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Corticospinal Excitability during Standing and Its Association with Postural Control Following Acute Lateral Ankle Sprain.

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Context: Individuals with acute lateral ankle sprain (ALAS) demonstrate balance deficits and altered neural excitability associated with acute injury symptoms. However, it is unknown whether corticospinal excitability is altered during standing after ALAS and which factors are associated with its neural change.

Objective: To determine the alteration of corticospinal excitability during single-leg standing and its relationship with postural control and acute injury symptoms following ALAS.

Design: Case-Control Study.

Setting: Research Laboratory.

Patients or Other Participants: Fourteen individuals with ALAS and 14 uninjured matched controls participated.

Main Outcome Measure (s): We measured the normalized motor evoked potential (MEP) in the fibularis longus using transcranial magnetic stimulation at 100%, 120%, and 140% of the active motor threshold (AMT) while maintaining a single-leg stance. Postural control during the same balance task was evaluated on a force plate by analyzing center-of-pressure (COP) parameters.

Results: Individuals with ALAS showed a higher normalized MEP at AMT100% (29%, P = 0.019) and greater COP velocities (total: 23%, P = 0.030; anterior-posterior: 20%, P = 0.013) and COP area (29%, P = 0.031) during single-leg standing compared to uninjured controls. Further, correlation analyses revealed that a higher normalized MEP was not associated with

acute injury symptoms (swelling: r_s = 0.387, pain: r = -0.084, P > 0.05) but moderately with a greater total COP velocity (r_s = 0.543, P = 0.048).

Conclusions: Following ALAS, corticospinal excitability in the fibularis longus is altered during a single-leg stance, and the level of excitability is associated with an increased rate of postural sways. These findings suggest a compensatory supraspinal mechanism for impaired postural control following ALAS. A future longitudinal study is warranted to determine whether these early neurobehavioral changes persist throughout the recovery period following the injury.

Word Count: 286

Key Words: acute injury, balance, neural adaptation, transcranial magnetic stimulation

Key points:

- Patients with ALAS had altered corticospinal excitability of the fibularis longus muscle, resulting in an increase in neural excitability.
- Increased corticospinal excitability of the fibularis longus was associated with impaired postural control during a single-leg balance in patients with ALAS.
- Patients with ALAS may adopt a compensatory supraspinal strategy to regulate postural instability experienced during a single-leg balance.

An acute lateral ankle sprain (ALAS) is a common injury affecting both general and sports populations worldwide.[1-4] ALAS has a substantial re-injury rate, with studies showing nearly 70% of individuals with an initial ALAS at risk of recurrence and one-fifth of them at high risk of developing persistent joint impairment, such as chronic ankle instability (CAI).[5] CAI is characterized by repeated episodes of the ankle giving way, a feeling of instability, and residual symptoms, which can impair daily activities and athletic performance [6]. The risk of intra-articular cartilage degeneration, such as post-traumatic ankle osteoarthritis, increases with repetitive ankle injuries and CAI.[7,8] Considering its long-term impact on our health community, there is a growing need for a clear understanding of the pathogenesis at the onset of ALAS to treat effectively and mitigate secondary consequences after ankle injuries.

Balance deficits have been indicators of motor-behavior impairments that affect joint stability after an ankle sprain, occurring in individuals with both ALAS and CAI.[9,10] Damage to the lateral ankle ligament complex accompanies acute inflammation, disrupted mechanoreceptors, altered afferent input, and impaired sensorimotor function.[11-13] Changes in balance control, such as an increased center of pressure (COP) excursions following ALAS,[14] may reflect ongoing neural deficits that interfere with injury recovery and rehabilitation.[15] Previous studies have suggested that the spinal reflex pathway may be related to these balance deficits after ALAS,[16-18] as it allows for reflex responses to postural disturbance, relaying sensory signals to spinal motoneurons and activating muscles to adjust posture.[19] However, our recent trial failed to find a significant relationship between changes in spinal reflex excitability during standing and impaired postural control after ALAS.[20] This observation indicates that other neural pathways may play a critical role in postural control deficits after ALAS.

While the traditional view of postural control during upright standing focused on the spinal level, sub-cortical regions, and cerebellum,[21] the importance of cortical control in postural control, particularly the role of the primary motor cortex via the corticospinal pathway, has been increasingly recognized in recent years.[22-24] Transcranial magnetic stimulation (TMS) studies have found that the corticospinal excitability is modulated in response to the demands of postural tasks.[23,25,26] Moreover, the corticospinal excitability of the fibularis longus, a critical muscle for joint stability after ALAS, was associated with the COP excursions during a standing position.[23] These data suggest that corticospinal excitability following ALAS may provide greater insights into the neural mechanisms responsible for sensorimotor deficits such as postural control.

Although emerging evidence has shown altered corticospinal excitability in patients with CAI, the evidence in ALAS patients is scarce. Additionally, most studies regarding corticospinal excitability have been conducted in non-balance conditions[27-29] and may not have fully addressed the impaired balance following ALAS. Since ALAS likely occurs in an unstable environment in which the body's postural control is challenged, it is crucial to examine this neural function in postural control, such as single-leg stance, in which ALAS patients often present postural instability.[14,30] While previous research[24] has found diminished corticospinal excitability during single-leg balance in CAI, it is still unknown whether the neural change arises from ALAS or is an indication of CAI.

