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Gait kinematics & kinetics at three walking speeds in individuals with chronic ankle instability and ankle sprain copers

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ABSTRACT

Background: Individuals with CAI have demonstrated a more inverted foot position during walking when compared to a healthy control group. Copers are individuals who have had an ankle sprain but learn to cope and return to pre-injury levels of function and may be a better comparison group than healthy controls because they have had the same initial injury.
Research question: A controlled laboratory study was performed to simultaneously analyze differences in lower extremity walking gait kinematics, kinetics, and surface electromyography (EMG) between individuals with CAI and copers at a preferred walking speed (PWS), 120% preferred walking speed (120WS), and standardized walking speed (SWS) of 1.34 m/s.
Methods: Thirty-six (18 coper, 18 CAI) physically active individuals participated. Three-dimensional kinematics and kinetics at the ankle, knee, and hip and EMG amplitude for fibularis longus, tibialis anterior, medial gastrocnemius, and gluteus medius muscles were analyzed. Ten consecutive strides from each speed were analyzed using statistical parametric mapping (SPM). A 2 × 3 group by speed ANOVA and post-hoc t-tests were used to compare differences between the coper and CAI groups.
Results: The CAI group had more ankle inversion at IC (PWS: MD = 4.2°, d = 1.08; 120WS: MD = 5.0°, d = 1.28; SWS: MD = 6.6°, d = 1.37) and greater peak inversion throughout swing at all three walking speeds (PWS: MD = 4.2°, d = 0.89; 120WS: MD = 4.4°, d = 0.91; SWS: MD = 6.2°, d = 1.21). The CAI group had greater peak hip adduction during swing (PWS: MD = 4.5°, d = 0.96; 120WS: MD = 4.1°, d = 1.04; SWS: MD = 3.6°, d = 0.98).
Significance: The CAI group demonstrated greater ankle inversion at IC and during the swing phase and greater peak hip adduction during the swing phase compared to the copers. As the speed increased, ankle inversion in the CAI group also increased which could be linked to greater risk of recurrent sprains. Therefore, modeling gait training programs after the coper mechanics may be advantageous.

1. Introduction

Lateral ankle sprains are common musculoskeletal injuries among physically active individuals [1–3] and the prevalence of recurrent sprain rates are estimated to be 70% [4]. While lateral ankle sprains are prevalent and recurrence rates are high, unfortunately, many individuals consider lateral ankle sprains to be an insignificant injury. Less than half of individuals with an ankle sprain seek care from a medical professional following their initial injury [5]. This is problematic because 40% of these individuals develop chronic ankle instability (CAI) [6] which is associated with feelings of "giving way," decreased function, and persistent symptoms [7]. Lack of appropriate treatment could contribute to the altered neuromuscular function, poor postural control, and altered gait patterns seen in individuals with CAI [8,9]. In addition, CAI is associated with several long-term consequences such as decreased physical activity across the lifespan [10], decreased quality of life [11], and an earlier onset of ankle osteoarthritis [12].

Individuals with CAI have previously demonstrated an inverted foot position at initial contact (IC) and toe-off, more lateral plantar pressures, and altered muscle activation patterns during walking compared to uninjured healthy controls [8,13–15]. The compromised foot position during the swing phase and lateral plantar pressure during the loading phase of gait may be a contributing factor to the high

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recurrence rate for reinjury. These adaptations may be an appropriate area for clinicians to target when treating someone with CAI, however, much of the literature in this area to date compared individuals with CAI to an uninjured control group. It has been suggested that comparing to a group with the same initial injury that has learned to successfully cope may be a more appropriate approach when considering potential treatment techniques [16]. Copers are individuals who have had a lateral ankle sprain but have learned to cope with the injury and return to pre-injury levels of function [16].

Only one study to date has identified differences in gait biomechanics between individuals with CAI and ankle sprain copers [17]. Other studies have been conducted to compare individuals with ankle instability and copers during gait, however, their inclusion criteria did not follow recently published guidelines [18,19] and should be interpreted with caution. Doherty et al. [17] compared gait biomechanics at IC and toe off between copers and CAI during barefoot walking. They found that individuals with CAI were more inverted during the toe off phase than the copers [17]. Changes up the kinetic chain were also identified where individuals with CAI had decreased hip extension and increased knee flexion at toe off and increased hip flexion at IC [17]. Kinetic profiles were similar between the two groups with the exception of the CAI group demonstrating a decreased knee flexion moment at toe off [17].

