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TITLE

Muscle activations during functional tasks in individuals with chronic ankle instability: a systematic review of electromyographical studies.

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Highlights

- Individuals with chronic ankle instability (CAI) show motor control abnormalities.
- EMG assessment is a useful tool to identify the features of movement abnormalities.
- Activation of the PL and TA is altered in a number of functional movements.
- It seems also that hip/spine muscles are activated first than ankle muscles.

Abstract

Background: It has been reported that individuals with chronic ankle instability (CAI) show motor control abnormalities. The study of muscle activations by means of surface electromyography (sEMG) plays a key role in understanding some of the features of movement abnormalities.

Research question: Do common sEMG activation abnormalities and strategies exist across different functional movements? **Methods:** Literature review was conducted on PubMed, Web-of-Science and Cochrane databases. Studies published between 2000 and 2020 that assessed muscle activations by means of sEMG during any type of functional task in individuals with CAI, and used healthy individuals as controls, were included. Methodological quality was assessed using the modified Downs&Black checklist. Since the methodologies of different studies were heterogeneous,

no meta-analysis was conducted. **Results:** A total of 63 articles investigating muscle activations during gait, running, responses to perturbations, landing and hopping, cutting and turning; single-limb stance, star excursion balance task, forward lunges, ball-kicking, y-balance test and single-limb squatting were considered. Individuals with CAI showed a delayed activation of the peroneus longus in response to sudden inversion perturbations, in transitions between double- and single-limb stance, and in landing on unstable surfaces. Apparently, while walking on ground there are no differences between CAI and controls, walking on a treadmill increases the variability of muscles activations, probably as a “safety strategy” to avoid ankle inversion. An abnormal activation of the tibialis anterior was observed during a number of tasks. Finally, hip/spine muscles were activated before ankle muscles in CAI compared to controls. **Conclusion:** Though the methodology of the studies herein considered is heterogeneous, this review shows that the peroneal and tibialis anterior muscles have an abnormal activation in CAI individuals. These individuals also show a proximal muscle activation strategy during the performance of balance challenging tasks. Future studies should investigate whole-body muscle activation abnormalities in CAI individuals.

Keywords: muscle activation; hip strategy; joint instability; surface electromyography; movement.

INTRODUCTION

Lateral ankle sprain is one of the most common musculoskeletal injuries among young and older individuals, during sport and daily activities[1,2]. A high percentage of individuals involved in a first episode of ankle sprain will undergo further injuries[2], developing chronic ankle instability (CAI).

CAI is a condition characterised by recurring episodes or perception of ankle sprains and ankle giving-way, accompanied by pain, weakness, reduced range of motion and a reduced self-reported function, persisting for more than one year after the first ankle sprain episode[3,4,5]. The persistence of ankle instability also leads, in the long term, to joint degenerative pathologies, such as osteoarthritis[6].

The causes of recurrent ankle sprains have been identified in both mechanical and neural factors. From a mechanical point of view, the first episode of lateral ankle sprain, characterised by hyper-supination and hyper-inversion of the foot, causes damage to the structures of the lateral foot-ankle complex, including ligaments, muscles, nerves, and tendons, which in turn leads to a mechanical increase of the ankle joint laxity[7,8]. At the same time, a number of neural factors have been identified in individuals suffering from CAI: primarily, a reduced spinal reflexive excitability of the soleus and peroneus longus muscles[9]. There is some evidence of reduced excitability of muscles acting on the ankle also at cortical level[10].

Spinal and supraspinal alterations which persist over time cause maladaptation in the control of movement. Abnormalities in movement control can be easily observed by investigating the kinetics and kinematics of functional movements. For example, in individuals suffering from CAI, a decreased knee flexion angle has been reported during landing tasks[11,12], or a higher degree of foot inversion and hip adduction during the swing phase of the gait cycle, with foot inversion increasing as walking speed increased[13]. All these results can be considered as risk factors for new ankle injuries[13], as well as for injuries in other lower limb joints[14].

Though the investigation of the kinetics and kinematics of functional movement is essential to identify movement alterations exposing CAI individuals to new injuries, the study of muscle activations by means of surface electromyography (sEMG) plays a key role in understanding the causes of movement abnormalities, since muscle activations mediate central and spinal neural events as well as the biomechanics of movement. A wide number

of tasks have been investigated by means of sEMG, but literature reviews have been mainly focused on walking, running and landing tasks[11,12,15]. It is not known whether common muscle activation abnormalities and common activation strategies exist across the different functional movements. The aim of this work is to provide a systematic review of studies based on sEMG investigating muscle activations during functional tasks in individuals suffering from CAI.

METHODS

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist[16] (Appendix 1). This study was registered in the PROSPERO database (N. CRD42020218867).

Search strategy

A systematic review of MEDLINE/PubMed, Cochrane Library database, and Web of Science was performed. The inclusion criteria were limited to articles published between 1 January 2000 and 31 December 2020. The terms and keywords used for searching articles were: (ankle instability OR chronic ankle instability OR CAI OR ankle sprain OR functional ankle instability OR FAI) AND (EMG OR electromyography OR muscle activations OR neuromuscular control OR motor control) located within the title and/or abstract and/or keywords. The algorithms used for the research strategy are reported in Appendix 2. Reference lists and citations of the included articles were manually screened to identify additional studies of interest.

Selection of the studies

After removing duplicates, the titles and abstracts of all studies were reviewed to determine their eligibility. The inclusion criteria were as follows: (1) participants with chronic/functional ankle instability; (2) performance of any kind of functional task, or responses to sudden perturbations; (3) description of amplitude and/or timing (onset/duration) of muscle activation of any muscle prior to or during the tasks assessed by EMG, and (4) presence of a control group constituted by healthy participants. Studies reporting a comparison between the CAI limb and the unaffected side with no control group of healthy participants were excluded, because it is known that unilateral CAI also impairs motor control and muscle function on the contralateral side[3]. Studies on participants suffering only from mechanical ankle instability were excluded. For studies with groups of participants with chronic/functional and mechanical instability, only the results of the chronic/functional groups were considered. Non-English language

publications, review articles, conference proceedings, editorials, case studies, letters, methodological studies, animal studies, and cadaveric studies were excluded. Studies in which EMG was used to record spinal reflexes or responses to electrically evoked contraction were excluded. No limitations were placed on the age or gender of the participants. Studies including an intervention were included only if the baseline measurements were available.

Risk of bias assessment

The risk of bias for each study included was assessed by means of the modified version of the Quality Index Checklist by Downs&Black[17], as in previous literature reviews, including non-intervention studies[18-20]. The number of items of the modified version was reduced from 27 to 14 (1,2,3,5,6,7,10,11,12,16,18,20,21,22), for avoiding questions that are only applicable for intervention studies. For each item on the checklist, an answer of “yes”, “no” or “unable to determine” was assigned. One point was given for each item with a “yes” answer, and the sum of all the scores is expressed as a percentage. Each study was classified in terms of “quality” according to the percentage of the items met: low quality (<60%), moderate quality (60–74%), or high quality ($\geq 75\%$)[21]. Detailed information on the risk of bias assessment is reported in Appendix 3. The risk of bias across the studies was also calculated for each item of the Downs&Black Checklist and was expressed as a percentage.

Data synthesis and analysis

Data extracted from the studies included were: (1) general paper’s information (author and year of publication), (2) participant information, (3) criteria for inclusion of CAI participants, (4) muscles tested, (5) task performed, (6) phase of the task analysed, (7) EMG analysis, (8) normalisation method and (9) results. Table 1 reports data extracted from the studies. Due to the heterogeneity of the parameters reported by the selected studies, a meta-analysis was not carried out. Therefore, a narrative synthesis of the results has been performed.

RESULTS

Search Results

The PRISMA flow diagram of the study selection process is shown in Figure 1. A total of 3669 articles were retrieved from the initial literature search. After the removal of duplicates and papers that did not satisfy inclusion and exclusion criteria, 63 articles remained[12,22-83]. Table 1 reports data extracted from each article.

Quality of the studies

The scores of the modified Quality Index for the studies considered ranged between 57.1% and 92.8%. A total of 38/63 studies were of moderate quality (score 60-74%), and 24 out of 63 were of high quality (score $\geq 75\%$). Only one study[22] was found to be of low quality (score below 60%). Appendix 3 shows the risk of bias score of each individual study. Figure 2 reports a graphic representation of the risk of bias across all the studies. The major sources of bias were represented by items 11 and 12 (external validity), and 21 and 22 (internal validity - confounding).

Participants

All the studies included a group formed of patients with chronic/functional ankle instability and a control group of healthy participants. Two studies also included participants with mechanical instability[23,24].

Muscle activations during gait

Table 1 reports data extracted from the 13 articles investigating gait. The studies investigating the magnitude of muscle activation during treadmill walking found that: PL activation during the post-heel strike time period was higher in CAI participants compared to controls[33]; TA activation was lower and PL, GM and MG activation was higher during a 100 ms time interval before the initial contact (IC) of the foot; GM also showed a higher activation during the final 50% of stance and first 25% of swing in CAI compared to controls[34]. Another study[35] reported a higher TA activation from 15% to 30% and 45% to 70% of the stance phase and a higher PL activation at initial heel contact and toe off. One study reported no differences between the groups for measurements of PL, TA, LG, RF, BF, and GM amplitude at either pre-initial or post-initial contact[36]. In addition, it was reported that CAI had lower PL EMG-amplitude variability from 1 to 10%, 32-38% and 56-100% of the gait cycle when compared to the controls[37]. In contrast, another study[38] found no difference between CAI and the controls in the PL, TA, MG, and GM activation variability in backward and forward treadmill walking, nor in the activation magnitude of the muscles. The timing of activation during treadmill walking was investigated in one study[36], which reported that PL, TA, LG, RF, BF, and GM were activated earlier in CAI than in the control participants and PL was activated for a longer time interval across the entire stride cycle in CAI compared to the controls[36].

Three studies on the magnitude of muscle activations in ground walking found no difference-between CAI and controls when considering EMG amplitude of PL, TA, MG, ST, RF, GM and Gmax[39], for TA and GM[40], and for PL and TA muscles[23], respectively. One study reported a reduced activation of GM from 6 to 9% and 99 to 100% of the stance phase in CAI compared to the controls, and no intergroup differences for PL, TA, LG, MG, and VL[41]. In contrast, another study reported significant differences between CAI and controls during various phases of the gait cycle for TA, PL, MG, VL, GM, and Gmax[42].

In the only study investigating the latency of TA and SOL muscles in response to an auditory stimulus for gait initiation, SOL was activated significantly sooner in the CAI group compared to controls, while no intergroup differences were found for TA[43].

During a planned gait termination, CAI participants showed a lower TA and a higher SOL activation of the injured limb than the uninjured in the leading limb[44]. In an unplanned condition of gait termination, controls showed a steady increase in activation of the TA throughout the stance phase, whereas considering CAI participants the TA activity increased very late in the stance phase[44].

No intergroup differences were found for walking over a sudden tilting platform[23].

Muscle activations during running

Muscle activations during running were investigated in one study[45]. PL, TA and LG muscle activations during running were not different in CAI participants compared to healthy individuals. Table 2 reports data extracted from this article.

