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Balance and Muscle Synergies During a Single-Limb Stance Task in Individuals With Chronic Ankle Instability

Marco Ghislieri, *Member, IEEE*, Luciana Labanca, Massimiliano Mosca, Laura Bragonzoni, Marco Knaflitz, *Member, IEEE*, Maria Grazia Benedetti, and Valentina Agostini

***Abstract*— The aim of this study was to investigate balance performance and muscle synergies during a Single-Limb Stance (SLS) task in individuals with Chronic Ankle Instability (CAI) and a group of healthy controls. Twenty individuals with CAI and twenty healthy controls were asked to perform a 30-second SLS task in Open-Eyes (OE) and Closed-Eyes (CE) conditions while standing on a force platform with the injured or the dominant limb, respectively. The activation of 13 muscles of the lower limb, hip, and back was recorded by means of surface electromyography. Balance performance was assessed by identifying the number and the duration of SLS epochs, and the Root-Mean-Square (RMS) in Antero-Posterior (AP) and Medio-Lateral (ML) directions of the body-weight normalized ground reaction forces. The optimal number of synergies, weight vectors, and activation coefficients were also analyzed. CAI group showed a higher number and a shorter duration of SLS epochs and augmented ground reaction force RMS in both AP and ML directions compared to controls. Both groups showed an increase in the RMS in AP and ML forces in CE compared to OE. Both groups showed 4 optimal synergies in CE, while controls showed 5 synergies in OE. CAI showed a significantly higher weight of knee flexor muscles in both OE and CE. In conclusion, muscle synergies analysis provided an in-depth knowledge of motor control mechanisms in CAI individuals. They showed worse balance performance, a lower number of muscle synergies in a CE condition and abnormal knee flexor muscle activation compared to healthy controls.**

***Index Terms*— Electromyography, motor control, motor modules, postural stability, unipedal stance.**

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This work involved human subjects or animals in its research. Approval of all ethical and experimental procedures and protocols was granted by the Institutional Ethics Committee under Application No. 193/2019-493/2020.

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I. Introduction

CHRONIC Ankle Instability (CAI) is a condition usually developed after a first ankle sprain injury and is featured by recurring episodes of ankle sprains and giving-way, typically accompanied by pain, weakness, a reduction of the joint range of motion, and a reduction of the self-reported function during daily and sporting activities [1], [2], [3], [4].

Among all the impairments, balance is one of the "abilities" majorly affected by CAI [5] for two main reasons. The mechanical instability of the ankle, resulting from the rupture or the damage of ankle ligaments, is the first factor affecting balance. The second factor is represented by the impaired ankle joint proprioception [3]. In fact, the rupture or the damage of ankle ligaments does not lead only to a mechanical ankle instability, but also to the loss of mechanoreceptors involved in the signaling of ankle position and movement [6], thus in turn affecting sensorimotor function and balance [5], [6], [7]. As a consequence, several studies have identified the genesis of CAI in both mechanical and neural factors [8]. From a mechanical point of view, the first episode of lateral ankle sprain causes damage to the structures of the lateral foot-ankle complex including ligaments, nerves, tendons, and muscles, which in turn leads to a mechanical increase of the ankle joint laxity. At the same time, from the neural point of view, there is evidence of reduced excitability of muscles acting on the ankle also at the cortical level causing changes in the motor control of the movements. In this framework, it might be interesting to investigate alterations in motor control strategies of individuals suffering from CAI, considering the overall coordination of muscles orchestrated by the Central Nervous System (CNS).

One of the tasks majorly used for the assessment and training of balance is Single-Limb Stance (SLS) since it is a challenging task requiring an efficient integration of somatosensory, visual, and vestibular information with the

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aim to generate a continuous and effective motor response to manage the reduced base of support [9], [10], [11], [12], [13], [14]. During the performance of the SLS, individuals with CAI have shown a greater postural instability compared to healthy controls, both in terms of normalized mean amplitude of the recorded EMG signals and Balance Error Scoring System (BESS) values [15], and a greater reliance on visual information when performing an open- vs. closed-eyes condition [16]. In addition, on the one hand, an abnormal cortical activity has been reported during the performance of SLS [17], thus showing that CAI affects also central control of movement. On the other hand, contrasting results have been reported regarding leg muscle activations during SLS [15], [18], [19]. In particular, the majority of the studies investigating muscle activations in CAI individuals has focused on muscles acting on the ankle [8]. Variability in the characterization of leg muscle activations during SLS may raise from the fact that the authors of conventional EMG studies typically analyzed and interpreted the activation of each leg muscle independently, without considering their coordination. Indeed, it is well known that the CNS organizes motor response in terms of motor modules (or muscle synergies) [20], [21], i.e., by means of the coordinated activation of a given number of synergistic muscles acting on a number of joints. Therefore, conventional analyses may fail to fully capture the way the CNS controls muscular activations during postural tasks. Given the contrasting results obtained through conventional EMG studies and the modular organization of the CNS during complex movements, it may be beneficial to use a research framework that examines neuromuscular activation patterns in people with CAI during such movements via muscle synergy analysis.