While that study was the first comparing copers and CAI gait biomechanics, it may be advantageous to analyze the entire stride cycle as well as muscle activation via surface electromyography (EMG) during gait between these groups. In addition, participants walked barefoot which may provide more precise laboratory measurements, however, many individuals wear shoes during walking or sporting activities and therefore, assessing walking in a shod condition may be more generalizable. Lastly, participants walked at a self-selected speed which may differ between individuals and thus impact the spatiotemporal aspects of the gait measures, but this issue can be abated by using a standardized walking speed. Therefore, the purpose of this study was to simultaneously collect gait kinematics, kinetics, and EMG during treadmill walking at three speeds (preferred walking speed (PWS), 120% of PWS (120WS), and a standardized walking speed (SWS)) between individuals with CAI and copers. We hypothesized that the CAI group would have a more inverted foot position during walking gait than the coper group. Our secondary hypothesis was that group differences would become larger as walking speed increased and became more challenging.

2. Methods

2.1. Study design

We performed a descriptive laboratory study to evaluate differences in walking gait biomechanics between copers and CAI groups. Our independent variables were group (CAI, coper) and walking speed (PWS, 120WS, SWS). We used 3-dimentional motion capture to simultaneously measure lower extremity kinematics, kinetics, and surface electromyography (EMG) amplitude.

2.2. Participants

Thirty-six (18 coper, 18 CAI) individuals volunteered for this study. All participants completed the International Physical Activity Questionnaire and self-reported being physically active for at least 1.5 h per week. Inclusion criteria for the coper group followed recommended guidelines. [16] Briefly, copers had a history of at least 1 significant lateral ankle sprain at least 12 months prior to study participation and did not have self-reported dysfunction (Foot and Ankle Ability Measure - Sport ≥ 97%) or feelings of instability. Instability was assessed using the Identification of Functional Ankle Instability (IdFAI) [20,21]. Copers were included if IdFAI scores were < 10 OR they a) answered "no" to the question "Do you frequently roll your ankle or feel like it gives way?" AND b) answered "never" or "once a year" for the following questions: 1) "During activities of daily life how often does your ankle feel unstable?" 2) "During sport or recreational activity how often does your ankle feel unstable?" Inclusion criteria for the CAI group followed recommendations of the International Ankle Consortium [7]. Individuals with CAI had a history of at least 1 significant lateral ankle sprain at least 12 months prior to study participation, self-reported dysfunction (Foot and Ankle Ability Measure - Sport ≤ 85%), and feelings of instability or "giving way" (IdFAI ≥ 11). When CAI was bilateral, the self-reported worse limb was chosen for data analysis. When the coper's had history of bilateral sprains, the self-reported worse sprained limb was chosen for data analysis.

Participants were excluded if they had history of lower extremity fracture or surgery, were currently seeking physical therapy, had any conditions known to affect gait, or were pregnant. Individuals with an ankle sprain in the last 6 weeks for CAI or in the last 12 months for copers were also excluded. This study was approved by the university's Institutional Review Board and all participants provided written informed consent prior to study enrollment.

2.3. Instrumentation

Three-dimensional kinematics were collected at 250 Hz using a 12-camera Vicon motion capture system (VICON motion systems, Lake Forest, CA). Kinetic data were collected at 1000 Hz using the Bertec™ Fully Instrumented Treadmill (Bertec, Columbus, OH) and a threshold of 20 N was utilized to identify initial contact and toe off. Surface EMG was collected using Trigno wireless EMG (Delsys, Boston, MA, USA) at 2000 Hz with a 10–500 Hz bandpass filter and 50-sample average moving window. All gait data were synchronized using Motion Monitor software (Innovative Sports Training, Inc., Chicago, IL).

2.4. Procedures

Participants were fitted with standard laboratory shoes (Brooks Defyance; Brooks Sports, Inc., Bothel, WA) with a posterior heel cutout that did not impact the integrity of the shoe and was used for marker placement on the calcaneus. Ten rigid clusters with reflective markers were secured on the participant's upper thorax, lumbar spine, and bilaterally on the lateral thighs, lateral shanks, posterior heel, and the dorsum of each foot. Segments were digitized to identify the joint centers for the C7/T1, T12/L1, L5/S1, anterior superior iliac spine, and medial and lateral knee joint lines, and medial and lateral malleoli for each limb.

For EMG, the participant's skin was prepared by shaving, exfoliating, and cleansing using isopropyl alcohol. Rectangular electrodes (37 × 26 x 15 mm) with parallel-bar electrodes were placed over the muscle belly of the tibialis anterior (TA), fibularis longus (FL), medial gastrocnemius (MG), and gluteus medius (GMed). Correct electrode placement was confirmed using manual muscle testing for each muscle. EMG data were only collected on the involved limb. Prior to walking, a quiet standing trial was conducted for 10-seconds for normalization purposes.