Muscle activations in response to ankle perturbations or postural perturbations

Ten studies investigating ankle/postural perturbations were included (Table 3). Studies investigating the latency of muscle activations in response to sudden inversion perturbations found a longer reaction time of the PL compared with controls[27]. A study investigating the magnitude of muscle activation before and in response to a sudden ankle inversion reported a decrease in the magnitude of the TA medium-latency reflexes in the support position, and increased magnitude of the PL short-latency and medium-latency reflexes in the perturbed position[47]. A second study reported decreased compensatory postural adjustments of the TA in the support position[48].

Two studies investigated muscle responses to ankle supination perturbation[28,49]. One study found no difference between CAI and controls in the response latency of the PL[49]. The other study[28], investigating ankle supination in active, passive, and reactive conditions, found no difference in EMG onset between-groups. A higher SOL and smaller TA activation during passive trials was found in CAI participants compared to the controls. In all groups, it was described a greatest activity in PL during active and reactive conditions, as well as a smaller activity in SOL during the reactive condition. SOL activation was faster than TA and PL in the passive condition. TA muscle activation was slower than PL and SOL in the reactive trials.

Ankle eversion and dorsiflexion response to an auditory stimulus was investigated in one study which reported a delayed PL activation[50].

One study investigating muscle pre-activation to a medial-lateral ankle sway, without brace and with two different types of braces, found no difference in PL, PB, TA and SOL activations between CAI participants and controls[51].

A study[52] investigating sudden ventral or dorsal perturbation loading with eyes open and eyes closed found that the onset time of muscle activity of PL and TA in patients suffering from CAI was longer than in controls. No differences were found in the timing of GM and Gmax activation. In both groups, the proximal muscles were activated before the distal muscles. Delayed muscle activations in CAI participants compared to controls were also found in RA and ES in response to sudden unloading trunk-flexion or trunk-extension perturbations[53].

Finally, no differences in PL and PB latency of activation after an inversion perturbation were reported between CAI and healthy participants after a fatiguing exercise[22].

Muscle activations during landing and hopping tasks

Muscle activations were recorded during landing/hopping tasks in 27 studies.

Single-leg landings

Table 4 reports data extracted from each article. One study reported lower activation of SOL after landing in CAI participants compared to controls and no other significant differences for PL, PB, TA, and LG before or after landing[54]. Two studies found a decreased PL activity before impact in CAI participants compared to controls[55,56]. No significant intergroup differences were found for SOL and TA[55,56], and RF[56]. Contrasting results were reported by another study where increased activation of MG, PL, ADD, VL, GM, and Gmax during initial landing was found in CAI participants compared to controls[57]. A study[58] using wavelet transformation for EMG data processing found that people suffering from CAI exhibited lower wavelet intensities across all the frequency ranges in all the muscles investigated (PL,TA,SOL,MG,LG).

Forward jump landings

In the three studies on forward-jump landing tasks, it was reported that CAI participants compared to controls showed a lower PL-EMG amplitude before impact, and no intergroup differences for SOL and TA[55]. It was also reported that in forward jumping after a visual stimulus, PL pre-motor time (i.e., the time between visual stimulus and the onset of EMG signal) was shorter in CAI participants, and PL motor time (i.e., the time between onset of the EMG and onset of the motion) was longer in CAI participants. No differences between-groups were found for PB, TA or SOL[59,60].

Single-limb rebound jump landings

During the single-limb-rebound jump landing[61], in the time period from 29 ms to 152 ms post-IC the CAI group showed a lower ADD-EMG amplitude than the control group. In the time period from 29 ms pre-IC to 300 ms post-IC and from 179 ms to 229 ms post-IC, the CAI group showed a lower PL- and PB-EMG amplitude than the control group, respectively.

Stop-jump landing

In the stop-jump landing task, athletes suffering from CAI had a significantly lower TA/PL co-contraction index during the pre-landing phase. In addition, the CAI group exhibited a lower PL-EMG amplitude in the post-landing phase[62]. A higher VM-EMG amplitude was found in the CAI group compared to the control one in one study investigating thigh muscle activation in a stop-jump landing task[63].

During the performance of a drop-jump, CAI participants had a higher PL-EMG amplitude than controls, from 17ms to 128ms post-contact[64].

Landings over tilting and inclined platforms

In jumping on a tilting platform, one study found that individuals suffering from CAI showed a significantly decreased pre-activation of the PL muscle compared to controls[23]. In contrast, two other studies found a significantly increased preparatory and reactive peroneal activation[25], and a higher LG-, PL- and SOL-EMG amplitude in pre-contact time (CT) and GM in post-CT[65] in CAI compared to the control, respectively. One study investigating the amplitude of muscle responses in time intervals corresponding to short and medium latency reflexes found that, following touchdown, the CAI group showed decreased short latency reflex responses in distal (PL and MG) and proximal muscles (GLM) on the affected side[66]. Muscle activation timing was investigated in one study reporting significantly longer PL and PB latencies in CAI participants compared to controls. The same study also reported lower TA-EMG amplitude during the post-landing phase following unexpected perturbations[12].

In the pre-landing phase on an inclined platform, CAI participants displayed a reduced PL-EMG amplitude, while in the landing phase, an increased TA activation was observed, as well as an increased co-contraction of ankle muscles in the sagittal and frontal planes[62].

Other types of landings

In a study investigating landing after a blocking movement, individuals suffering from CAI presented a lower pre-landing and a higher post-landing PL-EMG amplitude, while no significant differences between groups were found for the co-activation index[67]. The same study, during the investigation of timing of muscle activations, found that, while in controls PL and GL activated first and simultaneously, and TA presented a later activation, in CAI participants the three muscles activated simultaneously[67].

In one study investigating stepping down, TA-EMG amplitude activity was higher in CAI participants in the preparatory (pre-touchdown) as well as in the reactive (post-touchdown) phase when compared to controls[26].

Lateral hops

During lateral hopping tasks, CAI participants were shown to have an increase in pre- and post-IC RF, TA, and SOL activation[68]. In contrast, another study found lower PL, TA, LG, RF, BF, and GM pre-IC amplitude in CAI participants compared to the controls[69]. In lateral hopping over an obstacle, CAI participants showed higher TA-EMG amplitude at contact moving in the lateral direction[70]. In lateral hopping onto an elevated platform, CAI participants showed lower preparatory TA- and PL-EMG amplitude, and less TA and PL peak activation than the controls, regardless of the eyes-open or eyes-closed condition[71]. In a lateral hop over different surfaces, CAI participants exhibited a lower BF-EMG amplitude during the pre-activation and landing phases, and a lower GM-EMG amplitude during the pre-activation phase, compared to the controls[72]. Another study

on lateral hop found higher PL- and Gmax-EMG amplitude in the CAI group than in controls just before landing. TA-EMG amplitude was higher in a condition of fatigue in CAI participants, while no differences were present between-groups for the GM[73]. No differences between CAI and healthy participants were found in the forward hop to stabilisation, with or without cognitive load, for PL, TA, and LG muscle activations[74].

Muscle activations during cutting/turning tasks

Table 5 reports data extracted from the seven articles on cutting/turning tasks. In cutting after landing, a reduced EMG amplitude of TS, PL, MG, and GM was observed in one study[30], while in another study, an increased EMG amplitude of MG, PL, ADD, VL, GM, and Gmax[57] was observed in CAI participants compared to controls. The time frequency analysis performed with wavelet transformation and the principal component analysis revealed that compared to the control group, CAI participants exhibited lower PC1 scores in all muscles and across all tasks. Since PC1 captured the general magnitude of wavelet intensities across all frequencies, people suffering from CAI therefore exhibited lower wavelet power across all frequency ranges in all muscles and tasks[58].

For the side-cutting task, higher MG-EMG amplitude during the early stance phase[39], higher TA-EMG amplitude from 86-94% of the stance phase[74], and a higher PL-EMG amplitude before foot strike[24] were reported in CAI participants compared to controls during running. No differences between CAI participants and controls were observed for turning movements[24,39].

During lateral shuffle, timing of muscle activation was similar between CAI and control groups. CAI participants presented a lower PL-EMG amplitude during the 50ms time interval before initial ground impact and a lower PL and LG peak activation during the task[76].

Muscle activations during single-limb stance and transitions between double- and single-limb stance

Table 6 shows data extracted from the six articles considered. No differences between CAI participants and controls were found during single-limb stance on uniaxial and multidirectional surfaces[77]. During the single-limb eyes-closed balance task, a significantly lower TA- and RF-EMG amplitude was found in the CAI group compared to controls. For total lower extremity muscle activation (PL+TA+LG+RF+BF+GM), the CAI group had a significantly lower EMG amplitude compared to controls[68]. On the contrary, another study[32] found a higher PL-, TA- and MG-EMG amplitude activation in CAI participants.

In the study by Van Deun[78] investigating the transition between double- and single-limb stance, all muscles, i.e., PL, TA, MG, MH, TF, and GM, except for VL and VM, showed a significantly later onset in CAI participants compared to controls. In both groups, no differences were found between the eyes-open and eyes-closed conditions. In the control group, muscle activity appeared to begin before the start of the transition from double-leg stance to single-leg stance, except for the VM. In the CAI group, the onset of muscle activity was typically found after the beginning of the transition. Participants in this group tended to initiate muscle activity in the more proximal regions, while the ankle muscles were activated later in both conditions. The same was observed in a second study by Van Deun[79], in which CAI participants showed mainly a proximal strategy for all conditions (open eyes, closed eyes, with or without a balance pad), while controls mainly had a proximal strategy in the no vision and pad conditions, but showed a mixture of strategies in the vision condition. In the study by Levin[80], controls showed an earlier onset time of the TA and later onset time of the Gmax, with the remaining muscles positioned in between. No significant differences in onset times among muscles were observed during EC or between EO and EC. For CAI, the earliest EMG onsets were noticed in the TF. For the ankle muscles, onset times during EO were shorter than those observed during EC.

Muscle activations during star excursion balance task

Table 7 reports data extracted from the four articles. The study by Ahn[81] reported that, in CAI participants compared to controls, PL-, TA-, and BF-EMG amplitude was lower in the anterior direction, TA- and SOL-EMG amplitude were lower in the posterior direction, VL-EMG amplitude was lower in the lateral direction, PL- and TA-EMG amplitude were lower in the antero-medial and anterolateral directions, and TP and SOL-EMG amplitude were lower in the posterior-medial and posterior-lateral directions. Similarly, the study by Feger[36] reported a significantly lower TA-EMG amplitude in the CAI group during the anterior and posteromedial reach directions. However, no significant differences between CAI participants and controls were reported for PL, LG, RF, BF or GM. A significantly lower TA-EMG amplitude in the anterior direction was also found in the study by Jaber[31]. The latter, also reported a significantly lower Gmax-EMG amplitude in the posterolateral direction in CAI participants compared to controls. No differences between the groups were reported for PL and GM.

No significant differences in PL, TA, GM and Gmax activations between CAI participants and controls were found in the study by Pozzi[29].

Muscle activations during other tasks

Three studies investigated other types of tasks, i.e., forward lunges[69], ball-kicking over an unstable surface[82], y-balance test[83], and single-limb squatting[83]. Table 8 reports data extracted from each article.