In addition, an increasing number of studies is reporting that individuals with CAI show movement abnormalities also on joints more proximal than the ankle [8], [22] and on the upper part of the body [23], [24].

A recent study has reported no differences in the number of muscle synergies between CAI and healthy individuals [25]. However, this study was conducted only on leg muscles and only during a cutting task. To the best of the authors' knowledge, there are no other studies reporting muscle synergies in individuals with CAI and, more in detail, focusing on muscles acting on multiple joints and during the performance of balance tasks. Thus, the aim of this study was to assess balance and muscle synergies during a SLS task in both open- and closed-eyes conditions in individuals with CAI. Since CAI strongly affects postural stability [15], [26] and a low number of synergies has been observed in individuals with neuromuscular impairments [27], [28], it is hypothesized that CAI individuals will show a worse balance performance and a lower number of synergies in comparison with healthy controls.

II. Materials and Methods

A. Participants

Twenty patients with unilateral CAI (10 females and 10 males; age: 29 ± 9 years; height: 170 ± 10 cm; body mass: 69.6 ± 13.5 kg) were recruited to participate in the study. Inclusion criteria were: *a*) condition of CAI; *b*) age between 20 and 40 years; *c*) physical activity level of 2, 3, or 4 according to the Saltin and Grimby scale [29]. In particular, inclusion criteria to define the condition of CAI were based on the recommendations of the International Ankle Consortium [1]. Participants were excluded if: *a*) they had a history of previous surgeries to the musculoskeletal structures in either lower limb; *b*) they had a history of a fracture in either lower limb requiring realignment; *c*) they had an acute injury to the musculoskeletal structures of other joints of the lower extremity in the previous 3 months, which impacted joint integrity and function (i.e., sprains, fractures, etc.) resulting in at least 1 interrupted day of desired physical activity; *d*) they had a sedentary behavior, as defined by the level 1 of the Saltin and Grimby scale [29].

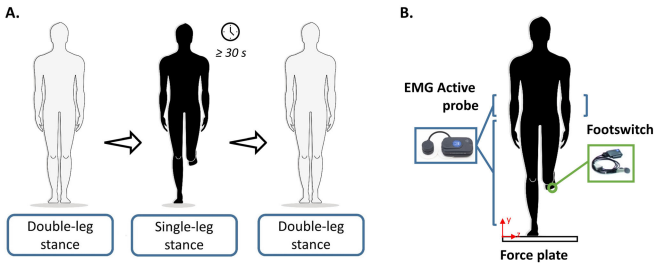


Fig. 1. (A) Schematic representation of the experimental protocol. (B) Acquisition system, EMG active probes are positioned over the main muscles of the lower limb sustaining the SLS and the trunk. A footswitch is positioned under the first metatarsal head of the contralateral foot to detect the onset/offset timing of SLS. A force plate is used to assess body sway during SLS.

Twenty healthy volunteers (10 females and 10 males; age: 24 ± 3 years; height: 180 ± 10 cm; body mass: 65.9 ± 12.2 kg) were recruited as a control group. Inclusion criteria were: *a*) absence of chronic ankle instability; *b*) age between 20 and 40 years; *c*) physical activity level of 2, 3, or 4 according to the Saltin and Grimby scale [29]. Exclusion criteria were: *a*) history of injuries or surgery to the lower limbs; *b*) abnormalities in lower limb and foot joints; *c*) sedentary behavior, as defined by the level 1 of the Saltin and Grimby scale [29].

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee (193/2019-493/2020). Each of the participants signed an informed consent before participating in the study.

B. Experimental Protocol and Data Recordings

The Single-Limb-Stance (SLS) task was conducted considering the experimental setup shown in Figure 1 [14], [30]. In each trial, the participant tried to perform a transition from double-leg stance to single-leg stance, afterwards maintaining unipedal balance as long as they could (up to a maximum of 100 s), and then returning back to double-leg stance. The experimenter checked that the subject could sustain the unipedal stance for at least 30 s. The task was performed under two different testing conditions: with Open Eyes (OE) and Closed Eyes (CE). In the CE condition, the subject closed the eyes right after reaching the SLS balance. Two repeated trials (each of them up to a maximum of 100 s) for each condition were performed in random order. The participants

were allowed to rest for two minutes between the trials. If during a specific trial the subject failed to maintain the SLS balance for at least 30 s, the test was repeated again, until a maximum of 4 times. In this case, the two best trials (with longest SLS epochs) were considered in the analysis.

Participants were asked to stand barefoot on a force platform (Dynamic Walkway P6000, BTS Bioengineering, Milan, Italy) with either the dominant limb (control group), or the injured limb (CAI group) and to maintain the contralateral knee joint flexed at approximately 90°. During the performance of the SLS task, participants were asked to look forward, to maintain their upper limbs aligned to the trunk, and to remain as still as possible for at least 30 s. They were not asked to count the time by themselves, but to stand still as long as they could (up to a maximum of 100 s). Minimal arm movements were allowed; however, participants were asked to minimize them as much as possible.