Therefore, the primary purpose of this study was to determine changes in the corticospinal excitability of the fibularis longus during single-leg standing in patients with ALAS. The fibularis longus is crucial for ALAS patients because it prevents excessive ankle inversion and further sprains while contributing to postural control.[31] If neural changes were found, we would be interested in their association with impaired postural control during the single-leg

stance. As a secondary purpose, we explored the relationship between these neural changes and acute symptoms (ankle pain and swelling) because previous research has demonstrated the association but was limited to neural excitability at the spinal level.[17,20] We hypothesized that reduced corticospinal excitability and impaired postural control would be present in ALAS, and these neural changes would be moderately associated with impaired postural control and acute symptoms.

Methods

Procedure

In this case-control study, 14 patients with ALAS and 14 healthy uninjured controls participated. The study was approved by the author's institutional review board. All the participants provided written informed consent prior to the initiation of study procedures. Participants visited the sports medicine laboratory twice first for eligibility screening and second for outcome measurements. During the screening, a licensed athletic trainer administered a standardized ankle injury evaluation consisting of but not limited to acute symptoms, palpation, special tests, and functional capability in accordance with previous guidelines.[32] Participants underwent assessments of single-leg balance and corticospinal excitability on a second day, at least 24 hours apart from the screening.

Participants

We enrolled patients with ALAS who presented acute symptoms of pain/tenderness, swelling, and loss of function. To evaluate the acute symptoms, we used the visual analog scale for pain assessment,[33] the figure-of-8 method for measuring ankle swelling,[34] and the Foot and Ankle Ability Measure (FAAM) for assessing self-reported ankle dysfunction during activities of daily living (ADL) and sports.[35,36] The experienced athletic trainer performed manual

ligamentous stress tests, including anterolateral drawer test, anterior drawer test, and inversion talar tile test, to examine the amount of anterior or lateral laxity of the ankle joint. We also identified and ruled out potential patients with other types of ankle sprains, such as syndesmotic and medial ankle sprains, or ankle/foot fractures through special tests or the Ottawa Ankle Rules, respectively. We categorized the severity of ALAS into grades I, II, and III based on the levels of discomfort and symptoms, ranging from mild to severe. General physical activity levels were evaluated using Global Physical Activity Questionnaire (GPAQ), which was divided into three activity levels: low, moderate, and high levels of physical activity prior to the study participation.[37]

Specific inclusion criteria for patients with ALAS are as follows: 1) a recent history of lateral ankle sprains within the past two weeks, 2) current self-reported ankle dysfunction quantified by scores of ≤ 90% in Foot and Ankle Ability Measure (FAAM) of daily living (ADL), and of ≤ 80% in FAAM-sports, and 3) no current lower extremity injury other than a recent acute lateral ankle sprain.

Uninjured controls were matched for sex, age, height, and weight of ALAS. They were included if they met the following criteria: 1) no history of an ankle injury, significant lower extremity injuries, or surgeries, 2) no episodes of ankle joint giving way, 3) no self-reported ankle dysfunction determined by scores of ≥ 95% in FAAM-ADL, and of ≥ 90% or greater in FAAM-S, 4) no current lower extremity injury, and 5) no history of injuries to the lower extremity joints in the previous six months, resulting in at least one interrupted day of physical activity.

Participants were excluded if they had 1) any history of musculoskeletal injuries to the lower extremity joints other than ankle sprain in the previous six months, resulting in at least one

interrupted day of physical activity, 2) any history of surgery in the lower extremity, 3) any history of low back pain in the previous six months, 4) any history of diagnosed neurological disorder, 5) any history of seizure disorder, 6) any metal/electrical/magnetic devices implanted in the body (e.g., cardiac pacemaker) except dental fillings, 7) a poorly controlled headache, and 8) hypersensitivity to magnetic stimulation.

Electromyography (EMG) Recordings

Participants lay prone on a table for surface EMG placement. The skin area was shaved with a razor and cleansed with skin preparation gel (Nuprep, Weaver and Company, Aurora, Colorado, USA). Bipolar EMG electrodes (Ag/AgCl EMG electrodes) were attached to the muscle belly of the fibularis longus, and the reference electrode was attached to the medial malleolus based on SENIAM guidelines.[38] Electrode impedance contact was assessed using an electrode impedance checker with an acceptable impedance level of < 5 kΩ. EMG signals were amplified at a gain of 1000, band-pass filtered of 10 Hz to 500 Hz, and sampled at 2 kHz. The analog-to-digital signal was converted using a 16-bit converter (MP160; BIOPAC Systems, Goleta, CA, USA), and EMG signals were displayed and recorded using Acknowledge software (Ver 5; BIOPAC Systems).