During forward lunge[69], a significantly lower proximal muscle EMG amplitude (RF+BF+GM) before IC was observed in CAI participants compared to controls. There was significantly lower EMG-amplitude of the TA in the CAI group compared to controls for post-IC amplitude. In addition, there were significantly less distal (PL+TA+LG) and total (PL+TA+LG+RF+BF+GM) lower extremity post-IC amplitudes in the CAI group compared with controls.

The study by Rios[82], analysing by means of a principal component analysis PL, TA, MG, LG, SOL, BF, RF, GM, ES, RA, and ADD activations during ball-kicking, showed that individuals suffering from CAI have reduced amplitude of EMG at the muscles around the ankle, while, around the hip, the amplitude is increased. The PCA revealed that CAI participants assemble different sets of muscle activations to compensate for their ankle instability, primarily activating hip/spine muscles.

The study by Fatima[83] recorded GM and Gmax activation during the y-balance test and single-limb squatting with and without a Swiss-ball. EMG amplitude of both muscles was lower in CAI compared to controls in all task and conditions.

DISCUSSION

This study systematically reviewed papers investigating muscle activations during the performance of functional motor tasks, recorded with surface EMG, in individuals with CAI. Due to the large number of different tasks investigated in the literature and the variety of muscles analysed, for several tasks and muscles it was not possible to draw definite conclusions. For muscles such as the PL and the TA, as well as for tasks such as walking, which has been widely investigated, some consistent results were observed across the studies.

During gait a high variability of muscle activations exists when walking on a treadmill in comparison with ground walking. For example, the PL showed a higher, longer and earlier activation in the majority of the studies investigating treadmill walking[33-36]. Accordingly, in the majority (4 out of 6) of the studies investigating ground walking, no differences in leg, thigh, and hip muscles were observed between CAI participants and controls[23,39-41]. The higher, longer, and earlier activation of the PL could be a compensatory strategy to avoid ankle inversion movements leading to ankle sprains, since a number of studies reported an increased ankle inversion[33,42] and plantar flexion[42], as well as an increased lateral COP displacement[35,37] during walking in CAI participants compared to controls. These differences are risk factors for lateral ankle sprains. In addition, it is likely that the abnormal PL activation is a strategy to compensate for neural alterations affecting ankle evertor muscles[9]. This could be further supported by the observation that all the studies investigating sudden ankle inversion perturbations found longer reaction times in the PL[27,46]. A longer reaction time of the PL was also found in the only study investigating ankle eversion/dorsiflexion after an auditory stimulus[50]. A longer latency of PL and TA was found in response to perturbations applied to the trunk[52]. Furthermore, the two studies investigating EMG amplitude in response to sudden ankle inversion perturbations reported a lower activation in CAI participants compared to controls for TA compensatory response, in particular in the time window corresponding to medium-latency reflexes[47,48]. Though few studies exist, it seems that similar alterations and the

same strategy are present for controlling the upper part of the body. In fact, delayed muscle responses to sudden trunk perturbations have been found in the ES and RA muscles[53]. At the same time, one study investigating ball-kicking by means of the principal component analysis showed that individuals suffering from CAI have reduced magnitude of EMG at the muscles around the ankle, while proximal muscle activity is increased[82]. Proximal muscles were also activated before distal muscles in response to trunk perturbations[52]. It follows that CAI participants primarily activate the hip/spine muscles during challenging tasks to compensate primarily for their ankle instability, but probably-also to compensate for trunk instability. The earlier and longer activation while walking on a treadmill, as well as the earlier activation of proximal muscles, can be interpreted as a “safety strategy” to compensate for an altered compensatory motor control system[84]. In other words, to stabilise posture, feedforward mechanisms are preferred to feedback mechanisms that are too slow to maintain a given posture, in response to sudden unpredictable events. In support of this observation, previous studies reported that when the compensatory/reactive responses to a perturbation are delayed, anticipatory control of movement is usually enhanced, for example in elderly individuals[85], or in other orthopaedic conditions affecting compensatory postural responses[86], probably with the aim to increase the stability of an unstable system.

Consistently, the same strategy was also reported for transition tasks from double- to single-limb stance[78,79], where CAI participants showed a more proximal strategy, with ankle muscles activating after the proximal muscles and with all the activations delayed compared to controls. This strategy does not seem to change by changing the conditions, i.e., on unstable surfaces or with the eyes open or closed[78-80]. No consistent results across studies were found for single limb stance tasks[69,77], as well as for muscle responses after sudden eversion perturbations or during mediolateral ankle sway, since only a few studies exist and report contrasting results[28,40,49].

Abnormal muscle activations have also been reported in landing and hopping tasks. It seems that CAI individuals have an abnormal timing of PL activation, since one study found a longer latency of activation while landing on a tilting platform[12]. Another study reported a longer PL motor time, i.e., the time between onset of the EMG and onset of the motion[59], and a third study found that CAI participants had a simultaneous activation of PL, TA and GL, while controls activated TA later than the other two muscles[67]. A large variability in the task execution, muscle investigated, and EMG analysis performed was found when considering the amplitude of the EMG signal in landing and hopping. This lack of homogeneity makes it difficult any comparison. However, in all the studies investigating pre-impact muscle activations during single-leg landings, forward-jump landings and single-limb rebound jump landings, a lower PL activation was reported[55,56,58,61]. It is not possible to draw conclusions on the other muscles, tasks or parameters investigated, since no consistent results were reported. It has been found that landing tasks have a higher risk of injury among sporting specific movements[87]. In CAI individuals the abnormal activation of the PL in the pre-impact phase of the landing might be related to the neural alterations of the peroneal muscles[9].-This condition, associated with abnormal movement kinematics, may therefore represent an injury risk factor, for both the ankle and the knee[11].

In the studies investigating muscle activation during the star excursion balance task, even if the muscles investigated were different among the studies, in 3 out of 4 studies a lower TA activation was found in the anterior direction of the task[31,69,73], and in two studies was also found in the posterior direction[69,73]. Only one study found no differences between CAI participants and controls[29]. Previous research reporting an altered cortico-spinal control of the TA during single-limb stance tasks could probably justify this result[88].

It is not possible to draw conclusions on running[45], cutting/turning tasks[24,30,39,57,58,75], forward lunges[69], y-balance test[83], and single-limb-squatting[83], the number of studies is too small and the variety of investigated muscles and analysis methodologies too large.

Thus, as a general summary, it appears that individuals suffering from CAI have a delayed activation of the PL in response to sudden inversion perturbations, in the transitions between double- and single-limb stances, and in landing on unstable surfaces. It seems that, while walking on ground no differences exist between CAI and healthy individuals, walking on a treadmill increases the variability of muscle activations, probably as a “safety strategy” to avoid ankle inversion. Finally, delayed responses have also been found in the muscles of the trunk in response to sudden perturbations. It seems however that the hip/spine muscles are activated before the ankle muscles in CAI individuals compared to healthy individuals.

It should be thus concluded that the altered sensitive information related to CAI leads to delayed muscle response in the ankle evtor muscles, and that the altered sensory information also leads to a compensatory postural strategy, mainly characterised by early activation of the hip/spine muscles. This observation is supported by previous research reporting the shift towards a hip strategy when sensory information arising from the ankle/foot complex is altered[89].

In light of these results, the investigation of the timing of muscle activations during functional tasks seems to lead to more consistent results across studies, while the investigation of the amplitude of the EMG signal leads to more heterogeneous results. This should be explained by the fact that sEMG timing analysis is more consistent across individuals than signal amplitude. This is due to the fact that activation time detection is less affected by the type of analysis and normalisation method adopted and by methodological issues in sEMG execution. The studies included in this review used a wide number of different normalization methods, with MVIC being the most used task. Methodological literature suggests that EMG data should be normalised using tasks as similar as possible to those under investigation to have the same neural drive and muscular coordination between tasks[90,91]. This is particularly important for dynamic tasks requiring fast and high intensity muscle contractions, such as running, jumping or cutting movements, which may result in higher EMG amplitudes and different frequency-domain characteristics than those recorded during MVIC[90,91].

Thus, even if MVIC is the most repeatable and reliable measurements, it seems unsuitable for EMG amplitude normalisation of high intensity dynamic tasks and it should be limited to the normalisation of low intensity tasks or longitudinal studies requiring multiple EMG assessments[90,91]. It is suggested that future studies be conducted strictly in accordance with the most updated EMG methodological guidelines.

Regarding the inclusion and exclusion criteria used by the studies to define CAI, it should be mentioned that a high percentage of studies included in this review were in line with the International Ankle Consortium (IAC) recommendations[92], which established well-defined criteria to improve the external validity of the studies. Though not all studies followed these criteria, no differences in the results were observed among the studies. This should be related to the fact that the selection of patients was quite rigorous even in studies not in line with the Ankle Consortium criteria. In fact, almost all the studies ensured chronicity of ankle instability by recruiting patients experiencing ankle sprains for at least one year, the absence of acute injuries, the consideration of mechanical stability, which was well-defined and assessed, and not used alone to define ankle instability. An important role also was played by the use of tools for self-reporting the perceived ankle instability. All these points are fundamental to the IAC's recommendations. However, the adherence of future studies to well-defined criteria for CAI definition is strongly recommended.

Some limitations should be mentioned for this study. First, the quality assessment of the studies performed with the Downs&Black checklist highlighted a) external and internal validity as the major sources of bias, since no information was reported regarding the population from which participants were recruited, b) the representability of the participants regarding their population, or c) the timing of recruitment of the participants (items 11,12,21,22). However, this information is mainly important for intervention studies (for which the checklist was originally created) where violation of these criteria may strongly skew the results of the study. The studies included in this review were mainly case-control non-intervention

studies, thus it is likely that the lack of such information did not have a high impact on the results. In line with this observation, no differences were observed in the results between studies with high scores and studies with lower scores.

Finally, it is known that neuromuscular and biomechanical alterations, which strongly affects muscle activations, are also a risk factor for lateral ankle sprain[93]. It should be mentioned that the studies included in this review were not longitudinal studies; and were only performed on individuals suffering from CAI. Thus, it is not known whether the alterations of muscle activations already existed before the first injury.

In conclusion, as a take-home message, this systematic review mainly highlighted abnormalities in the activation of PL and TA, which were the most investigated muscles in a number of functional tasks. In addition, during tasks that challenged balance, CAI individuals showed an earlier activation of proximal muscles (hip/spine) with respect to ankle muscles.

Future studies should further investigate muscles activation during functional and sporting tasks which are poorly investigated, with the aim to provide useful information for the training and rehabilitation of individuals suffering from CAI. Another important issue which should be further investigated is related to whole body muscle responses, which are to date unknown, since most the studies are focused on the ankle muscles. The analysis of whole-body muscle activations by means of new methodologies of data analysis, addressing muscle recruitment strategies for posture control, will provide excellent information on motor control abnormalities in CAI individuals. In addition, the investigation of muscle activations during functional tasks in longitudinal studies involving the pre-injury and post-injury timeframe is further recommended.

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Journal Pre-proof

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Figure 1. PRISMA flow-chart showing articles selection.