Muscle activations were recorded from 13 muscles of the dominant/injured lower limb and trunk through surface EMG probes (BTS FreeEMG 1000, BTS Bioengineering, Milan, Italy) fixed on EMG electrodes (Ag/AgCl) applied over Tibialis Anterior (TA), Peroneus Longus (PL), Peroneus Brevis (PB), Soleus (SOL), Lateral Gastrocnemius (LGS), Vastus Medialis (VM), Vastus Lateralis (VL), Rectus Femoris (RF), Lateral Hamstring (LH), Medial Hamstring (MH), Gluteus Medius (GMD), Longissimus Dorsii Ipsilateral to the dominant/injured lower limb (LD_I), and Longissimus Dorsii of Contralateral side (LD_C) in accordance with SENIAM recommendations [31]. Before electrode application, the skin was shaved and cleaned with ethyl alcohol to reduce impedance. A footswitch (FSW) was placed under the first metatarsal head of the contralateral foot (Footswitch Kit, BTS Bioengineering, Milan, Italy). Force platform (to record the ground reaction force for assessing postural sway), EMG probes (to record the electrical activity from muscles), and FSW (to detect the onset/offset timing of SLS) were part of the same integrated system. All the signals were synchronously acquired at a sampling rate of 1000 Hz and then imported into MATLAB® release R2022b (The MathWorks Inc., Natick, MA, USA) to be offline processed through custom routines.

C. Balance Assessment

The balance performance was evaluated, in both CAI and control subjects, with the following procedure:

1) SLS Task Segmentation: For each trial of each individual, the epochs of SLS were segmented by using the FSW signal, as detailed in [30]. When both feet of the subject are in contact with floor the FSW signal is equal to 1, while when one foot is raised from floor the FSW signal is equal to 0. The SLS epochs of each trial are defined as the longest 0-level of the FSW signal, excluding 5 seconds of signal following the 1-to-0 transition from bipedal to unipedal stance, and excluding 5 seconds of signals preceding the 0-to-1 transition from unipedal to bipedal stance. This was chosen to retain in the analysis only the central time samples of "pure" SLS. Examples of FSW signals and SLS task segmentations are shown in Figure 2 for a representative CAI subject.

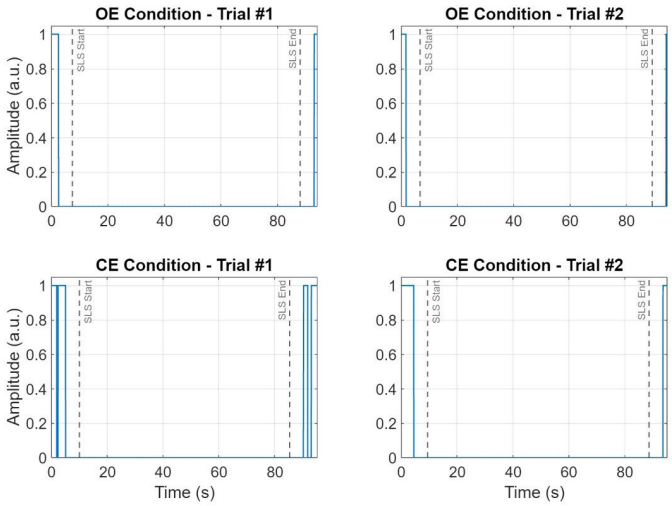


Fig. 2. Examples of footswitch (FSW) signals and Single-Limb Stance (SLS) segmentations for a representative CAI subject in each condition (OE and CE) and trial. FSW signals are equal to 1 when both feet of the subject are in contact with floor, while FSW signals are equal to 0 when one foot is raised from the floor.

2) Balance Outcome Measures: To assess the balance performance of each individual, in each trial, the following parameters were considered:

- Number of SLS epochs (N_{SLS}): number of attempts, within the same trial, required by each participant to perform a SLS task lasting longer than 30 s (if possible). High N_{SLS} values are associated with a reduced balance performance;
- SLS duration (T) (expressed in seconds): time duration of the longest SLS epoch within the trial. Low T values are associated with a reduced balance performance;
- The Root-Mean-Square reaction force in Antero-Posterior and Medio-Lateral directions ($F_{RMS,AP}$ and $F_{RMS,ML}$) (expressed in $N * kg^{-1}$): the RMS of the two ground reaction force components, body-weight normalized, was computed by time-windowing the low-pass filtered force signals (5th order zero-lag Butterworth digital filter with cut-off frequency at 10 Hz) through a 1-second window without overlap.

For each parameter, the parameter's mean value was calculated between the repeated trials performed by each individual, separately for OE and CE visual conditions. Then, the mean values (and standard errors) across the CAI and control populations were evaluated for the following statistical analysis (again separately for OE and CE conditions).