Participants completed 5 min of walking on a split-belt treadmill at a self-selected pace prior to data collection as a warm-up which was then used as their preferred walking speed (PWS). Participants were allowed to adjust the speed as many times as necessary during the warm-up until they verbally reported feeling comfortable. Data were collected for 60-seconds at the PWS, 120% of PWS (120WS), and a standardized walking speed (SWS) of 1.34 m/s (3.0 mph). The order in which the walking speeds were collected were randomized using a Latin square design. Kinematics, kinetics, and surface EMG were collected simultaneously for all walking trials.

2.5. Data processing

Ten consecutive strides from the beginning of each walking trial at each speed were analyzed for the test limb only. Data from each stride were reduced to 101 data points representing 0–100% of the gait cycle with 0% representing IC. The values from the 10 strides were averaged for each participant to evaluate three-dimensional ankle, knee, and hip kinematics and kinetics, and root mean square (RMS) EMG amplitudes of the TA, FL, MG, and GMed.

All kinematic data were processed using a low-pass fourth order Butterworth filter at 14.5 Hz. [22,23], The Euler rotation method (Y, X, Z or flexion/extension, abduction/adduction, internal/external rotation) was used to analyze ankle, knee, and hip joint rotations. Vertical ground reaction force (N) was normalized to the body mass (kg) of each participant. Internal joint moments were normalized to the height and mass (nm/kg) of each participant.

EMG amplitudes were normalized to the mean of a 10 s data epoch during quiet standing for each variable. Data processing was performed in Matlab version R2018a (MathWorks, Inc., Natick, MA).

2.6. Statistical analysis

An a priori sample size estimate revealed 18 participants per group were needed to identify large effects based on a between group mean difference of 2.5° of inversion and standard deviation of 2° following toe-off during walking between coper and CAI participants [17].

Independent t-tests were used to compare group demographic information in Statistical Package for Social Sciences (SPSS) version 24.0 (SPSS, Inc., Chicago, IL). Gait biomechanical data were analyzed using the spm1d Version 0.4 for one-dimensional statistical parametric mapping (SPM) analysis software package for Matlab [24,25]. Using SPM, 2 × 3 group by speed analyses of variance (SPM_{ANOVA}) and post-hoc SPM t-tests (SPMt-test) were used to compare differences between the coper and CAI groups. The a priori level of significance was set at $p \leq 0.05$. Cohen's d effect sizes were calculated to determine the magnitude of difference between the groups and were interpreted as large (≥ 0.80), moderate (0.50–0.79), small (0.20–0.49), or trivial (≤ 0.19). [26]

3. Results

There were no significant differences for age, height, mass, or physical activity levels between the two groups (Table 1). The CAI group walked at a faster PWS compared to the coper group (Mean difference (MD) = 0.1 m/s $p = 0.003$, $d = 0.63$) and thus the 120WS was also faster (MD = 0.1 m/s; $p = 0.004$, $d = 0.50$). The groups differed on ankle health status (total number of sprains, time since last sprain, FAAM-ADL, FAAM-Sport, IdFAI) as expected based on the guidelines for each of the groups [7,16].

3.1. Kinematics

Frontal plane kinematics are presented in Fig. 1 for all walking speeds. A significant group by speed interaction was identified at IC ($p = 0.050$) and group and speed main effects were identified during swing (group main effect $p = 0.035$, speed main effect $p = 0.027$) for ankle frontal plane motion using SPM_{ANOVA}. The CAI group had more ankle inversion at IC (PWS: MD = 4.2°, $d = 1.08$; 120WS: MD = 5.0°, $d = 1.28$; SWS: MD = 6.6°, $d = 1.37$) and throughout the majority of the swing phase at all three walking speeds for peak ankle inversion (PWS: MD = 4.2°, $d = 0.89$; 120WS: MD = 4.4°, $d = 0.91$; SWS: MD = 6.2°, $d = 1.21$)(Table 2). As the walking speed increased, the ankle inversion angle increased for the CAI group, but not the coper group. The CAI group had significantly greater ankle inversion angle during 71–100% of gait cycle PWS, from 69 to 100% during 120WS, and from 57 to 100% during SWS (Fig. 1). The groups were significantly different for a total of 34% of the gait cycle during PWS, for 61% during 120WS, and for 90% during SWS (Fig. 1).

Table 1
Participant demographics for coper and CAI groups.