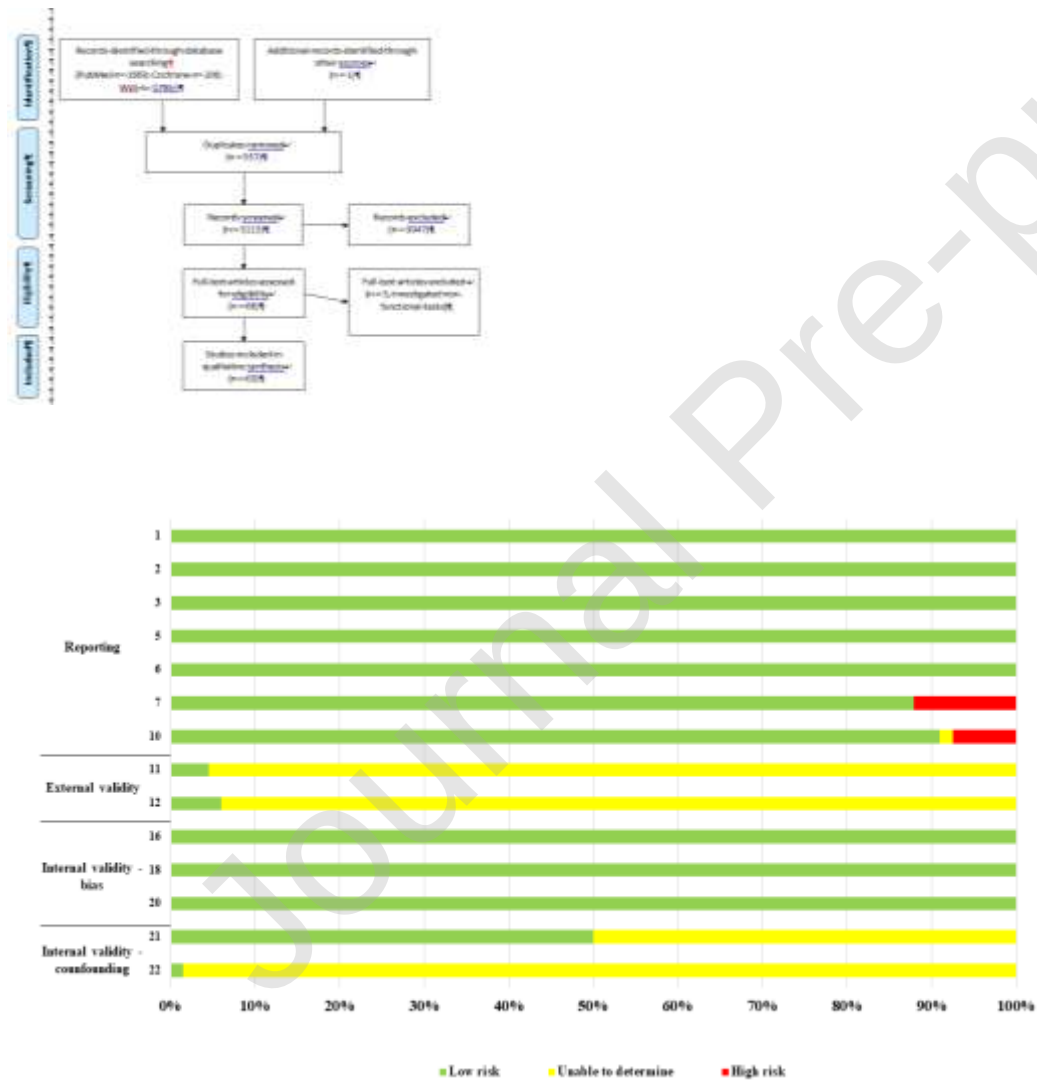


Figure 2. Risk of bias across studies express as a percentage. Data are provided for each item of each domain of the Downs and Black checklist[16].

GAIT							
Authors (year)	Participants	Criteria for CAI	Task	Muscles	EMG analysis	Normalization method	EMG Results
Balasukumaran et al. (2020)[38]	16 CAI (M 8, F 8; age: 25.4; PA: - ; height: 171 cm; body mass: 71.69 kg; AII: 6) 16 CG (M 7, F 9; age: 25.5; PA: - ; height: 172 cm; body mass: 68.36 kg)	In accordance with The International Ankle Consortium recommendations: 1) at least one ankle sprain in the 12 months prior to participate in the study, accompanied by inflammation and decreased physical activity; 2) at least two episodes of 'giving way' and feelings of ankle instability in the last 6 months; 3) the last ankle sprain occurred more than 3 months prior to participate in the study; 4) five positive responses to questions of the Ankle Instability Instrument (question 1, plus four others). Exclusion criteria were: 1) ankle fracture; 2) lower limbs' pathologies or surgeries within 1 year of study participation; 3) vestibular or neurological impairments.	Treadmill walking (forward and backward)	PL TA MG GM	The amplitude of 100 data-points for 15 strides was expressed as a percentage of the MVIC EMG value (%MVIC). The stride-to-stride variability was calculated as the SD of the EMG amplitude (as %MVIC) at each of the 100 data-points. The area under the curve of pre-IC (90–100% gait cycle) and post-IC (0–10% gait cycle) was calculated for all muscles activations.	MVIC of each muscle	There were no differences between CAI and CG for the %MVIC amplitude and activation variability (SD of %MVIC EMG) during forward and backward walking. A lower activation of TA and GM, and a higher activation of MG, were recorded during pre- and post-IC during backward walking in both groups.
Delahunt et al. (2006) [33]	24 FAI (14 M, 10 F; age, 26.6; PA: active; height: 1.7 m; body mass: 71.5 kg) 22 CG (14 M, 8 F; age 22.8; PA: active; height: 1.8 m; body mass: 70.5 kg)	1) At least 2 ankle sprains requiring reduced weightbearing and/or immobilization; 2) absence of fractures; 3) feelings of reduced ankle function, pain and weakness; 4) "giving-way" during sports practice; 5) subjective complaints secondary to ankle sprains; 6) lack of vestibular and neurological disorders.	Treadmill walking	PL TA SOL RF	EMG amplitudes of pre-HS (200 ms before HS) and post-HS HS (200 ms after HS) were normalized to peak EMG amplitude. For PL and SOL, EMG amplitudes were calculated also from HS to 40 ms after HS and from HS to 80 ms after HS.	Peak EMG amplitude mean from 10 records for each subject.	PL activation during the post-heel strike time period was higher in CAI subjects.

Feger et al. (2015) [36]	15 CAI (5 M, 10 F; age, 23; PA: active; height: 173 cm; body mass: 72.4 kg; FAAM-ADL: 87.2; FAAM-S: 68.5) 15 CG (5 M, 10 F; age 22.9; PA: active; height: 173 cm; body mass: 70.8 kg)	1) more than 1 ankle sprain. First sprain occurring more than 1 year before the study; 2) FAAM-S < 85%; 3) no history of lower limbs injuries within the 6 weeks before the study; 4) no history of lower limbs surgeries; 5) absence of balance or neurological disorders; 6) absence of diabetes or other disorders affecting balance.	Treadmill walking	PL TA LG RF BF GM	The point at which the magnitude of the RMS value exceeded 10 standard deviations above the EMG signal recorded during quiet standing (threshold value) was identified as the time of initial contact. A pre-activation was calculated if the muscle was activated before the initial contact, while a post-activation was calculated if the muscle was activated after the initial contact. The percentage of time in which each muscle exceeded the threshold value during the 5 stride cycles was calculated as an indication of percentage of activation time. Pre-IC (100 ms before heel strike) and post-IC (200 ms post heel-strike) amplitude were also calculated.	A 500-ms epoch during quiet standing was selected. The mean RMS value and SD were calculated and used to normalize activation measures during gait.	CAI participants activated earlier all the muscles in comparison with CG. PL had a longer activation during the stride cycle in CAI participants. No differences between CAI and CG were reported for pre-IC and post-IC EMG amplitude.
Gehring et al. (2013) [23]	19 CAI (FAI) (8 M, 10 F; age, 23.6; PA: active; height: 175.2 cm; body mass: 65 kg; CAIT: 18.1) 19 CAI (FAI+MAI) (-; age, 23.7; PA: active; height: 177 cm; body mass: 75 kg; CAIT: 19.8) 18 CG (-; age 22.8; PA: active; height: 175 cm; body mass: 70 kg)	FAI+MAI group: 1) feelings of instability and/or 'giving way' in at least one ankle; 2) CAIT score ≤ 24; 3) at least two ankle sprains with the last occurring within 2 years before the study; 4) diagnosis of MAI after manual examination. FAI group: all features of FAI+MAI group except the diagnosis of MAI. Exclusion criteria were: 1) lack of participation in risk sports; 2) individuals with MAI only; 3) individuals who could not be clearly rated for FAI and MAI.	Standing, walking and jumping on a tilting platform	PL TA	Muscle activations in terms of RMS were calculated for 5 time intervals: - EMGPre, i.e. the 100 ms before ground contact; - EMGPrep, i.e. from ground contact until the tilt of the platform; - EMGTilt, i.e. the phase of platform tilting; - EMG 60–90 ms, i.e. an early response phase after tilting the platform; - EMG90–120 ms, i.e. a late response phase after tilting the platform.	EMG values were individually normalised at the RMS value of a normal gait cycle (=100%), which was determined on the basis of 20 consecutive gait cycles on a treadmill.	No differences between the groups were reported for muscles activations after the tilting of the platform, for plantar flexion and for internal rotation. The PL pre-activation during the jumping phase was lower in FAI+MAI group compared with FAI group.
Hopkins et al. 2012[35]	12 CAI (M 5, F 7; age, 23; PA: active; height: 174 cm; body mass: 71.6 kg) 12 CG (M 5, F 7; age 23; PA: active; height: 176 cm; body mass: 71.4 kg)	1) FAAM-ADL: 90% or less; 2) FAAM-S: 80%; 3) MAII: 2 "yes" answers on questions 4–8 on the MAII; 4) negative talar tilt test.	Treadmill walking	TA PL	EMG data were smoothed using an RMS algorithm with a time window of 50 ms, and then time normalized to 100% of the stance phase.	EMG amplitudes were normalized to the EMG signal during quiet standing.	When compared to CG, CAI showed: a higher TA activation from 15% to 30% and 45% to 70% of the stance phase; a higher PL activation at initial heel contact and toe off; and a trend to lower PL activation from 20% to 40% of stance phase.