3) Muscle Synergy Extraction and Analysis: Muscle synergies were extracted and sorted in accordance with a previous study [30]. Briefly, EMG signals corresponding to SLS epochs were high-pass filtered by means of an 8th order zero-lag Butterworth digital filter with a cut-off frequency of 35 Hz, full-wave rectified, low-pass filtered through a 5th order zero-lag Butterworth digital filter with a cut-off frequency of 12 Hz, and normalized in amplitude to the global maximum activation of each muscle separately for each trial of each condition [32].

Pre-processed EMG signals were then factorized into low-dimensional components through the Non-Negative Matrix Factorization (NNMF) algorithm [33]. The NNMF algorithm allows for modelling the original EMG data as the linear combination of two low-dimensional elements: the time-independent weight vectors (W) and the time-dependent activation coefficients (C) matrices, of each subject, in each trial modelling the spatial and temporal component of the motor control, respectively. The MATLAB® function "*nnmf*" was used to extract muscle synergies, setting the function's input parameters as defined in [30]. To test different factorization solutions, the factorization process was run several times on the same EMG signals, changing the factorization rank (or number of muscle synergies) from 1 to 13. Muscle synergies extracted from different participants and SLS conditions were sorted in the same order by implementing a k-means clustering approach to the weight vectors (W). k-means clustering was applied setting the number of k-means clusters to the final number of muscle synergies selected, the maximum number of iterations to 1000, the number of replicates to 15, and selecting cosine similarity as distance metric [30]. The activation coefficients (C) were then sorted accordingly.

Usually, muscle synergies are extracted averaging or concatenating a certain number of repeated cycles of the same movement, such as gait cycles [34]. However, given the non-cyclical nature of the postural task under consideration, it is difficult to define what should be considered as a single movement cycle. During one trial, participants performed several postural adjustments to maintain their unipedal stance, each of them characterized by different durations and patterns. Hence, muscle synergies were extracted from the whole SLS epoch ($>$ 30 s, if possible), representing the concatenation of all the postural adjustments performed by the subject.

Since the SLS motor task is not characterized by any distinctive cyclic pattern (differently from motor tasks such as walking or pedaling [35], [36]), any direct interpretation of the activation coefficients C is difficult [21]. Therefore, while W are directly compared between groups (CAI and controls) and conditions (OE and CE), the average recruitment levels ($Recr$) are calculated from the activation coefficients C [21], [30], and compared between groups and conditions.

In addition to the study of the composition of muscle synergies ($Recr$ and W), specific parameters can be evaluated related to the muscle synergy model reconstructing EMG data. One of the most widely used outcome measure is the optimal number of muscle synergies (N) required to reconstruct the original EMG data. Considering a variable number of muscle synergies, the percentage of variance explained by the model is estimated through the R-squared (R^2) value, obtaining the R^2 vs. Number of synergies curve. An example of R^2 curve is represented in Figure 3 for a representative CAI subject. N is defined as the point at which the highest change in slope (the "elbow") is observed in the R^2 vs. Number of synergies curve [37].

The curvature was iteratively computed from every three consecutive R^2 vs. Number of synergies curve's points (i.e., the first curvature is computed considering $N = 1$, 2, and 3; the second considering $N = 2$, 3, and 4; etc.). For each participant and condition, the final number of synergies (N) was defined as the number of synergies in correspondence of the highest curvature among the computed ones. Finally, we selected the number of muscle synergies for each population and condition based on the mode of the number of muscle synergies selected from each trial separately. Variance Accounted For (VAF) related to the selected number of synergies was also estimated. VAF was defined as the uncentered Pearson's correlation coefficient expressed in percentage [21].

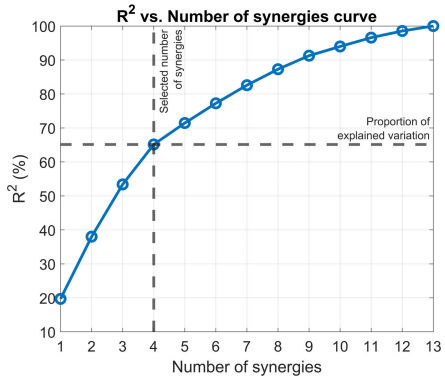


Fig. 3. Example of R^2 vs. Number of muscle synergies curve for a representative CAI subject in OE condition. For each tested number of synergies, the proportion of explained variation (R^2) measures the reconstruction quality of the muscle synergy model. The optimal number of muscle synergies (N=4 in this case) is selected as the point at which the highest change in slope is observed.

The parameter values obtained from repeated trials performed by the same individual, in the same visual condition, were averaged. Then, the mean values (and standard errors) across the CAI and control populations were evaluated, separately for OE and CE conditions, for the following analysis.

4) Statistical Analysis: The statistical analysis was performed through the IBM® SPSS® Statistical Software (SPSS Inc., Chicago, IL) setting the significance level (α) equal to 0.05. All analyzed parameters were expressed as estimated mean values and standard errors.