Corticospinal Excitability:

Participants were instructed to stand barefoot on a force plate with their arms crossed over their chest. They first maintained a bipedal stance as a reference position and then transitioned to a single-leg stance for testing the corticospinal excitability induced by transcranial magnetic stimulation (TMS). One to two minutes of rest was given every 5 to 10 minutes to prevent fatigue and discomfort during the testing. In a single-leg stance, participants stood with a slightly bent knee, while keeping the non-stance leg off the ground. A double-coned coil (D110,

Magstim Company Ltd, Wales, UK) connected to a single-pulse TMS stimulator (Magstim® 200^2, Magstim Company Ltd, Wales, UK) was used. A monophasic current was applied to the contralateral side of the injured or involved side with the current traveling in the posterior-anterior direction. Participants wore a Lycra swim cap with a dot grid line of 1cm x 1cm squares and a straight line in the mid-sagittal plane. A series of TMS stimuli of 1.0 Tesla was delivered to identify the hotspot location of the motor cortex related to fibularis longus, where the largest amplitude was observed.[39] Once the hotspot was determined, the location was marked with a pen on the swim cap, and the cap was secured with elastic wrap. The active motor threshold (AMT) was defined as the lowest stimulation intensity required to elicit a peak-to-peak motor-evoked potential (MEP) amplitude above 3SD of mean background EMG activity that was measured during a 10-sec single-leg stance. This approach was adopted to improve the existing criterion that used a fixed cut-off value[40,41] or 2 SD above the peak-to-peak amplitude,[24] which is believed to be a robust method for detecting MEP response during high levels of muscle activity during a single leg balance. The AMT was determined using software that runs the maximum-likelihood threshold tracking algorithm, Parameter Estimation by Sequential Testing (PEST).[42] Ten trials of TMS were delivered at three different TMS intensities of 100%, 120%, and 140% of AMT. TMS tests were performed while participants maintained a single-leg balance. Any trials not maintaining proper single-leg stance were discarded and repeated, including noticeable adjustment of the ankle, knee, hip, or trunk or contact of the non-test limb with stance limb or locked knee. The background EMG (bEMG) activity of fibularis longus was measured by calculating the root mean square of EMG signals of 50 ms before the onset of the TMS stimulus to normalize each MEP amplitude (normalized MEP/bEMG).[24] The normalization method was

used to minimize the effects of bEMG activity on MEP.[24] The averages of normalized MEP at each TMS intensity were used as an outcome variable representing the corticospinal excitability.

Single-leg Balance

For assessment of single-leg balance, we utilized the previously used methods[14,30] with a force plate (AccuSway Plus, AMTI, Waterfront, MA). Briefly, the foot positions were outlined with blue tape on the center of the force place in a rectangular manner, along with their foot sizes, to ensure consistent foot positioning on the force place. Participants were asked to stand as still as possible during testing. Participants completed three trials of single-leg stance with eyes open for 10 seconds during each trial at a sampling rate of 50 Hz. If a participant touched down with the opposite limb, contacted the stance limb, or was unable to maintain a standing posture during the 10-second trial, the trial was terminated and repeated. Balance Clinic Software (Advanced Mechanical Technology, Inc) and customized programming software (MATLAB) were used to compute center of pressure (COP) excursion outcomes including total COP velocity (cm/s), medial-lateral (ML) COP velocity (cm/s), anterior-posterior (AP) COP velocity (cm/s), and COP area (cm^2), defined as a 95% COP confidence ellipse.[14,43] A higher COP parameter reflects poorer postural control during a single-leg stance. Three successful trials of each balance test were recorded and averaged for statistical analysis.

Statistical Analysis

The Shapiro-Wilk test was used to determine the normality of data. Robust Regression and Outlier Removal Method (ROUT) with a detection parameter of 0.1% was used to rigorously identify and remove statistical outliers.[44] One outlier from COP data in the uninjured control group was removed from the statistical analyses. Independent-sample *t*-tests were conducted to determine group differences in normally distributed data, and Mann-Whitney *U*-tests were

conducted in non-normally distributed data. Effect sizes were assessed to determine the magnitude of the effect on group differences using Cohen's *d* effect sizes with 95% confidence intervals for data that were normally distributed, and *r* effect size ($Z/\sqrt{n}$) was conducted for data that were not normally distributed.[45] Cohen's *d* effect sizes were interpreted as small (0.2), moderate (0.5), and large (≥ 0.8),[46] and the *r* effect size was interpreted as small (0.10-0.29), moderate (0.30-0.49), large (0.50-0.69), and very large (>0.70).[47] Pairwise relationships between corticospinal excitability and postural control outcomes, as well as between corticospinal excitability and acute symptoms in patients with ALAS, were evaluated using Pearson Product-Moment Correlations (*r*) for data that were normally distributed and Spearman rho correlation tests (r_s) for data that were not normally distributed. The correlation coefficient for both tests was interpreted as follows: negligible (0.00-0.30), weak (0.30-0.50), moderate (0.50-0.70), strong (0.70-0.90), and very strong (0.90-1.00).[48] The level of significance was set *a priori* at 0.05. All statistical analyses were performed using SPSS 25.0 statistical software (SPSS Incorporated, Chicago, IL, USA) and GraphPad Prism 10.

Results

Participants: Participant demographics are presented in Table 1. Both groups had similar demographics in age, height, weight, and sex ($P > 0.05$). A majority of patients with ALAS (79%) had grade I ankle sprains with an average of 7 days past since the injury onset. All patients with ALAS had the presence of acute injury symptoms demonstrating a significantly large joint swelling, pain, and loss of function compared with uninjured controls ($P < 0.001$). Both ALAS and control groups maintained a similar physical activity level with more than 70% of them involving high physical activity prior to the study participation, indicating that the level of physical activity did not appear to affect group comparisons in the current study.

Background EMG activities of fibularis longus. There were no significant differences in bEMG activity of fibularis longus, recorded over 50ms before TMS stimulus, eliciting each MEP. These results revealed that similar fibularis longus muscle activities between groups did not affect the group difference in MEP at 100% of AMT (Table 2).