Koldenhoven et al. (2016) [34]	17 CAI (M 6, F 11; age, 20; PA: active; height: 170.2 cm; body mass: 77.4 kg; FAAM-ADL: 92.9; FAAM-S: 75; idFAI: 21.3; sprains: n 5) 17 CG (M 6, F 11; age 21.8; PA: active; height: 167 cm; body mass: 75.9 kg)	1) at least one ankle sprain, more than 1 year prior to participate in the study; 2) FAAM-S score < 85 %; 3) idFAI score ≥ 10 ; 4) absence of sprains in the last 6 weeks; 5) absence of lower limbs' injuries; 6) neurological or vestibular impairments; 7) other pathologies affecting gait performance.	Treadmill walking	PL TA MG GM	EMG RMS was calculated for epochs 100 ms pre-IC and 200 ms post-IC. sEMG RMS amplitudes during the entire stride cycle were condensed to 100 data points. Stance phase was represented by the first 60 data-points. Swing phase by the last 40 points. The 60 data points were analysed in windows of 10%. The 40 data points were analysed in windows of 25%.	Mean value of quiet standing RMS amplitude for each muscle.	EMG RMS was significantly lower for TA and significantly higher for PL, MG and GM in CAI compared to CG during the 100 ms pre-IC. During the last 50% of the stance phase and the first 25% of the swing phase, a higher GM RMS was reported in CAI compared to CG.
Koldenhoven et al. (2018) [37]	17 CAI (M 6, F 11; age, 20; PA: active; height: 170.2 cm; body mass: 77.4 kg; FAAM-ADL: 92.9; FAAM-S: 75; idFAI: 21.3; sprains: n 5) 17 CG (M 6, F 11; age 21.8; PA: active; height: 167 cm; body mass: 75.9 kg)	1) at least one ankle sprain, more than 1 year prior to participate in the study; 2) FAAM-S score < 85 %; 3) idFAI score ≥ 10 ; 4) absence of sprains in the last 6 weeks; 5) absence of lower limbs' injuries; 6) neurological or vestibular impairments; 7) other pathologies affecting gait performance.	Treadmill walking	PL TA	sEMG amplitudes during the stride cycle were transformed to 100 data points for each of the 15 consecutive strides. The SD of the EMG amplitude was calculated for each of the points. SD and COV were calculated for the area under the EMG RMS curve for the pre-IC (100 ms) and the post-IC (200 ms).	Mean value of quiet standing RMS amplitude for each muscle.	The CAI group showed a lower PL sEMG amplitude variability from 1 to 10%, 32–38% and 56–100% of the gait cycle when compared with the CG.
Koshino et al. (2015) [39]	10 CAI (M 9, F 1; age, 21; PA: active; height: 174 cm; body mass: 65.9 kg; CAIT: ≤ 25) 10 CG (M 9, F 1; age 20.8; PA: active; height: 174 cm; body mass: 66.5 kg)	Partially in accordance with the recommendations of the International Ankle Consortium: 1) at least one sprain requiring protected weight bearing and/or immobilization; 2) a history of two or more lateral sprains to the same ankle; 3) ankle "giving-way"; 4) CAIT score ≤ 25 ; 5) absence of lower limbs fractures or surgical interventions; 6) absence of swelling and inflammation; 7) absence of any kind of injury in the last 3 months.	Walking; walking side-turning; running side-cutting	PL TA MG ST RF GM Gmax	Initial contact and toe-off were identified as the instant the vertical GRF first exceeded 10 N, and the first time the vertical GRF fell below 10 N after IC, respectively. EMG amplitude was calculated during the 200 ms before IC and the stance phase, i.e. from IC to toe-off. Mean values of the normalized EMG data were calculated by averaging the pre-IC phase and every 10 % windows of stance phase, respectively.	The maximum amplitudes during the MVICs were used to normalize the EMG data during all movement trials.	No between-groups differences were found during walking. During the side-cutting task, CAI showed a higher MG activity during the early stance phase when compared with CG.
Moisan et al. (2020) [41]	32 CAI (M 4, F 17; age, 26.3; PA: active; height: 165 cm; body mass: 64.9 kg; FAAM-ADL: 86.4; FAAM-S: 69.6) 31 CG (M 4, F 17; age 25.1; PA: active; height: 167 cm; body mass: 61.7 kg)	In accordance with The International Ankle Consortium recommendations: 1) at least one ankle sprain in the 12 months prior to participate in the study, accompanied by inflammation and decreased physical activity; 2) FAAM: < 80%; 3) FAAM-S: < 90%; 3) at least two episodes of 'giving way' and feelings of ankle instability in the last 6 months; 4) absence of lower limbs fractures or surgeries; 5) absence of injuries in the last 3 months; 6) pathologies affecting gait performance. Ankle Instability Instrument, Cumberland Ankle Instability Tool or Identification of Functional Ankle Instability were not used since not available in French.	Walking at a comfortable or fast speed	PL TA LG MG VL GM	EMG amplitude was calculated as RMS of the data using a moving window of 100 ms width with an overlap of 50 ms.	RMS data of each muscle were normalized with the mean peak RMS amplitude of all FW trials	In CAI group a lower GM activation was observed from 6 to 9% and 99 to 100% of the stance phase.

Northeast et al. (2018) [40]	18 CAI (M 13, F 5; age, 22; PA: active; height: 176.8 cm; body mass: 74.1 kg; IdFAI: 19.1) 18 CG (M 14, F 4; age 22.4; PA: active; height: 177.8 cm; body mass: 70.4 kg)	In accordance with The International Ankle Consortium recommendations: 1) at least one ankle sprain in the 12 months prior to participate in the study, accompanied by inflammation and decreased physical activity; 2) at least two episodes of 'giving way' and feelings of ankle instability in the last 6 months; 3) the last ankle sprain occurred more than 3 months prior to participate in the study; 4) five positive responses to questions of the Ankle Instability Instrument (question 1, plus four others). Exclusion criteria were: 1) ankle fracture; 2) lower limbs' pathologies or surgeries within 1 year of study participation; 3) vestibular or neurological impairments.	Walking barefoot comfortable speed	TA GM	The EMG RMS was calculated by means of a moving window of 100 ms.	MVIC	No differences between CAI and CG were reported for EMG RMS during stance and swing phases of gait.
Son et al. 2019[42]	100 CAI (M 49, F 51; age, 22.2; PA: active; height: 174 cm; body mass: 70.8 kg; FAAM-ADL: 82.5; FAAM-S: 62.2; MAII: 3.6; sprains: n 4.6) 100 CG (M 55, F 45; age 22.5; PA: active; height: 173.1 cm; body mass: 72.6 kg)	In accordance with the International Ankle Consortium: 1) at least 2 recurrent unilateral ankle sprains, the most recent 3 months before participating in the study; 2) at least 2 episodes of giving way in the 6 months before participating in the study; 3) MAII: at least 2 responses of yes on questions 4 to 8; 4) FAAM-ADL less than 90%; 5) FAAM-S less than 80% on the FAAM-Sports; 6) absence of fractures or surgery to lower limbs; 7) acute sport-related injury.	Walking at a self-selected speed	TA PL GM VL MG Gmax,	The EMG RMS was calculated by means of a moving window of 125 ms.	EMG recorded during a 3-second isometric double-legged squat position	It was observed in CAI with respect to the CG: a 7% less TA activation during brief parts of early stance and 5% more during pre-swing; a 3% to 4% less PL activation between brief parts of early stance and midstance; a 4% less MG activation during early stance and 14% more throughout most of stance; a 4% less VL activation throughout most of stance; a 4% less GM activation throughout most of stance; a 5% to 10% less Gmax activation during brief parts of early stance, midstance and pre-swing.
Wikstrom et al. 2010[44]	20 CAI (-; age, 20.5; PA: active; height: 169 cm; body mass: 74.2 kg) 20 CG (-; age 20.8; PA: active; height: 164 cm; body mass: 64.2 kg)	1) at least one unilateral lateral ankle sprain requiring immobilization for at least 3 days; 2) at least one episode of giving way in the last year; 3) at least one recurrent ankle sprain between 3 and 6 months before participating in the study; 4) ankle pain, instability, and/or weakness; 5) attributing these signs to their initial ankle injury; 6) failure to resume all preinjury level of activities; 7) no previous ankle fractures; 8) no previous head and acute lower extremity injury within the past 3 months; 9) no formal rehabilitation.	Planned and unplanned gait termination	TA SOL GM	The EMG average was calculated by means of a moving window of 25 ms.	EMG activity of normal gait	In the leading limb during the planned gait termination TA SOL GM had a higher activation in CG compared to CAI. No differences were observed for the swing limb. CAI showed a lower TA and a higher SOL activation of the injured limb than the uninjured during phase 4 in the leading limb. In the swing limb, SOL activation was higher in the uninjured limb. In the unplanned condition, CG showed a steady increase in activation of the TA throughout the stance phase, whereas in CAI the TA activity increased very late during the stance phase.
Yousefi et al. (2020) [43]	17 CAI (M; age, 24.3; PA: active; height: 175 cm; body mass: 71.15 kg; CAIT: <90%; FAAQ: <80%) 17 CG (M; age 23.4; PA: active; height: 176.32 cm; body mass: 72.25 kg)	1) at least one episode of sprain requiring protected weight-bearing or immobilization for at least 3 days between 12 and 3 months before to participate in the study; 2) CAIT score: <90%; 3) FAAQ score: <80%.	Gait initiation after auditory signal	TA SOL	TA and SOL latency of activation was calculated in response to the auditory signal. Response time was defined as the time at which the EMG signal exceeded three standard deviations of the corresponding muscle EMG activity recorded at baseline.		The CAI group showed an earlier activation of the SOL when compared to CG. There were no differences between the two groups for the TA.

Table 1. Data extracted from each article for qualitative synthesis. CAI: chronic ankle instability; FAI: functional ankle instability; CG; control group; PA: physical activity level; MVIC: maximal voluntary isometric contraction; RMS: root mean square; EMG: electromyography; PL: Peroneus longus; TA: Tibialis anterior; PB: Peroneus brevis; SOL: Soleus; LG: Lateral gastrocnemius; MG: Medial gastrocnemius; VL: Vastus lateralis; RF: Rectus femoris; ST: semitendinosus; GM: Gluteus medium; Gmax: Gluteus maximum; AII: Ankle Instability Instrument score; FAAM-ADL: Foot and Ankle Ability Measure - Activities of Daily Living score ; FAAM-S: Foot and Ankle Ability Measure - Sport scale; idFAI: Identification of Functional Instability scale; CAIT Cumberland Ankle Instability Tool; FAAQ: Foot and Ankle Ability Questionnaire; MAII: Modified Ankle Instability Index.

RUNNING							
Authors (year)	Participants	Criteria for CAI	Task	Muscles	EMG analysis	Normalization method	EMG Results
Lin et al. (2011)[45]	15 CAI (M 6, F 9; age, 21.6; PA: active; height: 166.8 cm; body mass: 62.6 kg; CAIT: 18.1; sprains: 3.5 x year) 15 CG (M 7, F 8; age 21.5; PA: active; height: 164.5 cm; body mass: 59.2 kg;)	1) at least 1 ankle sprain leading to swelling, pain, and protected weightbearing and/or immobilization; 2) ankle “suddenly giving way”; 3) at least 2 ankle sprains within the past 2 years; 4) ankle sprain at least once in the past 6 months; 5) CAIT score < 27; 6) history of lower extremity fractures or injuries; 6) absence of inflammation.	Running; stop-jump landing	PL LG TA	Each of the tasks was divided into 2 phases: the pre-landing phase, i.e., the 200 milliseconds immediately prior to foot strike, and the post-landing phase, i.e., the 200-millisecond immediately after foot-strike. The pre-landing phase was in turn divided into 2 phases: phase I (200-100 milliseconds prior to foot-strike) and phase II (100 milliseconds to foot-strike). Similarly, the post-landing phase was divided into phase III (foot-strike to 100 milliseconds after) and phase IV (100-200 milliseconds after foot-strike). Coactivation index and EMG RMS were calculated for all the phases. Then data were normalized.	MVIC of each muscle	No differences between CAI and CG were found during running. CAI group showed a lower TA/PL co-contraction index during the pre-landing phase, and a lower activation of the PL in the postlanding phase.