Before the beginning of the experimental sessions, the sample size was computed (with a statistical power of 0.8) through a dedicated power analysis procedure, considering as main outcome the final number of muscle synergies based on a previous study [30]. We found that a minimum of 16 subjects is required. Hence, we decided to enroll 20 subjects for each population.

To determine if there are statistically significant differences in the computed balance outcome measures, a two-way multivariate analysis of variance (2-way MANOVA) for repeated measures followed by *post-hoc* analysis with Bonferroni adjustment for multiple comparisons was performed setting *Group* (CAI and controls) and *Condition* (OE and CE) as independent variables and N_{SLS}, T, $F_{RMS,AP}$, and $F_{RMS,ML}$ as dependent variables.

To analyze muscle synergies' composition ($Recr$ and W) during SLS task, first, the normal distribution hypothesis was tested through the Lilliefors test. Then, if the normal distribution hypothesis was accepted, a two-tailed

TABLE I

BALANCE AND MUSCLE SYNERGY OUTCOME MEASURES FOR CHRONIC ANKLE INSTABILITY (CAI) PATIENTS AND CONTROLS

	CAI patients		Controls		2-way MANOVA (p-value)	
	OE Condition	CE Condition	OE Condition	CE Condition	Group	Condition
Balance outcome measures						
N_{SLS}	**3.9 ± 0.3**	**5.0 ± 0.4**	**3.3 ± 0.2**	**3.8 ± 0.9**	**0.005**	**0.008**
T (s)	**72.6 ± 5.4**	**33.4 ± 4.7**	**91.2 ± 1.2**	**68.6 ± 5.6**	**<0.0001**	**<0.0001**
$F_{RMS,AP}$ $(N*kg^{-1})$	**0.13 ± 0.04**	**0.17 ± 0.06**	**0.07 ± 0.02**	**0.13 ± 0.04**	**<0.0001**	**0.001**
$F_{RMS,ML}$ $(N*kg^{-1})$	**0.09 ± 0.03**	**0.10 ± 0.03**	**0.04 ± 0.01**	**0.07 ± 0.04**	**<0.0001**	**<0.0001**
					2-way ANOVA (p-value)	
					Group	**Condition**
Muscle synergy outcome measures						
N	**4.9 ± 0.2**	**4.5 ± 0.1**	**5.4 ± 0.1**	**4.8 ± 0.1**	**0.008**	**0.003**

Values of parameters are reported as mean ± standard deviation over the sample populations. Statistically significant differences ($p < 0.05$) between Group or Condition are represented in bold. Group: CAI patients vs. controls. Condition: Open Eyes (OE) vs. Closed Eyes (CE).

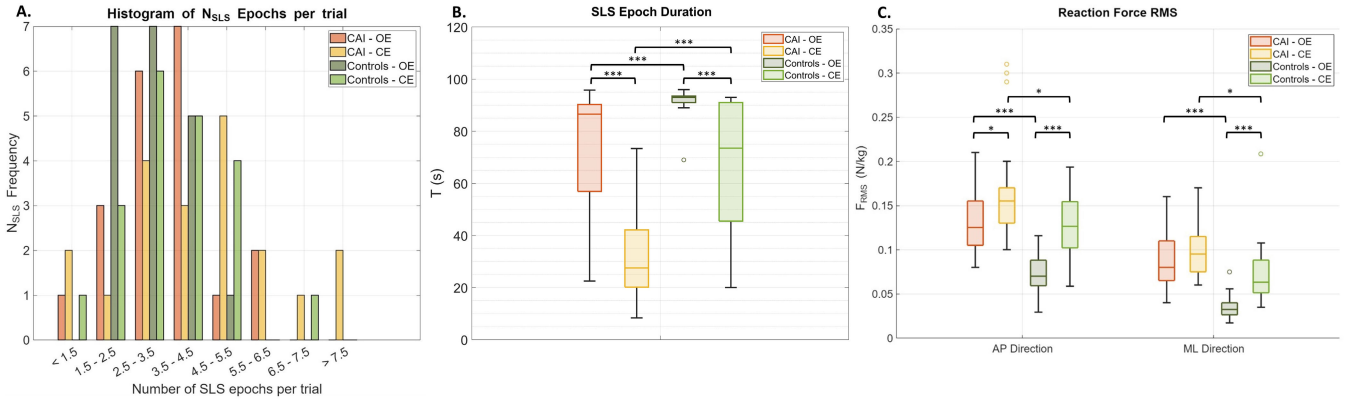


Fig. 4. Comparison of the balance performance of Chronic Ankle Instability (CAI) patients and controls, in each testing condition, i.e., with Open Eyes (OE) and Closed Eyes (CE). (A) Histogram of the Number of Single-Limb-Stance epochs (N_{SLS}) counted within each trial (the averaging between two trials was performed for each subject), (B) boxplots representing the SLS duration (T), and (C) boxplots representing the reaction force Root-Mean-Square (F_{RMS}) in the Antero-Posterior (AP) and Medio-Lateral (ML) directions. Significant differences are marked by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Student's *t-test* was performed, otherwise, a Wilcoxon test was performed.