Corticospinal excitability: Group comparisons. Group comparisons of corticospinal excitability are presented in Figure 1. Compared with controls, patients with ALAS had 29.2% greater corticospinal excitability in normalized MEP at 100% of AMT ($t(26)=2.50$, $P=0.019$) with a large effect size ($d=0.94$, 95% CI=0.14, 1.70). At higher TMS intensities, there was no statistically significant difference between groups (MEP at 120% of AMT, $t(26)=0.96$ $P=0.346$, $d=0.36$, 95% CI=-0.39 to 1.10; MEP at 140% of AMT, $U=75.0$, $P = 0.306$, effect size $r=0.21$).

Postural control: Group comparisons. Group comparisons of postural control are presented in Figure 2. Compared with controls, patients with ALAS had 22.5% higher total COP velocity ($U=46.5$, $P=0.030$, effect size $r=0.42$), 19.7% higher AP COP velocity ($U=40.0$, $P=0.013$, effect size $r=0.48$), and 29.0% greater COP area ($t(25)=2.29$ $P=0.031$, $d=0.88$, CI=0.07, 1.64). There was no statistically significant difference in ML COP velocity between groups ($U=51.0$, $P=0.054$, effect size $r=0.37$)

Relationship between altered corticospinal excitability and impaired postural control. Correlation plots between altered corticospinal excitability and impaired postural control are presented in Figure 3. Corticospinal excitability in the normalized MEP at 100% of AMT was moderately and positively associated with total COP velocity (Spearman $r_s = 0.543$, $P=0.048$), indicating that as individuals with ALAS made a larger and more frequent postural control adjustment due to impaired postural control, they tend to utilize greater corticospinal excitability at the low TMS stimulation during the single-leg balance. There were no other statistically

significant associations between MEP at 100% of AMT with AP COP velocity and COP area ($P > 0.05$).

Relationship between altered corticospinal excitability and acute symptoms. The correlation of corticospinal excitability with acute symptoms is presented in Table 3. Altered corticospinal excitability in the normalized MEP at 100% of AMT was not associated with any of acute symptoms in patients with ALAS ($P > 0.05$).

Discussion

The primary finding of the study is increased corticospinal excitability, which is against our hypothesis, based on previous ankle studies.[24,27-29] Our findings of increased corticospinal excitability during single-leg standing in patients with ALAS appear to align with previous studies in acute knee pathological conditions[49,50] but not with studies involving ankle injuries.[24,27-29] MEP of the quadriceps during knee extension increased by about 26% after effusion injection into the intra-articular space of the knee.[49] Similarly, patients with anterior cruciate ligament (ACL) injury exhibited a marked increase in MEP at two weeks post-ACL reconstruction.[50] In contrast, patients with CAI have reported reduced cortical excitability during sitting[27-29] and single-leg standing.[24] Although there are methodological differences existing between our study and previous studies, such as joint involved (e.g., knee vs. ankle), experimental effusion settings,[49] chronic injury,[24,27-29] targeted muscle,[24,50] and testing position,[27-29] it is evident that corticospinal excitability is altered after joint injuries, but patients with ALAS demonstrate a distinct facilitation of the corticospinal excitability.

Our result of the increase in MEP (29% greater than uninjured controls) at 100% of AMT, while there were no group differences in other levels, aligns with a previous study that

reported selective changes in MEP at 100% and 105% of AMT in patients with CAI.[29] However, they observed decreased MEP at lower TMS intensities, possibly due to a restricted motor area resulting in a small amplitude response to external stimuli.[51] The increased MEP response at lower AMT reflects the direct activation of corticospinal axons in the cortical grey matter, which has a lower threshold to be excited by TMS intensities.[52,53] Several possible factors may be associated with this neural alteration after the acute joint injury. Firstly, this increased excitability could be attributed to the altered cortical inhibition associated with joint swelling. Reduced cortical silent period, related to the suppression of cortical inhibitory interneurons (GABA), has been reported following joint effusion, along with facilitated MEP response,[49] implicating dysregulation of neural inhibition, influencing the level of corticospinal excitability after the injury.[54] This mechanism is not feasible in our findings that altered excitability was not associated with ankle joint swelling (Table 3). Additionally, there could also be a state-dependent change in corticospinal excitability. The motor response to lower stimulus intensity is dependent on the brain's state during the stimulation.[53] Joint injuries can cause profound changes in the neural state, associated with altered sensory input to the CNS, disrupted efferent control, pain sensation, restricted motion, emotional stress, fear of reinjury, and/or cognitive demands.[55-57] Electroencephalography studies have demonstrated increased cognitive processing of the motor area during the movement, associated with joint injury or pain,[58,59] indicating greater attentional effort and motor demands in performing the task, which may contribute to increased corticospinal excitability. The increased activity in the primary motor cortex might be necessary for performing the task, but it could also indicate neural inefficiency, overusing neural resources for a simple behavioral task.[60]