	Copers (n = 18)	CAI (n = 18)	P-value
Sex (female, male)	16, 2	16, 2	–
Age (years)	20.5 ± 1.9	21.5 ± 3.4	0.27
Height (cm)	168.2 ± 6.0	167.5 ± 9.1	0.78
Mass (kg)	66.2 ± 11.3	66.9 ± 14.4	0.86
FAAM-ADL (%)	99.9 ± 0.3	86.1 ± 9.7	< **0.001**
FAAM-Sport (%)	98.9 ± 1.7	68.7 ± 17.3	< **0.001**
IdFAI	10.6 ± 3.6	20.6 ± 3.6	< **0.001**
TSK	30.8 ± 3.6	35.0 ± 6.1	**0.02**
IPAQ	5014.3 ± 2210.0	5133.9 ± 3327.2	0.92
Total number ankle sprains	1.3 ± 0.6	3.5 ± 1.1	< **0.001**
Time since first sprain (months)	70.6 ± 42.1	98.1 ± 52.0	0.10
Time since last sprain (months)	63.2 ± 45.7	18.1 ± 27.4	**.001**
Preferred Walking Speed (m/s)	0.9 ± 0.1	1.0 ± 0.2	**.003**
120% Preferred Walking Speed (m/s)	1.1 ± 0.2	1.2 ± 0.2	**.004**

Abbreviations: Foot and Ankle Ability Measure (FAAM), Activities of Daily Living (ADL), Identification of Functional Ankle Instability (IdFAI), Tampa Scale of Kinesiophobia (TSK), International Physical Activity Questionnaire (IPAQ).

A significant group by speed interaction was identified during early stance phase ($p = 0.049$) for hip frontal plane, however, no group or speed differences were found upon post-hoc analysis at this time point. During the swing phase, significant differences were identified between the groups at all three speeds upon post-hoc t-test analyses (Table 2). The CAI group demonstrated increased peak hip adduction during the swing phase during all three walking speeds (PWS: MD = 4.5°, $d = 0.96$; 120WS: MD = 4.1°, $d = 1.04$; SWS: MD = 3.6°, $d = 0.98$). The CAI group had significantly greater hip adduction from 61 to 84% of gait cycle during PWS, from 62 to 85% during 120WS, and from 66 to 88% during SWS (Fig. 1). No other group differences were identified for any other kinematic variables.

Significant speed main effects were identified for all variables suggesting that walking speed impacted kinematics, however, this was not a primary focus of this study. Briefly, walking speed primarily impacted the timing of the movement, but did not change the kinematic motion (Fig. 2). The changeover from dorsiflexion to plantarflexion, which takes place during late stance when transitioning to toe-off, occurred earlier when walking at the faster speeds. Additionally, there was increased peak plantarflexion at toe-off at the faster walking speeds.

3.2. Kinetics

The CAI group demonstrated an increased ankle peak plantarflexion moment during 120WS from 42 to 50% of the gait cycle and during SWS from 50 to 60% of the gait cycle (120WS: MD = 0.7 Nm/kg, $d = 0.99$; SWS: MD = 0.6 Nm/kg, $d = 0.86$) compared to copers (Table 2). No other significant differences were found between the groups for kinetics (Fig. 3).

3.3. EMG

No group differences were identified for EMG variables; however, the coper group trended towards higher GMed RMS amplitude compared to the CAI group during the stance phase for all walking speeds and higher FL RMS amplitude during late swing compared to the CAI group during the SWS (Fig. 4).