To assess statistically significant changes in the optimal number of muscle synergies (N), a two-way univariate analysis of variance (2-way ANOVA) for repeated measures followed by *post-hoc* analysis with Bonferroni adjustment for multiple comparisons was performed setting *Group* (CAI and controls) and *Condition* (OE and CE) as independent variables and N as the dependent variable.

III. RESULTS

The balance and motor performance (muscle synergies) of CAI and control populations during the maintenance of unipedal stance were compared, considering both visual conditions (i.e., tests performed with eyes open and closed).

A. Balance Assessment

The balance performance of the two groups (CAI and controls) was compared in terms of: (*i*) the number of SLS epochs (N_{SLS}), (*ii*) the epoch duration (T), and (*iii*) the RMS reaction force in AP and ML directions ($F_{RMS,AP}$ and $F_{RMS,ML}$), in the two visual conditions (OE and CE).

Balance outcome measures of CAI patients and controls are reported in Table I, with the indication of the statistically significant differences ($p < 0.05$).

Overall, a statistically significant 2-way repeated measures MANOVA effect was obtained for both Group (Pillai's trace = 0.58, F(4, 69) = 23.44, $p < 0.0001$, $\eta^2 = 0.58$) and Condition (Pillai's trace = 0.55, F(4, 69) = 21.09, $p < 0.0001$, $\eta^2 = 0.55$). No statistically significant interaction effect between Group and Condition was detected. Between-subject analysis revealed statistically significant main effects for both Group ($p < 0.005$) and Condition ($p < 0.008$) considering all the dependent variables. Results of the *post-hoc* analysis for multiple comparisons are reported in Table I.

Figure 4A reports, separately for each population (CAI and controls) and condition (OE and CE), the histogram of the N_{SLS} values obtained within each trial, averaged across the two trials of each subject. CAI histograms are shifted toward higher values of the number of SLS epochs with respect to controls, a behaviour that becomes even more evident when considering the CE condition.

Overall, the SLS epoch duration (T) is shorter in CAI patients with respect to controls (Figure 4B), while their reaction force RMS (F_{RMS}) is augmented, both in the AP and in the ML directions (Figure 4C).

On average, CAI patients show a conspicuous reduction of T (by 54.0%) when passing from OE to CE condition ($T_{OE} = 72.6$ s; $T_{CE} = 32.4$ s), while the analogous reduction of T in controls is only by 24.5% ($T_{OE} = 91.2$ s; $T_{CE} = 68.8$ s). On average, CAI patients

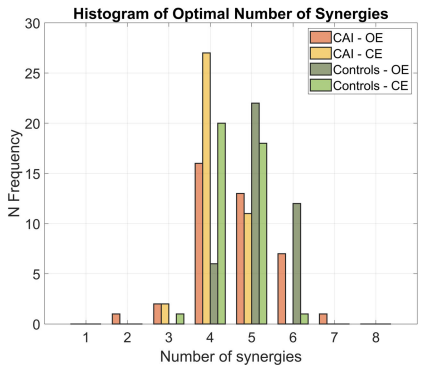


Fig. 5. Histogram of the optimal number of muscle synergies (N) expressed during the Single-Limb-Stance (SLS) task for each group (patients suffering from Chronic Ankle Instability and controls) and testing condition (Open Eyes and Closed Eyes).

significantly increase their $F_{RMS,AP}$ when passing from OE to CE condition (from 0.13 $N*kg^{-1}$ to 0.17 $N*kg^{-1}$), while no significant change was observed considering $F_{RMS,ML}$. On average, controls significantly increase their reaction force RMS when passing from OE to CE condition in both AP and ML.

B. Muscle Synergy Analysis

The motor performance of the two groups (CAI and controls), in the two visual conditions (OE and CE), were compared in terms of optimal number of muscle synergies (N) and composition of muscle synergies ($Recr$ and W).

1) Optimal Number of Muscle Synergies (N): Two-way ANOVA for repeated measures revealed statistically significant main effects between Group ($p = 0.008$, $\eta^2 = 0.09$) and Condition ($p = 0.003$, $\eta^2 = 0.11$). No statistically significant interaction effects between Group and Condition were detected ($p = 0.50$).

Figure 5 reports the histogram of the optimal number of muscle synergies (N) of each group (CAI and controls), in each condition (OE and CE). CAI histograms are shifted toward smaller values of N with respect to controls, a behavior that becomes even more evident when considering the CE condition. In the following, the average VAF values obtained considering the selected number of muscle synergies are reported: 89.8 $\pm$ 2.3% and 89.2 $\pm$ 2.5% considering CAI patients during OE and CE conditions, respectively; 92.1 $\pm$ 1.3% and 90.4 $\pm$ 1.9% considering control subjects during OE and CE conditions, respectively.