296 The positive associations between corticospinal excitability and total COP velocity in the
297 current study suggest a compensatory mechanism of corticospinal excitability in impaired
298 postural control in patients with ALAS. It appears that the higher demand for postural control
299 requires greater corticospinal excitability of fibularis longus. The previous work[23] measured
300 MEP of fibularis longus and COP velocity during four different balance positions from wide
301 double-leg, narrow double-leg, tandem, and single-leg stance and assessed the relationship
302 between MEP and COP velocity. They found that MEP increased as the balance task became
303 more challenging. In addition, MEP linearly correlates with medial-lateral COP velocity
304 (r_s=0.5).[23] Although the previous study involved healthy individuals, these findings agree upon
305 the critical role of corticospinal excitability of fibularis longus in modulating the greater and
306 faster rate of postural sway. However, we observed a significant correlation between MEP and
307 total COP velocity, but not with medial-lateral COP velocity. The discrepancy could be
308 attributed to a constrained postural strategy in which individuals with ALAS make a shift
309 towards anterior-posterior postural sway (Figure 2-C) to avoid additional stress on damaged
310 lateral ankle ligaments and prevent further sprain. Enhanced descending motor drive to the
311 fibularis longus during a single-leg stance could contribute to less exaggerated postural sway or
312 increased rigidity in the medial-to-lateral axis. The corticospinal pathway, which provides faster
313 and predictive motor control, enables immediate neural conduction and increases excitability
314 when balance instability is anticipated.[61] Our results provide an important role of corticospinal
315 excitability of fibularis longus in maintaining balance following an ankle sprain.

316 Acute injury symptoms, including swelling and pain, have long been regarded as a major
317 contributing factor to altered neural excitability through a change in joint afferent input on the
318 surrounding muscles of the injured joint.[62] However, the theory behind atherogenic muscle

inhibition regarding acute joint injury has limitations, as much of the evidence is based on animal and effusion models.[15,62] Although studies have shown that soleus can be affected by acute injury symptoms,[17,20] it involves spinal-level adaptation. No studies have examined corticospinal excitability after ALAS. In our study, the ankle swelling and pain level were not associated with corticospinal excitability. A prior study with post-surgical knee found increased MEP two weeks after ACL reconstruction.[50] However, the corticospinal excitability was not associated with joint effusion; the excitability remained elevated even after the patients achieved a full range of motion, minimal effusion, and normal walking gait.[50] Interestingly, the corticospinal excitability was indeed related to muscle strength, in which higher corticospinal excitability was associated with lower muscle strength.[50] This suggests that acute joint symptoms, often considered a major contributing factor to altered neuromuscular control, do not directly influence corticospinal excitability after ALAS.

Our study is not without limitations. We selectively assessed the fibularis longus muscle as the muscle is critical for ankle stabilization after ALAS. The study may not fully capture the comprehensive view of neural adaptation as other ankle muscles, such as the soleus and tibialis anterior, also play important roles in postural stability after ALAS. However, assessing three different muscles with the current TMS investigation during single-leg standing might lead to discomfort and fatigue due to increased data collection duration associated with more standing trials, potentially affecting the quality of data. Another limitation is that we did not investigate the contralateral uninjured side, as acute symptoms are present on the injured side. It is possible that the contralateral side of the injured limb may also be affected by ALAS.[9] Future studies should also examine both sides of the ankle for corticospinal excitability and postural control. Lastly, while the current case-control study design is efficient and appropriate for identifying the

presence of altered excitability in relation to postural control, it does not establish clear causality. A future longitudinal study is recommended to determine how the corticospinal excitability and postural control change over the course of recovery after ALAS.

Conclusion

Individuals with ALAS demonstrated altered corticospinal excitability of the fibularis longus at 100% of AMT during single-leg stance compared with uninjured controls. Moreover, a significant association was found between corticospinal excitability and the rate of overall postural sway. However, ankle swelling and pain, commonly observed after ALAS, were not associated with the altered corticospinal excitability. These findings suggest an acute increase in supraspinal demands to compensate for impaired postural control during a single-leg balance following ALAS. A future longitudinal study is warranted to determine whether these early neurobehavioral changes persist even over the course of the recovery period following the injury.

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Figure captions.

Fig. 1. Scatter plots with bar graphs illustrate individual values of normalized MEP during single-leg balance, with the bar graph representing the mean and the error bar representing the standard deviation between the ALAS and control groups for 1A and 1B, while 1C shows the median and interquartile range. Figures 1A, 1B, and 1C illustrate normalized MEP at 100% AMT, 120% AMT, and 140% AMT, respectively. MEP at 100% AMT during single-leg balance was higher in ALAS compared with control (Figure 1A). The asterisk (*) indicates a statistically significant difference ($P \leq 0.05$).