Table 2. Data extracted from each article for qualitative synthesis. CAI: chronic ankle instability; CG; control group; PA: physical activity level; MVIC: maximal voluntary isometric contraction; RMS: root-mean-square; EMG: electromyography; PL: Peroneus longus; TA: Tibialis anterior; LG: Lateral gastrocnemius; CAIT: Cumberland Ankle Instability Tool; RMS: Root Mean Square.

ANKLE PERTURBATIONS AND POSTURAL PERTURBATIONS							
Authors (year)	Participants	Criteria for CAI	Task	Muscles	EMG analysis	Normalization method	EMG Results
Kavanagh et al. (2012) [50]	12 CAI (6 M, 6 F; age: 26; PA: -) 12 CG (6 M, 6 F; age: 26; PA: -)	1) at least one ankle sprain within the previous 2 years which lead to swelling, pain and temporary loss of function at the time of injury, and self-reported ankle instability; 2) CAIT < 27.	Ankle eversion and dorsiflexion in response to an auditory stimulus	PL TA	The Standard Error of the Slope was used to determine the onset of muscle activation by means of a linear model analysis. Premotor time was calculated and defined as the duration from the audio cue to the onset of muscle activity. Motor time was calculated as the onset of muscle activity to the force sensing resistor being released.		Motor time of the PL was significantly slower in CAI compared to CG.
Kazemi et al. (2017) [52]	16 FAI (F; age: 26; PA: -; height: 165 cm; body mass: 61.2 kg) 18 CG (F; age: 26; PA: -; height: 163 cm; body mass: 56.5 kg)	1) ankle sprain within the last year; 2) absence of ankle fracture; 3) ankle “giving way”; 4) no rehabilitation; 5) no evidence of mechanical instability as assessed by anterior drawer and Talar tilt tests; 6) absence of current injuries; 7) absence of previous surgeries; 8) absence of pregnancy; 9) absence of pain; 10) absence of any condition affecting movement patterns.	Sudden ventral or dorsal perturbation loading with eyes open and eyes closed	PL TA GM Gmax	Onset timing of activity and muscle activity amplitude and RMS EMG parameters were calculated.	MVC of each muscle	PL and TA onset of activation was longer in FAI than CG. No differences between the two groups were found for GM and Gmax. In both groups proximal muscles were activated before distal muscles.
Marshall et al. (2009) [53]	12 FAI (M 5, F 7; age: 29.1; PA: active; height: 173 cm; body mass: 70.9 kg; FADI: 92.9, FADI -S: 84.2; CAIT: 17.3) 12 CG (M 5, F 7; age: 23.7; PA: active; height: 171 cm; body mass: 67.9 kg)	1) at least one ankle sprain in the last year; 2) ankle “giving way” symptoms; 3) absence of pain; 4) no rehabilitation; 5) no signs of mechanical instability; 6) absence of pathological joint laxity (Beighton hypermobility scale); 7) absence of any neurological or vestibular disorder; 8) absence of any previous lower limb surgery, use of orthotics, any open foot wound, any obvious bony or musculoskeletal deformity or asymmetry between the limbs; 9) absence of back disability or had experienced an episode of back pain.	Sudden unloading trunk-flexion or -extension PERTURBATIONS	ES RA	Onset times of ES (in response to flexion unloading) and RA (in response to extension unloading) were calculated as the point after which the RMS of a 25-ms period of activity of the rectified EMG signal was increased by 1.5 SD above the pre-release level of activity.		Trunk muscle onsets were delayed in FAI subjects when compared with CG in both flexion and extension.
Mendez Rebolledo et al. (2015) [27]	10 FAI (M; age: 29.1; PA: active; height: 180 cm; body mass: 81.5 kg) 10 CG non-instability (M; age 23.7; PA: active; height: 180 cm; body mass: 81.8 kg) 11 CG healthy (M; age 23.7; PA: active; height: 180 cm; body mass: 84.5 kg)	CAI group: 1) at least one ankle sprain in the last 12 months; 2) the last episode of lateral ankle sprain must be between three and 12 months prior to participate in the study; 3) pain, instability, and/or weakness in affected ankle; 4) resumed all pre-injury activities without limitations; 5) AJFAT score < 22; 6) took part in programs for prevention and/or treatment for ankle injuries. CG non instability group: 1 AJFAT score > 22; exclusion criteria: 1) had two or more lateral ankle sprain; 2) pain, instability and/or ankle weakness at the moment of evaluation. CG healthy: 1) participating in 30 minutes of Moderate physical activity at least 3 times a	Sudden ankle inversion	PL TA PB	Muscle reaction time was analysed and identified as the moment at which the sEMG activity passed the threshold of at least three standard deviations from the average of the signal at rest for 150 ms and maintained this threshold for at least 25 ms. The muscle was considered “switched off” when the sEMG signal fell below this threshold for more than 50 ms.		FAI group showed a longer PL reaction time in comparison with CG and non-instability group, and a longer PB and TA reaction time when compared to the non-instability group.

		week; 2) AJFAT score < 22.; 3) do not practice sports that involve jumping as a principal motor skill; 4) absence of a history of two or more lateral ankle sprain; 5) absence of pain, instability and/or ankle weakness at the moment of the participation in the study.					
Needle et al. (2015) [28]	19 CAI (- ; age, 22.3; PA: active; height: 171 cm; body mass: 73.6 kg; CAIT: 18.3; sprains n 4.3) 20 COPERs (- ; age, 23.1; PA: active; height: 172 cm; body mass: 73.4 kg; CAIT: 29.4; sprains n 1.4) 20 CG (- ; age 22.5; PA: active; height: 170 cm; body mass: 68.9 kg)	CAI: 1) history of 1 or more ankle sprains; 2) CAIT < 25. COPERS: 1) history of 1 or more ankle sprains; 2) CAIT > 25. CG: 1) no history of ankle sprain; 2) CAIT > 27. All subjects had no history of lower limbs' fracture or surgery, and no neurological disorders. CG & COPERs participants were free of all lower extremity injury for 12 months before the participation in the study.	20° supination perturbation	PL TA SOL	EMG onset was calculated for each muscle by identifying the peak activity and searching backwards to find the point EMG went below 10% of peak activity. The average EMG activity (%MVIC) was calculated for 250ms prior to the perturbation, 250ms from the start of the perturbation and 250-500ms from the start of the perturbation.	The ensemble peak EMG from the 3 MVIC trials was used for normalization of stiffness trials.	SOL and TA activation was lower in participants with instability when compared to CG in passive trials. It was observed that a higher and earlier TA activation led to a higher stiffness in the CG, and a higher SOL activation led to a higher stiffness in subjects with instability.
Rodrigues et al. 2019[22]	12 CAI (F ; age, 27.; PA: active; height: 166 cm; body mass: 62.5 kg; CAIT: 19.5) 11 CG (F ; age 27; PA: active; height: 161 cm; body mass: 57.6 kg)	1) ankle instability assessed with the CAIT score; 2) no history of fracture or surgery of the lower limbs within the last six months; 3) absence of vestibular and/or neurological disorders.	Sudden ankle eversions before and after fatigue	PL PB	The latency of PL and PB activation was calculated and identified as the value of latency immediately after the beginning of the fall of the platform, which exceeded the average signal of approximately 50 ms by 3 standard deviations prior to the fall of the platform. The RMS of the EMG signal was calculated up to 200 ms after the fall of the platform.	The RMS value was normalized by the average interval of 4 s of the MVIC.	There were no differences between CAI and CG for the latency of the PL and PB in response to the sprain simulation before and after the induction of fatigue. A lower activation of PL and PB was observed under fatigue condition.
Sierra Guzman et al. (2018) [46]	50 CAI (- ; age, 22.6.; PA: active; height: 172 cm; body mass: 69.1 kg; CAIT: 19.5) 55 CG (- ; age 20.9; PA: active; height: 172 cm; body mass: 66.5 kg)	1) at least 1 significant ankle sprain (the most recent injury must have occurred >3 months prior to study); 2) 2 or more episodes of the ankle giving way in the last 6 months; 3) CAIT ≤ 24; 4) absence of previous surgeries to the musculoskeletal structures; 5) absence of history of a fracture in either lower limb; 6) absence of acute injury to musculoskeletal structures.	Inversion perturbation	PL PB	Reaction Time to the perturbation was defined as the time from the platform opening to electromyographic onset determined by an increase greater than twice the noise level in the EMG signal.		Participants with CAI showed prolonged peroneal reaction time in comparison with CG.
Sousa et al. (2018) [47]	24 CAI (FAI + MAI) (M 18, F 6; age: 22.2; PA: active) FAI: (height: 175 cm; body mass: 69 kg; sprains n 3.5)	In accordance with the criteria set by the International Ankle Consortium: 1) at least one significant unilateral ankle sprain; 2) initial sprain occurred at least 12 months prior to enrolment in the study; 3) at least one ankle sprain was associated with inflammatory symptoms; 4) at least one ankle sprain created	Sudden ankle inversion	PL PB TA SOL	RMS was analysed at two epochs in relation to the first deflection of the accelerometer signal (T0): 50 to 200 ms (subcortical CPA), and 200 to 350 ms (voluntary CPA).	The magnitude of the EMG signal in each interval was normalised by baseline values (from -500 to -350 in relation to T0) to assess the degree of	TA compensatory postural adjustments were decreased in participants with FAI with respect to CG in both limbs, while in SOL were decreased in the uninjured limb. SOL and PB compensatory postural adjustments were lower in MAI with respect to the other two groups.

	MAI: (height: 177 cm; body mass: 70.5 kg; sprains n 2.7) 20 CG (M 17, F 3; age 22.5; PA: active; height: 178 cm; body mass: 73.8 kg)	at least one day of interruption of desired physical activity; 5) most recent injury more than three months prior to enrolment in the study; 6) ankle joint “giving way”; 7) absence of previous surgeries to the musculoskeletal structures in either limb of the lower extremity; 8) absence of lower limb fracture requiring realignment; 9) absence of acute injuries; 10) absence of neurological impairments.				magnitude modulation of each muscle during compensatory responses in relation to upright standing.	
Sousa et al. (2018) [48]	24 CAI (M 18, F 6; age, 20.6; PA: active; height: 176 cm; body mass: 70 kg; sprains: n 3.1) 20 CG (M 17, F 3; age 21.8; PA: active; height: 178 cm; body mass: 73.8 kg; sprains)	In accordance with the criteria set by the International Ankle Consortium: 1) at least one significant unilateral ankle sprain; 2) initial sprain occurred at least 12 months prior to enrolment in the study; 3) at least one ankle sprain was associated with inflammatory symptoms; 4) at least one ankle sprain created at least one day of interruption of desired physical activity; 5) most recent injury more than three months prior to enrolment in the study; 6) ankle joint “giving way”; 7) absence of previous surgeries to the musculoskeletal structures in either limb of the lower extremity; 8) absence of lower limb fracture requiring realignment; 9) absence of acute injuries; 10) absence of neurological impairments.	Sudden ankle inversion	PL PB TA SOL	The latency of muscle response was detected in a time window from - 200 ms to + 200 milliseconds with respect to the first deflection of the accelerometer signal, and it was defined as the 50 milliseconds (or more) when the EMG amplitude was higher than baseline value plus 3 standard deviations, from - 500 to + 450 milliseconds. Short latency reflexes were identified as the first 20 ms of the reflex response, while medium latency reflexes were identified as a 20 ms window starting 30 ms after the onset of muscle activation.	Baseline signal obtained in upright standing posture.	When compared with CG, CAI showed a delayed activation of TA and SOL, and a lower activation of TA medium latency reflexes. The showed also a higher activation of the PL in the short- and medium-latency reflexes windows.
Vaes et al. (2002) [49]	40 CAI (M 18, F22; age, 18 to 23; PA: -) 41 CG (M 9, F32; age 15 to 29; PA: -)	1) at least one ankle sprain followed by pain, swelling, and inability to participate in recreational or other activities for at least 3 weeks; 2) complaints of instability causing pain and swelling; 3) ankle “giving way”; 4) absence of ankle sprain or surgery in the last 3 months; 5) full weight bearing on the tested limb; absence of inflammation.	50° ankle supination	PL	The onset of PL EMG activity during the sudden inversion was identified as an increase in the signal more than twice the noise level. The electromechanical delay was measured in an additional experimental set-up.		The latency was not significantly different between CAI and CG.