*2) Composition of Muscle Synergies (*Recr *and* W*):* Figure 6 compares the recruitment levels ($Recr$) and the weight vectors (W) between groups and conditions. Notice that the information conveyed by the two panels (A and B) of the figure is similar, however, Figure 6A directly compares the groups, separately for each visual condition, while Figure 6B directly compares the visual conditions, separately for the two groups. We decided to keep both representations to help the reader focusing either on the group comparison (CAI vs. controls) or in the condition comparison (OE vs. CE).

It can be observed (Figure 6A, CE condition) that the muscle recruitment levels $Recr_1$, $Recr_2$ and $Recr_3$ are increased in CAI patients with respect to controls. Considering the weight vectors, in the second synergy (W_2) the weights of TA and SOL muscles are increased in CAI patients with respect to controls, while the weight of GMD is decreased. In the third synergy (W_3), the weights of TA and SOL muscles are decreased in CAI patients with respect to controls. In the fourth synergy (W_4), besides the decrease of the TA weight, there is an increase in the weights of LGS, LH, MH and GMD in CAI patients with respect to controls. In particular, the weight increase of the LH and MH muscles are noticeable.

IV. Discussion

In literature, the study of muscle synergies in upright stance is mainly focused on the evaluation of balance recovery after a perturbation [38], [39], [40], [41], [42].

The main result of this study is that CAI patients show a worse balance performance, and lower number of muscle synergies in the open-eyes condition during a SLS task when compared with controls participants, thus confirming the initial hypothesis of the study.

With regards to balance, the results observed in this study are in line with those reported by previous literature highlighting a worse performance of CAI compared to healthy individuals [4], [43]. The worse balance performance should be ascribed to the mechanical instability given by the rupture/damage of the ankle ligaments [4] and to the loss of proprioceptors located in ligaments, thus to a failure in transmitting an ongoing information to CNS on the ankle positions and movements [16], and in turn to an abnormal sensorimotor control and balance [5], [7], [16]. These abnormalities are not a peculiarity of CAI individuals, but they have been also observed in case of other muscle skeletal injuries/pathologies [44], [45], [46], [47], thus showing the key role of joint integrity for the control of balance and posture.

Furthermore, it has been reported that CAI individuals strongly rely on visual information to control posture and balance [16]. Thus, it was not surprising to observe in this study that CAI group not only had worse performance compared with the control group, but also a marked worsening of the balance performance in the closed-eyes compared to the open-eyes condition, as showed by the higher number and the shorter duration of SLS epochs. Previous literature has largely reported that there is a transition to the predominance of vision above other senses in case of loss of peripheral sensitive information [48], [49].

The higher postural instability of CAI individuals was observed also in the higher AP and ML ground reaction forces when compared to healthy controls in both open- and closed-eyes conditions.

The first two common synergies (W_1, W_2) observed in both CAI and healthy controls are highly representative of the role of the ankle joint for the control of balance during the SLS task. In fact, they clearly show a consistent activation of all the leg muscles acting on the ankle and involved in the control of ML and AP sway. The other two common synergies (W_3, W_4) are features by a mixed activation of ankle, knee, hip and back muscles.