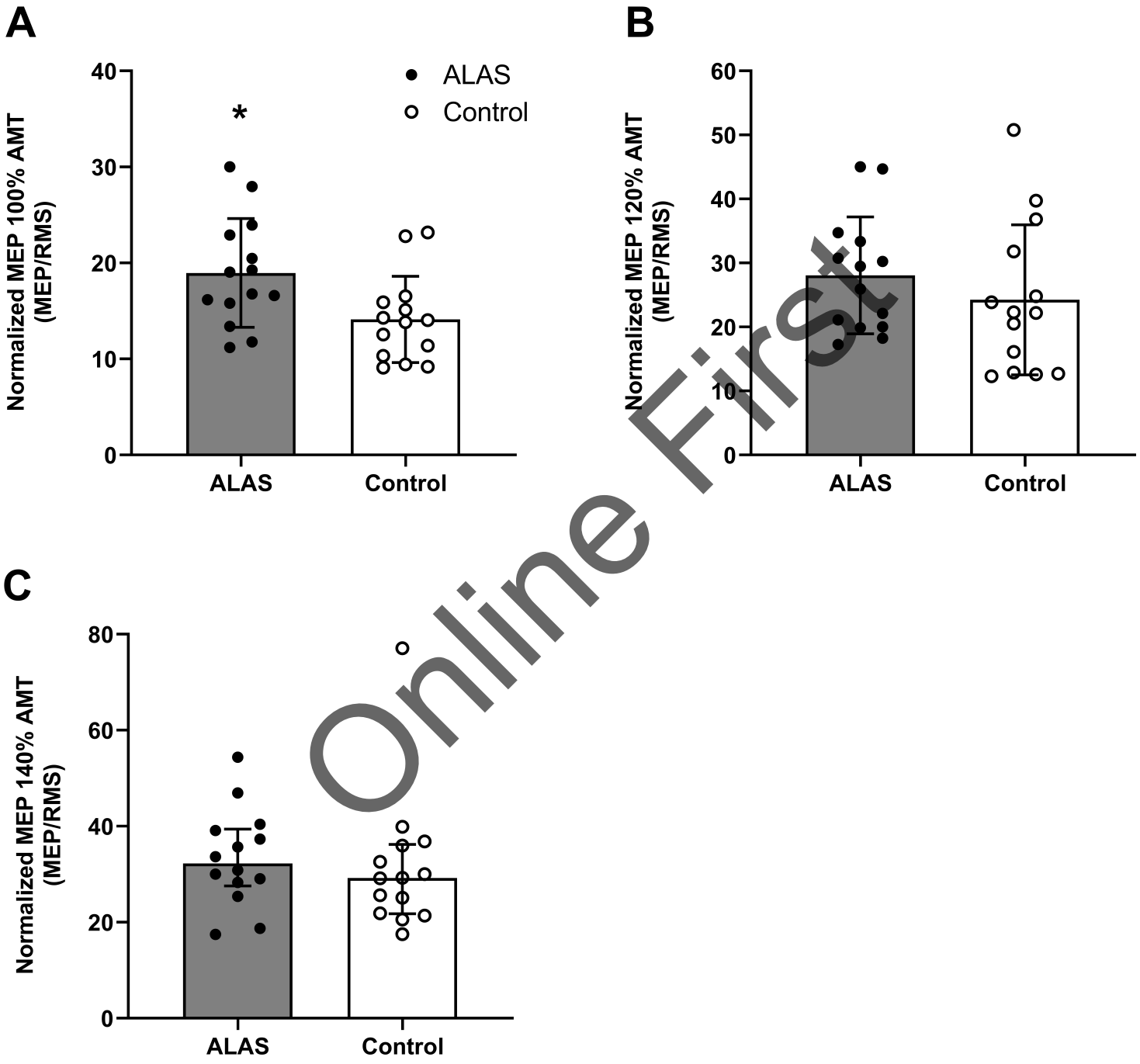
Abbreviations: MEP, motor evoked potential; RMS, root mean squared of electromyographic (EMG) activity; AMT, active motor threshold; ALAS, acute lateral ankle sprain.