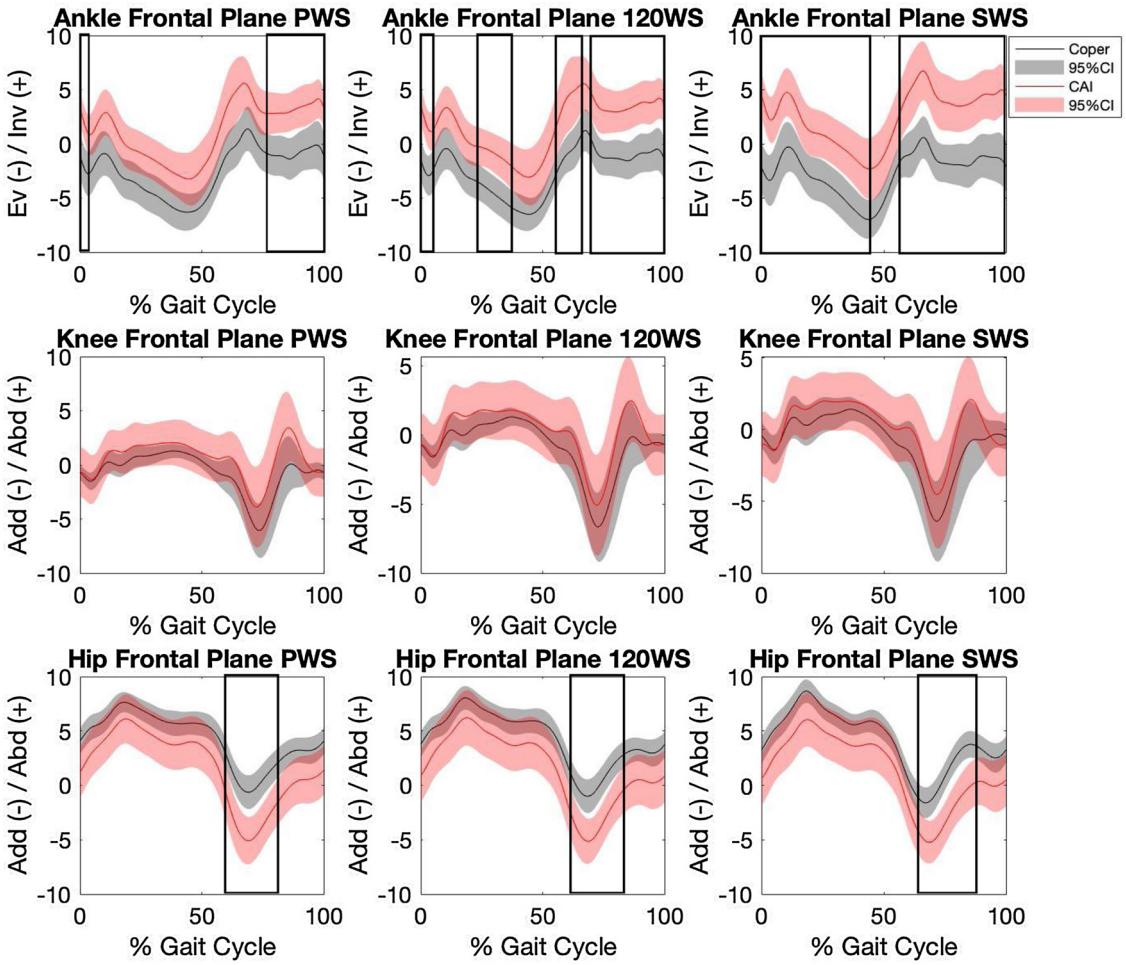

Fig. 1. Means ± 95% confidence intervals for frontal plane motion at the ankle, knee, and hip between copers and CAI during the preferred, 120%, and standardized walking speeds. Boxes indicate significant differences.

Table 2
Results for significant kinematic and kinetic differences between the CAI and coper groups.

Variable	CAI (mean ± SD)	Coper (mean ± SD)	p-value	Effect Size (*d*)
Ankle Inversion at IC (°)				
PWS	3.3 ± 3.4	−1.1 ± 4.6	0.048	1.08
120WS	3.6 ± 3.7	−1.4 ± 4.1	0.044	1.28
SWS	4.4 ± 4.6	−2.2 ± 5.0	0.017	1.37
Ankle Inversion Peak during Swing (°)				
PWS	5.6 ± 5.1	1.4 ± 4.3	0.039	0.89
120WS	5.6 ± 5.4	1.2 ± 4.2	< 0.001	0.91
SWS	6.8 ± 5.9	0.6 ± 4.2	< 0.001	1.21
Hip Adduction Peak during Swing (°)				
PWS	−5.1 ± 5.7	−0.6 ± 3.4	0.019	0.96
120WS	−5.1 ± 4.5	−1.0 ± 3.3	0.022	1.04
SWS	−5.2 ± 4.2	−1.6 ± 3.1	0.017	0.98
Ankle Peak Plantarflexion Moment (Nm/kg)				
120WS	−1.7 ± 0.6	−1.0 ± 0.8	0.040	0.99
SWS	−1.8 ± 0.7	−1.2 ± 0.7	0.049	0.86

Abbreviations: Newton meters per kilogram (Nm/kg), standard deviation (SD).

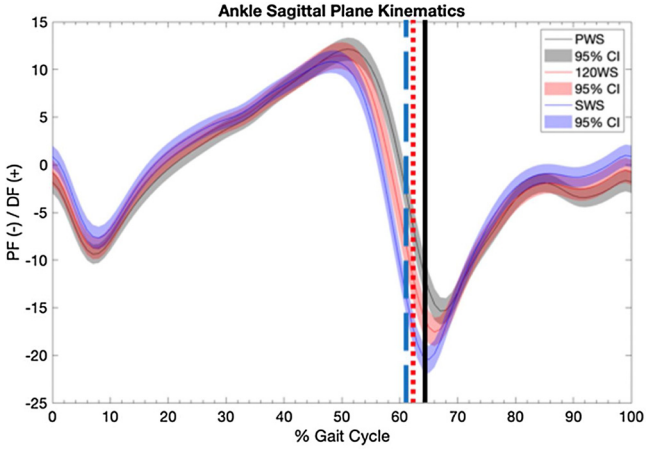

Fig. 2. Means ± 95% confidence intervals for ankle sagittal plane motion during the preferred, 120%, and standardized walking speeds. Vertical lines in corresponding colors indicate toe-off for each speed. Significant differences between the speed are indicated where 95% confidence intervals do not overlap.