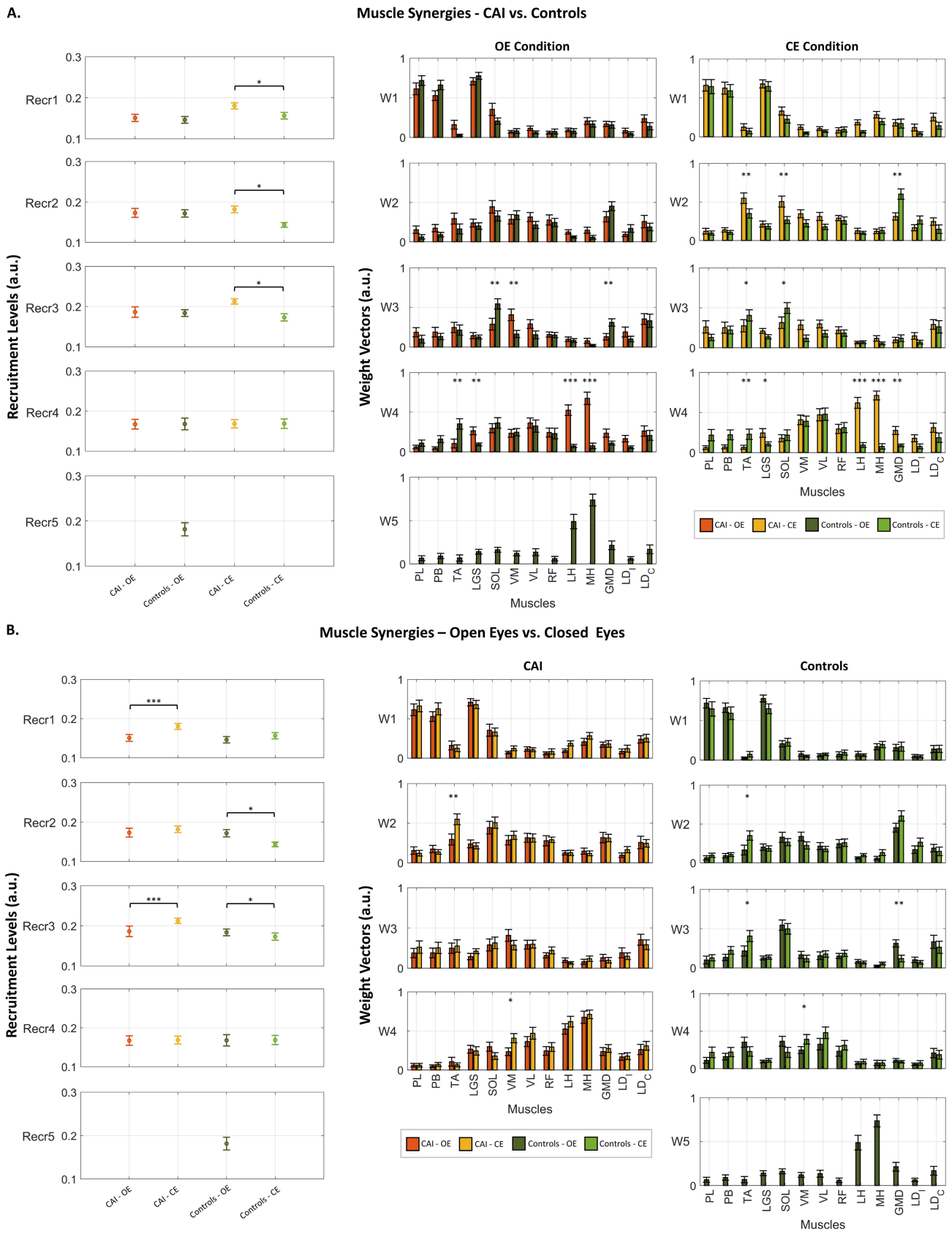


Fig. 6. Comparison of muscle synergies in Chronic Ankle Instability (CAI) patients and controls, in each testing condition, i.e., with Open Eyes (OE) and Closed Eyes (CE). Panel A directly compares the two groups (CAI and controls), separately for each condition, while panel B directly compares the visual conditions (OE and CE), separately for each group. For each muscle synergy (k = 1,. . .,N), the recruitment level (*Recrk*,k = 1,. . . , N) and weight vector (*Wk*, k = 1,.., N) are displayed (mean value and standard error across the population). Significant differences are marked by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

It is interesting to notice that no significant differences between the groups were observed in the recruitment level of all the synergies during the open-eyes condition, thus confirming that visual feedback may compensate for proprioceptive deficit in CAI individuals. In line with this, during the closed-eyes condition, CAI individuals showed a high recruitment level of the first three synergies compared to controls. In these latter synergies, the contribution of the muscles acting on the ankle is consistent; thus, it is plausible to think that the higher reliance on these synergies is a strategy to counteract mechanical instability of the ankle.

Noteworthy, in CAI individuals, knee flexor muscles showed a prominent role in the fourth synergy. Since this latter synergy is mainly featured by the activation of muscle acting on the knee, it could be speculated that it is used during major sway events, where the role of the ankle is not enough to control balance [50]. Furthermore, it has been shown that CAI instability also leads to an abnormal control of muscles acting on more proximal joints, i.e., the knee and the hip [8], [22], and that an arthrogenic inhibition affects knee flexor muscles [51]. Considering these abnormalities, it is plausible to think that CAI individuals need a higher coactivation of knee extensor/flexor muscle to stabilize the knee joint. It should be also noted that the fourth synergy is the one featured by the highest activation of the quadriceps, also in healthy controls. However, in healthy controls the higher activation of the quadriceps was not accompanied by an activation of the knee flexors, which instead are consistently activated in a fifth additional synergy (W_5). Further studies are needed to better understand the abnormal control of more proximal joints in individuals affected by CAI, as well as the reason why a synergy with the predominance of knee flexors was observed only in the open-eyes condition in healthy controls.

From a practical point of view, the results of this study are of importance for rehabilitation interventions of individuals with CAI since they point out that deficit in balance are related to motor control abnormalities which not only affects muscles acting on the unstable ankle, but also those acting on more proximal joints. Furthermore, the high reliance on vision of CAI individuals highlights the considerable deficit in ankle joint proprioception. Thus, training interventions should focus on whole body motor control as well as on the enhancement of the ankle joint proprioception in particular in closed-eyes conditions.

V. Conclusion

In conclusion, individuals with CAI show worse balance during a SLS task when compared with healthy controls and a lower number of muscle synergies when visual information is lacking, thus showing a high reliance on vision to compensate for alterations in proprioception affecting the ankle. CAI individuals also show a higher activation of the knee flexor muscles regardless of the presence of visual information, probably as a strategy to stabilize the knee joint. The analysis of muscle synergies provides an in-depth knowledge of motor control mechanisms in CAI patients that cannot be obtained through traditional approaches of balance analysis and posturography. Future studies should focus on mechanisms leading to abnormal control of muscles acting on lower limb joints in individuals with CAI.

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