of the lateral malleolus

Posture for EMG Normalization Method for measured muscles.

Muscles	Standard Posture for EMG Normalization Method
Vastus medialis, vastus lateralis	(1) Preparatory position: The participant short sits with a pad under the distal thigh to maintain the horizontal position of the femur. The hands rest on the table on either side of the body for stability. The participant may grasp the table edge. Their knee is flexed at approximately 30°. (2) While giving verbal encouragement, resistance is applied in the direction of knee flexion. The participant is instructed to try to extend the knee for 5 s.
Biceps femoris	(1) Preparatory position: The participant stands in the orthostatic position with the hands on the table for stability. The knee is flexed at 90°, the other leg is straight, and the pelvis is horizontal. (2) While giving verbal encouragement, resistance is applied in the direction of knee extension. The participant is instructed to try to flex the knee for 5 s.

Tibialis anterior	(1) Preparatory position: The participant short sits with a pad under the distal thigh to maintain the horizontal position of the femur. The hands rest on the table on either side of the body for stability, and the participant may grasp the table edge. The tibia is aligned with the vertical direction, and the ankle is flexed at 0°. (2) While giving verbal encouragement, resistance is applied in the direction of ankle plantarflexion. The participant is instructed to try to dorsiflex the ankle for 5 s.
Gastrocnemius medialis, gastrocnemius lateralis	(1) Preparatory position: The participant sits with a pad under the distal thigh to maintain the horizontal position of the femur. The hands rest on the table on either side of the body for stability, and the participant may grasp the table edge. The tibia is aligned with the vertical, and the ankle is flexed at 0°. The forefoot touches the wall. (2) While giving verbal encouragement, the participant is instructed to try to step on the wall with the forefoot for 5 s.

Peroneal longus	(1) Preparatory position: The participant sits with a pad under the distal thigh to maintain the horizontal position of the femur. The hands rest on the table on either side of the body for stability, and the participant may grasp the table edge. The tibia is aligned with the vertical direction, and the ankle is flexed at 0°. (2) While giving verbal encouragement, resistance is applied in the direction of ankle inversion. The participant is instructed to try to evert the ankle for 5 s.