4. Discussion

The primary findings of this study were the large meaningful differences in frontal plane kinematics demonstrated at the ankle and hip joints between the groups at all three walking speeds. The CAI group

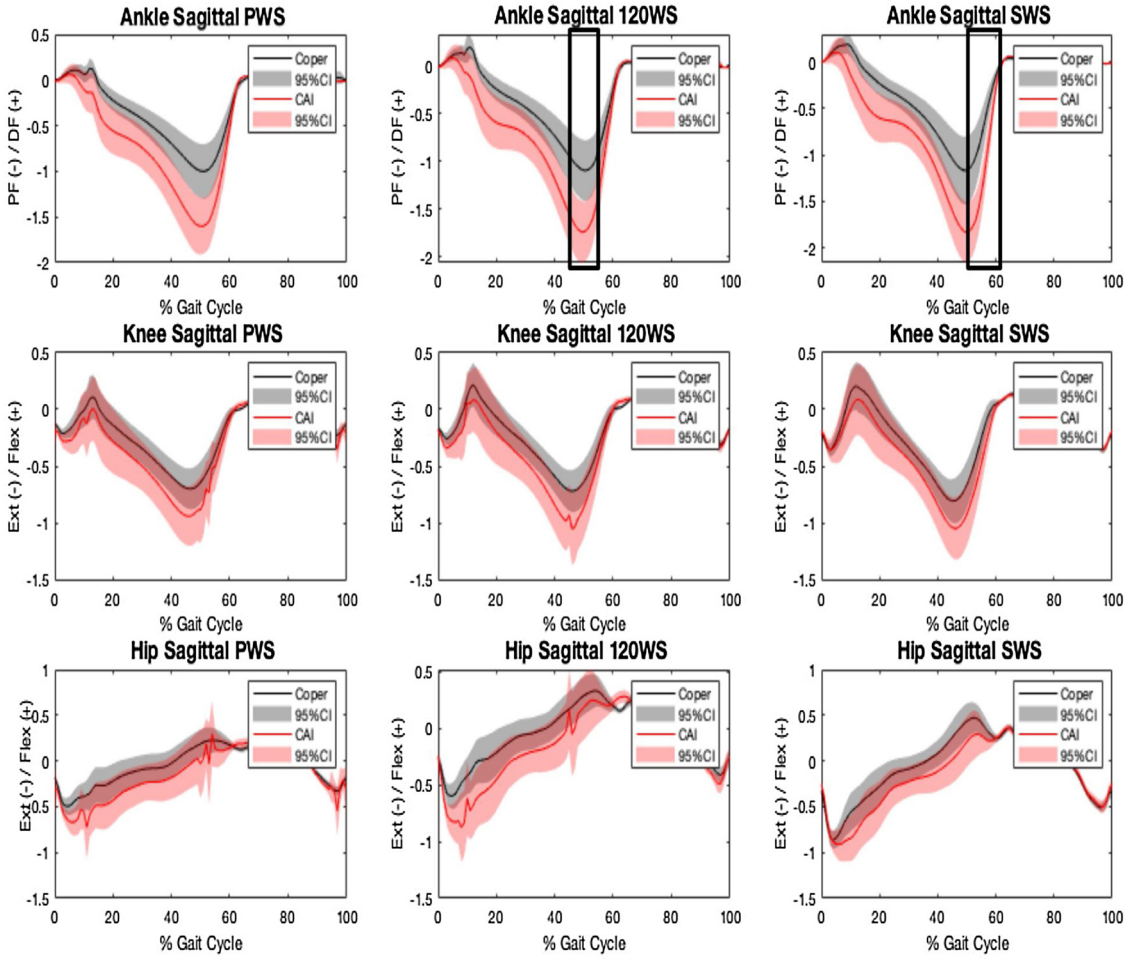

Fig. 3. Means ± 95% confidence intervals for internal sagittal plane moments during the preferred, 120%, and standardized walking speeds. Boxes indicate significant differences.

displayed 4-6° more ankle inversion than the coper group at IC and during the swing phase of gait during all three walking speeds. As the speed increased, ankle inversion in the CAI group increased, with the greatest differences seen at SWS which was also the fastest speed. The coper group did not change in ankle inversion angle as the speeds increased. At initial contact, participants with CAI have been shown to be approximately 6-7° more inverted than a healthy control group [13] which is a greater difference between groups than seen in our study. A gait training protocol aiming to reduce ankle inversion by 4-6° as demonstrated by the copers may be a reasonable initial goal because they have had the same initial injury and learned to cope with the injury and return to pre-injury levels of function. In addition, the CAI group demonstrated 4° more hip adduction during the swing phase of gait compared to the copers but the difference between groups remained relatively consistent as the speed increased.