Table 3. Data extracted from each article for qualitative synthesis. CAI: chronic ankle instability; FAI: functional ankle instability; CG; control group; PA: physical activity level; MVIC: maximal voluntary isometric contraction; RMS: root mean square; EMG: electromyography; SL: single limb; SLJ: single limb jumping; SEBT: star excursion balance test; PL: Peroneus longus; TA: Tibialis anterior; PB: Peroneus brevis; SOL: Soleus; GM: Gluteus medium; Gmax: Gluteus maximum; ER: Erector spinalis (ER); RA: Rectus abdominis; FADI: Foot and Ankle Disability Index; FADI-S: Foot and Ankle Disability Index - Sport; CAIT: Cumberland Ankle Instability Tool.

LANDING AND HOPPING							
Authors (year)	Participants	Criteria for CAI	Task	Muscles	EMG analysis	Normalization method	EMG Results
Brown et al. (2004) [54]	10 FAI (-; age 22.5; PA: active; height: 176.5 cm; body mass: 82.6 kg; AJFAT < 20) 10 CG (-; age 21.9; PA: active; height: 181.8 cm; body mass: 72.8 kg)	1) at least 2 ankle sprains in the last year; 2) ankle “giving-way” during activity; 3) AJFAT < 20; 4) absence of current and past lower limbs’ injuries.	SLJ landing downward	PL PB TA LG SOL	The onset of muscle activation was identified when EMG signal was higher than 3 SD above baseline signal and lasted more than 10 ms. EMG amplitude was calculated 200 ms before to 1000 ms after the impact.	Maximal voluntary activation mean amplitude of double-limb maximal vertical jump.	The only difference between CG and FAI, was the observation of a lower SOL activation after landing in the latter.
Caulfield et al. (2004) [55]	12 FAI (6 M, 6 F; age, 26.4; PA: active; height: 171 cm; body mass: 72 kg) 10 CG (5 M, 5 F; age 24.9; PA: active; height: 167 cm; body mass: 69 kg)	1) at least 2 ankle sprains requiring protected weight bearing and/or immobilization; 2) absence of current and past lower limbs fractures; 3) self-reporting of weakness, pain and reduced function to the ankle; 4) subjective complaints secondary to past an inversion sprain.	SLJ landing downward and for distance	PL TA SOL	Vertical component of ground-reaction force was analysed to detect the time of impact. EMG amplitude was calculated for 300 ms pre-impact and 300 ms post-impact. EMG amplitude was calculated as the average of 5 records and then normalized.	Maximum EMG amplitude averaged from the 5 records for each jump activity for each participant.	There were no differences between the two groups for SOL and TA activations. CAI group showed a lower PL activation in the pre-impact of the two types of jumps.
Delahunt et al. (2006) [56]	24 FAI (15 M, 9 F; age, 25; PA: active; height: 174 cm; body mass: 72.08 kg) 24 CG (16 M, 8 F; age 22; PA: active; height: 176 cm; body mass: 70.87 kg)	1) at least 2 ankle sprains requiring protected weight bearing and/or immobilization; 2) absence of current and past lower limbs fractures; 3) self-reporting of weakness, pain and reduced function to the ankle; 4) subjective complaints secondary to past an inversion sprain; 5) lack of neurological or vestibular diseases.	SL drop jump	PL TA SOL RF	EMG amplitude was calculated as the integral of the signal for the 200-ms pre-initial contact (IC) and for the 200-ms post-IC of 10 EMG records.	Peak EMG amplitude averaged from 10 records for each subject.	PL activation was lower in FAI during the pre-impact when compared with CG.

Delahunt et al. (2007) [68]	26 FAI (16 M, 10 F; age, 25.6; PA: active; height: 173.7 cm; body mass: 72.14 kg) 24 CG (15 M, 9 F; age 22.6; PA: active; height: 175.8 cm; body mass: 70.28 kg)	1) at least 2 ankle sprains requiring protected weight bearing and/or immobilization; 2) absence of current and past lower limbs fractures; 3) self-reporting of weakness, pain and reduced function to the ankle; 4) subjective complaints secondary to past an inversion sprain; 5) lack of neurological or vestibular diseases.	Lateral hopping	PL TA SOL RF	EMG amplitude was calculated as the integral of the signal for the 200-ms pre-initial contact (IC) and for the 200-ms post-IC of 10 EMG records.	Peak EMG amplitude averaged from 10 records for each subject.	The activation of RF, TA and SOL was higher in FAI than CG during pre- and post- IC. FAI group showed also a less everted position from 45 ms pre-IC to 95 ms post-IC.
Dundas et al. (2014) [26]	11 CAI (-; age, 26; PA: - height: 170 cm; body mass: 76.77 kg; CAIT: 18; sprains: n 5.2) 9 COPERS (-; age, 26; PA: - height: 173.7 cm; body mass: 69.31 kg; CAIT: 28; sprains: n 2.1) 13 CG (1-; age 26; PA: - height: 170 cm; body mass: 68.08 kg)	Participants were divided according to the CAIT score. Those with scores greater than 27 were assigned to the COPERS group, while those with scores less than 25 were assigned to the CAI group.	Stepping down	PL TA	Preparatory and reactive EMG activity was calculated as the area under the processed EMG signal 200 ms before and after touchdown, respectively, in the TA and PL muscles.	Maximal activation for each muscle during the gait trials.	COPERS showed a higher TA activity in the preparatory (pre-touchdown) and reactive (post-touchdown) phases when compared to CAI and CG.

Feger et al. (2014) [69]	15 FAI (5 M, 10 F; age, 23; PA: active; height: 173. cm; body mass: 72.4 kg; FAAM-ADL: 87.2; FAAM-S: 68.5; sprains: n 4.5) 15 CG (5 M, 10 F; age 22.9; PA: active; height: 173 cm; body mass: 70.08 kg)	1) more than 1 ankle sprain with the initial sprain occurring more than 1 year before the study onset; 2) self-reported impairments in function and symptoms; 3) absence of ankle sprains within the 6 weeks before the study; 4) absence of lower extremity injury or surgery; 5) absence of balance disorders, neuropathies, diabetes; 6) absence of other conditions affecting balance.	SL stance eyes closed; SEBT; forward lunges; lateral hops	PL TA LG RF BF GM	<u>Forward Lunge</u>. Pre-IC and pot-IC EMG amplitude were calculated as the RMS of a 50-millisecond epoch immediately before IC and a 100-ms epoch immediately after IC, respectively. <u>Single-limb Eyes-closed Balance</u>. A 3-s epoch during the middle of the single-limb eyes-closed balance trial was analysed. <u>SEBT</u>. A 500-millisecond epoch before maximum excursion was averaged over 3 trials for each of the 3 directions. <u>Lateral Hops</u>. Pre-IC and pot-IC EMG amplitude were calculated as the RMS of a 50-millisecond epoch immediately before IC and a 100-ms epoch immediately after IC, respectively. Data were analysed also as distal lower limb (TA, PL, LG), proximal lower limb (RF, BF, GM) and entire lower limb (all the muscles).	Amplitudes were normalized to respective MVIC	<u>Forward Lunge</u>. CAI showed: a lower proximal muscle activation before IC, a lower TA activation during post-IC, a lower distal and total post-IC activation when compared with CG. <u>Single-limb Eyes-closed Balance</u>. CAI showed a lower activation of TA and RF, and a lower activation of the entire lower limb in comparison with CG. <u>SEBT</u>. CAI showed a lower activation of the TA during the anterior and posteromedial directions. <u>Lateral Hops</u>. CAI showed a lower activation of the distal and the entire lower limb during pre-IC in comparison with CG.
Fereydounnia et al. (2018) [59]	8 CAI (6 M, 2 F; age, 23.7; PA: active; height: 173.6 cm; body mass: 60.4 kg) 11 CG (9 M, 2 F; age 24.7; PA: active; height: 175.2 cm; body mass: 66.6 kg)	1) History of ankle sprain; 2) the first sprain occurred within 6 months and 1 year; 3) occurrence of successive ankle sprains; 4) feelings of ankle "giving way"; 5) absence of pain and restriction in both ankles during the test; 6) negative talar test.	Forward jump landing with visual stimulus	PL PB TA G-SOL	<u>Premotor time</u> was calculated as the time interval between activation of the visual stimulus and onset of the EMG activity. <u>Motor time</u> was calculated as the time between the visual stimulus and onset of the EMG (mean + 3 sd at rest). <u>Reaction time</u> was calculated as the time interval between activation of the visual stimulus and onset of the motion. <u>Anticipation</u> was defined as the minimum and the maximum of the EMG activity of the off time of the contact switch and calculate it from the onset of off time to the moment when the EMG signal reached minimum + 10% maximum.		PL pre-motor time was shorter in CAI. PL motor time was longer in CAI. No differences in other parameters.