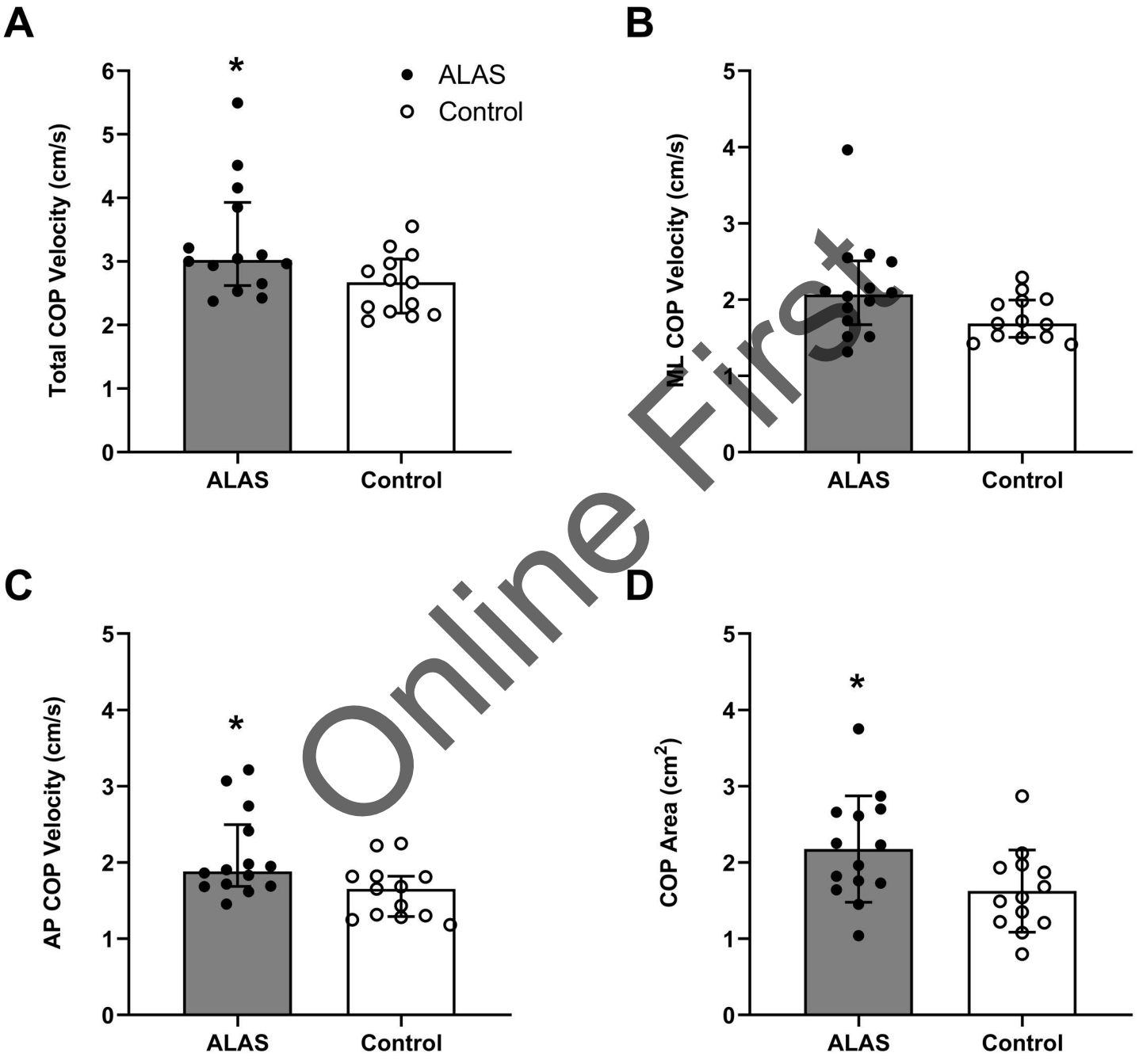
Fig. 2. Scatter plots with bar graphs illustrate individual values of COP during single-leg balance, with the bar graph representing the median and the error bar representing the interquartile range between the ALAS and control groups for 2A, 2B, and 2C while 2D shows the mean and standard deviation. Fig. 2A, 2B, 2C, and 2D illustrate total COP velocity, ML COP velocity, AP COP velocity, and COP area, respectively. The total velocity (Fig. 2A) and AP COP velocity (Fig. 2B) were significantly higher in ALAS compared with control. The COP area (Fig. 2D) was significantly greater in ALAS compared with control. The asterisk (*) indicates a statistically significant difference ($P \leq 0.05$).

Abbreviations: COP, center of pressure; ML, medial-lateral; AP, anterior-posterior; ALAS, acute lateral ankle sprain.

Fig. 3. Normalized MEP at 100% of AMT significantly correlates with total COP velocity in patients with ALAS (Fig. 3A). However, the specific directional COP velocities of AP directions (Fig. 3B), which showed impaired balance compared to the control group, does not correlate

566 with the normalized MEP at 100% of AMT. Moreover, the COP area also does not correlate with
567 normalized MEP at 100% of AMT (Fig. 3C). r = Pearson correlation, r_s = Spearman rho
568 correlation. The asterisk (*) indicates a statistically significant difference ($P \leq 0.05$).
569 *Abbreviations*: MEP, motor evoked potential; RMS, root mean squared of electromyographic
570 (EMG) activity; AMT, active motor threshold; COP, center of pressure; AP, anterior-posterior.