This compromised kinematic position seen at both IC and throughout the swing phase in the CAI group could contribute to repetitive bouts of instability during simple activities of daily living. The inverted foot position throughout the stride cycle, in addition to hip adduction during the swing phase demonstrated by those with CAI, may also explain the more lateral plantar pressure identified by previous studies [14,27,28]. Of additional concern, as the task became more demanding with the increase in speed, the CAI group became increasingly more inverted. The coper group maintained a consistent amount of ankle inversion during all 3 speeds which potentially allows them to avoid recurrent ankle sprains and the development of CAI by placing the foot in a safer position prior to and following IC. Therefore, modeling gait training after the copers' biomechanics may be an appropriate rehabilitation intervention for individuals with CAI.

In addition to kinematic changes, we identified an increased ankle plantarflexion moment in the CAI group during late stance to toe-off which occurs when the ankle transitions from a dorsiflexed position to a plantarflexed position in preparation for the swing phase. An increased plantarflexion moment may be a compensatory mechanism used by those with CAI to increase their propulsion forces and overcome a more inverted foot position prior to toe off. Our results are in contrast to those of Doherty et al. [17], but this may be due to differences in study methods. In our study, participants walked on a split belt treadmill at a constant pace and wore standard laboratory shoes, whereas, in the other study, individuals were barefoot and walked across a walkway. Their study identified a decreased knee flexion moment prior to toe off [17]. The authors speculated that the reduced knee flexion moment was representative of a more rigid strategy to accommodate for 'push-off' [17] which may also be supported by our findings of an increased plantarflexion moment in the CAI group. Both studies have identified kinetic alterations in the sagittal plane for the CAI group which verifies the need for further investigation in this area in the future.

It was surprising that there were no differences in muscle activation between the two groups since we saw differences in kinematics and kinetics between the two groups. Although not significant, the coper group appeared to use more GMed activation during the loading response and the CAI group appeared to use more FL activation during late stance. We believe statistically significant results were not obtained for these measures due to the relatively small sample size and naturally

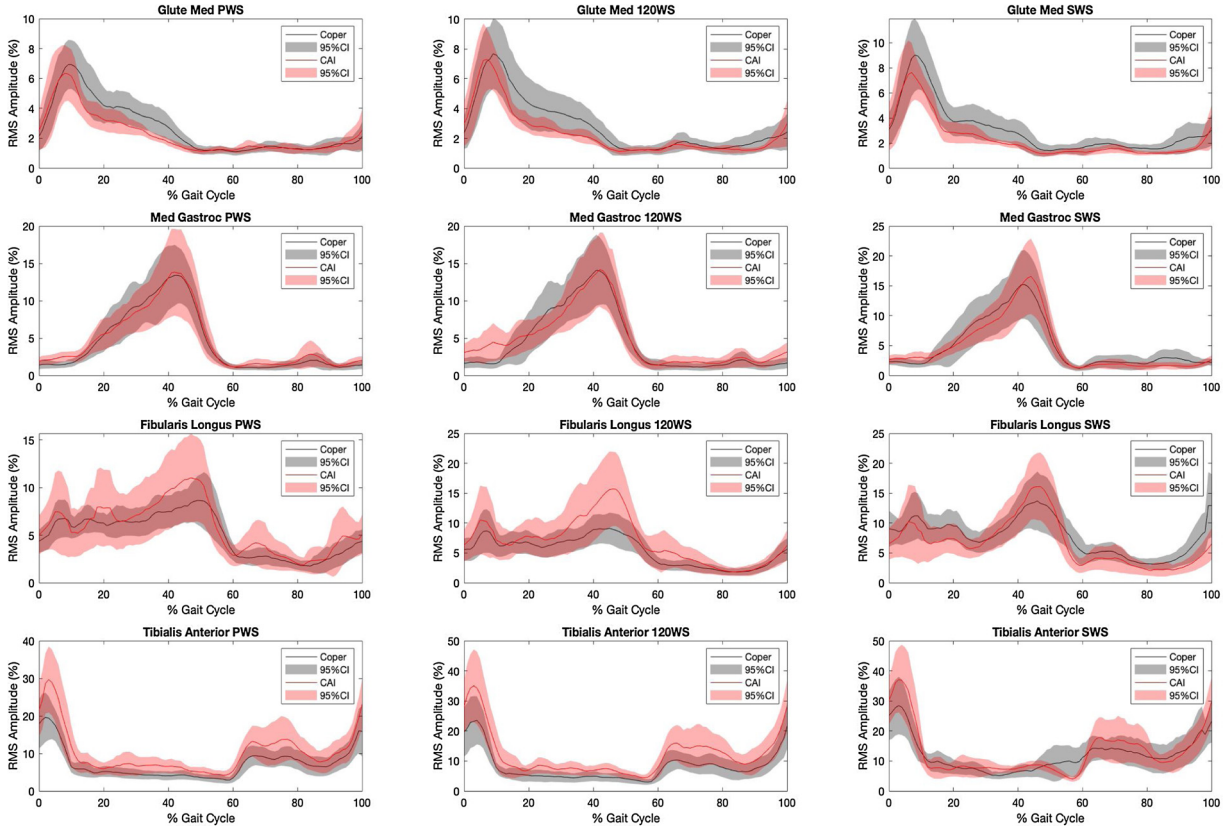

Fig. 4. Means ± 95% confidence intervals for the EMG RMS amplitudes (% above quiet standing) during the preferred, 120%, and standardized walking speeds. No significant differences were identified between the groups.

high variability when measuring EMG activity. Previous studies have demonstrated that individuals with CAI have had earlier and increased activation of the FL muscle compared to health controls [14,29], but it is possible that copers must also adapt their walking by using increased FL activation. More work needs to be done in this area to better understand the role of muscle activation in each of these groups during walking and other functional activities.

The increases in walking speed magnified the differences between the groups for ankle inversion and hip adduction kinematics. When considering the spatiotemporal aspect, toe off occurred earlier in the stride cycle when speed increased (PWS = 64%, 120WS = 62%, SWS = 61%) and therefore increases the importance of standardizing walking speeds when measuring differences between groups or overtime during gait analyses. Standardizing the speed would also improve the reproducibility of results between studies, however, PWS is an individualized component of gait and should be considered in addition to a standardized speed when analyzing gait in a laboratory or clinical setting. When using individualized non-standardized speeds, results should be interpreted with caution. In addition, we recommend using a faster walking speed to reveal adverse gait patterns that exist but may not be identified at slower walking speeds. This may be beneficial for clinicians attempting to assess ankle motion to establish the need for gait training. In our study, the ankle inversion in the CAI group increased as speeds increased, but the coper group did not change their ankle inversion as the speeds increased. If the task is not challenging enough, it may be difficult to detect meaningful changes between two groups.

Our study had several limitations. The speed at which individuals walked may have been impacted by lack of exposure to a split belt treadmill and research setup regardless of having a 5-minute familiarization period. The PWS for both groups was significantly slower than the SWS, however, participants in our study walked at similar speeds to individuals with CAI from a similar study that reported PWS ranging from 0.98 to 1.68 m/s while walking barefoot over a 10-meter walkway [13]. Secondly, the study was originally powered to identify differences between the groups for ankle inversion kinematics and did not account for kinetic and EMG variables. A larger sample size may be needed to identify differences for EMG. Upon calculation for a new sample size estimate using data from our study, we estimate 29 individuals per group would be needed to identify differences between CAI and coper groups. Additionally, data for EMG was only collected on the involved limb so we could not make comparisons between limbs. Lastly, EMG normalization techniques range among studies with some studies normalizing to a quiet measure and others normalizing to the maximum voluntary isometric contraction or peak activation during a functional movement. Our data processing technique may have contributed to lack of significant differences between the groups.

In conclusion, individuals with CAI had alterations in frontal plane kinematics at all speeds compared to the coper group which may explain why CAI patients experience recurrent ankle sprains. The mean difference for the ankle inversion angle between the groups got larger and lasted for more of the gait cycle as the speed increased. The coper group used a strategy that resulted in less inversion throughout the stride cycle and more hip abduction during the swing phase. Furthermore, the copers exhibited an everted foot position at IC (1–2°) while the CAI group had an inverted foot position at IC (3–4°). The kinematic profile of the coper group should be used for gait retraining of individuals with CAI to potentially reduce the risk for subsequent ankle sprains.

Disclosure

This work was performed in partial fulfillment of the requirement for the doctor of philosophy degree and is archived in the Online Archive of University of Virginia Scholarship (DOI: 10.18130/v3-zv0r-mj47).

Declaration of Competing Interest

There are no conflicts of interest to declare in relation to this manuscript.

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