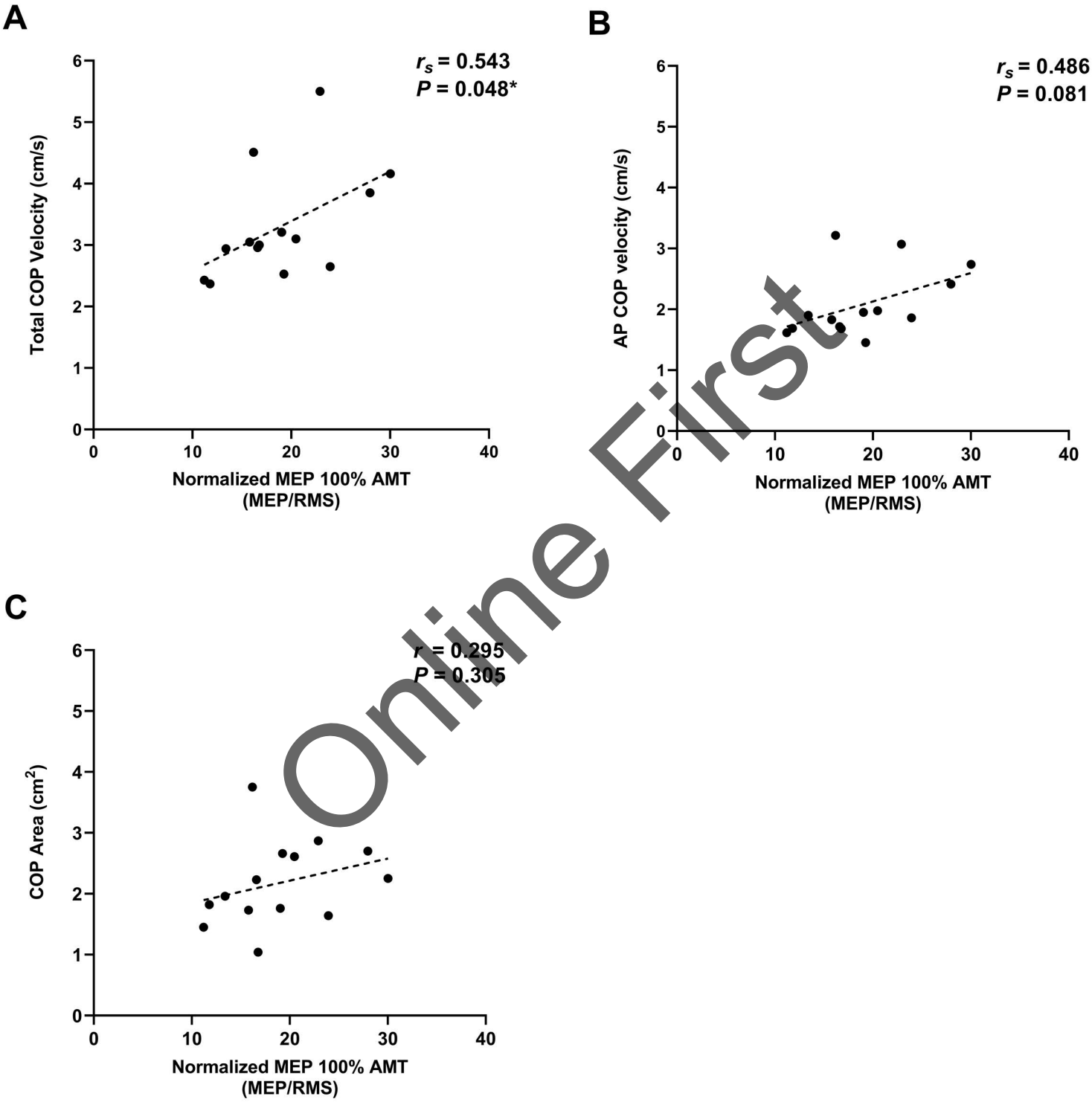


Table 1. Participant Demographics (Mean ± Standard Deviation).

Variables	ALAS	Control	*P* value
Participants	14	14	N/A
Sex	Male:5, Female:9	Male:5, Female:9	N/A
Age (years)	19.8±2.0	20.7±2.3	*P* = 0.204
Height (cm)	171.9±8.2	174.2±8.8	*P* = 0.487
Mass (kg)	69.7±8.2	69.5±14.9	*P* = 0.960
Injury grade	Grade I:11, Grade II:3	N/A	N/A
Time since injury (days)	7.3±4.2	N/A	N/A
Ankle swelling (cm)	1.1±0.8	0.1±0.2	*P* <0.001
Pain-current (VAS score, 0-10cm)	2.6±1.3	0.0±0.0	*P* <0.001
Pain-24h (VAS score, 0-10cm)	5.0±1.7	0.0±0.0	*P* <0.001
FAAM-ADL (0-100%)	63.1±11.5	99.9±0.3	*P* <0.001
FAAM-Sports (0-100%))	38.3±14.2	100.0±0.0	*P* <0.001
Ligament laxity (anterolateral drawer test)[a]	Grade II:1, Grade III:1, Grade IV:9, Grade V:3	N/A	N/A
Ligament laxity (anterior drawer test)	Grade II:2, Grade III:4, Grade IV:5, Grade V:3	N/A	N/A
Ligament laxity (talar tilt test)	Grade II:1, Grade III:4, Grade IV:7, Grade V:2	N/A	N/A
Physical activity level (GPAQ)	High:10, Moderate:4	High:11, Moderate:2, Low:1	N/A

[a] Ligament laxity tests were graded into five scales: Grade I: very hypomobile; Grade II: slight to moderately hypomobile; Grade III: normal; Grade IV: slight to moderately hypermobile; Grade V: very hypermobile. Abbreviation: ALAS, acute ankle sprain; VAS, visual analogue scale; FAAM, foot and ankle ability measure; ADL, activity of daily living; GPAQ, global physical activity questionnaire.

Table 2. Background EMG activities of fibularis longus collected prior to a TMS stimulus, eliciting each MEP (Mean ± Standard Deviation).

Variables	ALAS	Control	*P* value
bEMG during $MEP_{100\%}$ (mV)	0.49±0.29	0.51±0.17	*P* = 0.407
bEMG during $MEP_{120\%}$ (mV)	0.51±0.26	0.50±0.18	*P* = 0.777
bEMG during $MEP_{140\%}$ (mV)	0.54±0.26	0.49±0.13	*P* = 0.480

Abbreviation: bEMG, background EMG; ALAS, acute lateral ankle sprain; MEP, motor evoked potential; AMT, active motor threshold. There were no significant group differences in bEMG of fibularis longus ($P > 0.05$).

Table 3. Correlation of corticospinal excitability with acute symptoms in patients with ALAS

	Normalized MEP AMT 100%	
	***r* / r_s**	***P***
Ankle swelling (cm)	r_s = 0.387	0.171
Pain-current (VAS score, cm)	*r* = -0.084	0.776
Pain-24h (VAS score, cm)	*r* = -0.095	0.748

Abbreviations: MEP, motor evoked potential; AMT, active motor threshold; FAAM, foot and ankle ability measure; ADL, activity of daily living. *r* = Pearson correlation, r_s = Spearman rho correlation.

Normalized MEP at 100% of AMT does not correlate with any of the symptoms (*P* > 0.05).