Fereyounnia et al. (2018) [60]	8 CAI (6 M, 2 F; age, 23.7; PA: active; height: 173.6 cm; body mass: 60.4 kg) 11 CG (9 M, 2 F; age 24.7; PA: active; height: 175.2 cm; body mass: 66.6 kg)	1) History of ankle sprain; 2) the first sprain occurred within 6 months and 1 year; 3) occurrence of successive ankle sprains; 4) feelings of ankle “giving way”; 5) absence of pain and restriction in both ankles during the test; 6) negative talar test.	Forward jump landing	PL PB TA G-SOL	<u>Premotor time</u> was calculated as the time interval between activation of the visual stimulus and onset of the EMG activity. <u>Motor time</u> was defined as the time span between onset of the EMG activity and APTTS. <u>Reaction time</u> was calculated as the time interval between activation of the visual stimulus and onset of the motion.		The CG showed a longer pre-motor time of the G-SOL in the dominant leg compared to non-dominant leg. The CAI group showed a significantly longer pre-motor time of the G-SOL and PB of the injured leg compared to non-injured leg.
Gehring et al. (2013) [23]	19 CAI (FAI) (8 M, 10 F; age, 23.6; PA: active; height: 175.2 cm; body mass: 65 kg; CAIT: 18.1) 19 CAI (FAI+MAI) (-; age, 23.7; PA: active; height: 177 cm; body mass: 75 kg; CAIT: 19.8) 18 CG (-; age 22.8; PA: active; height: 175 cm; body mass: 70 kg)	FAI+MAI group: 1) feelings of instability and/or ‘giving way’ in at least one ankle; 2) CAIT score ≤ 24; 3) at least two ankle sprains with the last occurring within 2 years before the study; 4) diagnosis of MAI after manual examination. FAI group: all features of FAI+MAI group except the diagnosis of MAI. Exclusion criteria were: 1) lack of participation in risk sports; 2) individuals with MAI only; 3) individuals who could not be clearly rated for FAI and MAI.	Standing, walking and jumping on a tilting platform	PL TA	Muscle activations in terms of RMS were calculated for 5 time intervals: - EMGPre, i.e. the 100 ms before ground contact; - EMGPrep, i.e. from ground contact until the tilt of the platform; - EMGTilt, i.e. the phase of platform tilting; - EMG 60–90 ms, i.e. an early response phase after tilting the platform; - EMG90–120 ms, i.e. a late response phase after tilting the platform.	EMG values were individually normalised at the RMS value of a normal gait cycle (=100%), which was determined on the basis of 20 consecutive gait cycles on a treadmill.	No differences between the groups were reported for muscles activations after the tilting of the platform, for plantar flexion and for internal rotation. The PL pre-activation during the jumping phase was lower in FAI+MAI group compared with FAI group.
Gutierrez et al. (2011) [25]	19 CAI (AI) (8 M, 10 F; age, 20.9; PA: active; height: 171 cm; body mass: 68.3 kg; CAIT: 19.9; sprains: n 2.9) 9 LAS (-; age, 21.2; PA: active; height: 179 cm; body mass: 69.2 kg; CAIT: 28.1; sprains: n 1.8) 18 CG (-; age 20.6; PA: active; height: 175.2 cm; body mass: 65 kg)	CAI: 1) history of ankle sprain; 2) repeated “ankle giving-way”; 3) CAIT: < 24; 4) absence of fractures or injuries in the las 6 months. LAS: 1) history of ankle sprain; 2) absence of instability; 3) CAIT: < 28; 4) absence of fractures or injuries in the las 6 months.	Landing on an ankle supinating device	PL TA	Preparatory and reactive EMG amplitude and EMG patterns for the pretouchdown and posttouchdown were calculated. The preparatory time period was identified as the time window of 200 ms before touchdown, while the reactive time period was defined as 200 ms after the touchdown.	The EMG data were normalized to the maximum activity during the unknown, non-supinating trials in each respective muscle.	PL activation was increased in preparatory and reactive phases In AI compared the other groups. The LAS group showed a trend toward increased preparatory TA activation.

Herb et al. (2018) [64]	24 CAI (- ; age, 21.4; PA: active; ; height: 169 cm; body mass: 60.7 kg; FAAM-ADL: 86.7; FAAM-S: 66.5; sprains: n 4.6) 23 CG (- ; age 21.7; PA: active; height: 165.4 cm; body mass: 63.3 kg)	1) 1 or more lateral ankle sprains at least 12 months before the study; 2) persistence of instability; 3) FAAM-S < 85%; 4) IdFAI > 10; 5) absence of fractures or surgeries to lower limbs; 6) pathologies affecting balance.	Drop vertical jump	PL PB TA MG	Trials were divided into 100 points representing 100 milliseconds before initial contact on the force plate to 200 milliseconds post-contact. Surface EMG amplitudes were root mean square rectified and then normalized.	Mean EMG during quiet-standing.	The patients with CAI had greater peroneal activity from 17 to 128 milliseconds post-contact than the control participants.
Kim et al. (2019) [57]	100 CAI (M 54, F 46; age, 22; PA: active; height: 174 cm; body mass: 72 kg; FAAM-ADL: 82.6; FAAM-S: 62.2; MAAI: 3.6; sprains: n 4.5) 100 CG (M 54, F 46; age 22; PA: active; height: 173 cm; body mass: 71 kg)	1) At least two sprains in the past 6 months; 2) FAAM- ADL <90%; 3) FAAM- S <80%; 4) at least two “yes” answers on questions 5-9 of the AII; 5) absence of lower- extremity musculoskeletal injuries in the previous 3 months; 7) no lower- extremity surgery and/or fracture in lifetime.	Single leg jump landing/cutting task	PL TA MG VL MH GM Gmax	A rigid link model (foot, shank, thigh, and pelvis segments) was created using the static calibration, and this model was assigned to all landing and jumping trials, in order to calculate ankle, knee, and hip joint kinematics. Ankle, knee, and hip joint angles were calculated using a Cardan rotation sequence.	EMG data collected from eight muscles during 3 seconds of an isometric squatting with 45° of knee flexion and 30° hip flexion.	CAI showed a higher activation of MG, PL, ADD, VL, GM, and Gmax during initial landing in comparison with CG.
Kim et al. (2020) [58]	11 CAI (- ; age, 22.4; PA: active; height: 168 cm; body mass: 69 kg; FADI: 90.3; FADI-S: 88.6; MAAI: 3.6; sprains: n 4.5) 11 CG (- ; age 22.6; PA: active; height: 174 cm; body mass: 66.8 kg)	The Ankle Instability Instrument, the FADI and FADI-S questionnaires were used to identify individuals with CAI.	1) double-leg forward jump with single-leg landing, 2) double-leg forward jump with single-leg landing and anticipated cutting, and 3) double-leg forward jumping with single-leg landing and unanticipated cutting.	PL TA SOL MG LG	The wavelet transformations analysis was performed. It allow for the simultaneous analysis of EMG signals in the time and frequency domains. The intensity of the EMG signal was calculated with a wavelet intensity analysis in which EMG data was transformed in the time-frequency domain with a set of 11 nonlinearly scaled Cauchy wavelets (w1-w11). The center frequencies of the wavelets were 6.90, 19.29, 37.71, 62.09, 92.36, 128.48, 170.39, 218.08, 271.50, 330.63, and 395.46 Hz to capture the full range of the EMG signal spectrum. The intensities were time-normalized to make 0–100 % of stance phase. The intensity from w1 was excluded for further analyses because it was considered to reflect movement artifacts. The total intensities of each wavelet (w2-w11) were compiled into a matrix. The matrix had 915 rows representing (183 trials × 5 muscles) and 10 columns representing total intensity from w2-w11. A principal component analysis was applied to the matrix to find the principal components (PC) that accounted for 90 % of the total variance (VAF).	-	CAI group showed smaller PC1 scores than CG across all muscles and tasks. CAI group exhibited also smaller PC2 scores than CG during only anticipated cutting.

Kunugi et al. (2018) [61]	15 CAI (M; age, 19.8; PA: active; height: 172.6 cm; body mass: 66.59 kg; CAIT: 21.13) 15 CG (M; age 20; PA: active; height: 173.6 cm; body mass: 68.31 kg)	1) at least 2 ankle sprains, occurring at least 12 months prior to study participation; 2) at least one day of modified PA because of the sprain; 3) “giving-way” symptoms; 4) absence of previous fractures or surgical interventions to lower limbs; 5) Injuries in the previous 3 months.	Diagonal single leg rebound jump	PL PB MG TA AD GM	Data were averaged across the three successful trials completed by each participant. All time averaged data were identified in the periods from 300ms pre-IC to 300ms post-IC.	The 3 s data of maximum voluntary isometric contraction (MVC) for each lower leg muscle against manual resistance was recorded.	The CAI group had reduced hip adductor and peroneus muscle activations.
Levin et al. (2015) [80]	9 CAI (M 4; F 5; age, 23.7; PA: active; height: 172cm; body mass: 70.8 kg) 9 CG (M 5, F 4; age 21.1; PA: active; height: 178 cm; body mass: 67.6 kg)	1) history of ankle sprains; 2) complaints of repetitive lateral ankle sprains for at least 6 months; 3) ankle “giving way”; 4) decreased performance level of recreational, competitive, or professional activities.	Landing on a stable or unstable surface causing ankle inversion	PL TA MG GM	RMS was calculated at three time windows within a 500 ms period of interest, ranging from 200 ms prior to TD (pre-impact period) to 300 ms after TD (post-impact period). The first time window corresponded to the pre-impact period (i.e., 200 to 0 ms). The remaining two time windows of interest were set within the post impact period (0 to +300 ms). The first reflex window (+30 to +60 ms) corresponded to SLR and the second window (+80 to +135 ms) corresponded to LLR.	RMS of the EMG signal within the 500 ms window of interest (i.e., from 200 ms before to +300 ms after TD) during the non-inverting condition	In CAI pre-landing muscle activation was higher in contralateral limb. After the impact, SLR were decreased in all muscles in CAI compared to CG.
Li et al. (2018) [62]	21 CAI (F; age, 21; PA: active; height: 164 cm; body mass: 64.4 kg; idFAI: 22.2; CAIT: 19.3) 21 CG (F; age 21; PA: active; height: 165 cm; body mass: 64.4 kg)	The inclusionary and exclusion criteria established by the International Ankle Consortium were utilized for identifying those with CAI.	Drop landing on a 25° inclined force plate	PL TA LG RF VL BF	EMG amplitude was calculated for the pre-landing phase, defined as the interval from 50 ms prior to contact (-50 ms) to the instant of initial contact (vertical GRF > 10 N) and the landing phase, defined as the first 100 ms after initial contact. Cocontraction index (CCI) was calculated between tibialis anterior and gastrocnemius lateralis, and between tibialis anterior and peroneus longus. Time to peak EMG linear envelope was calculated in order to reveal the neuromuscular control and muscle activation strategy during landings.	MVIC of each muscle.	In the pre-landing phase, compared to controls, CAI participants showed a reduced ankle evtor activation during the pre-landing when compared with CG. A higher TA activation was recorded during the landing phase in CAI, and thus a higher co-contraction.

Lin et al. (2011) [45]	15 CAI (M 6, F 9; age, 21.6; PA: active; height: 166.8 cm; body mass: 62.6 kg; CAIT: 18.1; sprains: 3.5 x year) 15 CG (M 7, F 8; age 21.5; PA: active; height: 164.5 cm; body mass: 59.2 kg;)	1) at least 1 ankle sprain leading to swelling, pain, and protected weightbearing and/or immobilization; 2) ankle “suddenly giving way”; 3) at least 2 ankle sprains within the past 2 years; 4) ankle sprain at least once in the past 6 months; 5) CAIT score < 27; 6) history of lower extremity fractures or injuries; 6) absence of inflammation.	Running; stop-jump landing	PL LG TA	Each of the tasks was divided into 2 phases: the pre-landing phase, i.e. the 200 milliseconds immediately prior to foot strike, and the post-landing phase, i.e., the 200-millisecond immediately after foot-strike. The pre-landing phase was in turn divided into 2 phases: phase I (200-100 milliseconds prior to foot-strike) and phase II (100 milliseconds to foot-strike). Similarly, the post-landing phase was divided into phase III (foot-strike to 100 milliseconds after) and phase IV (100-200 milliseconds after foot-strike). Coactivation index and EMG RMS were calculated for all the phases. Then data were normalized.	MVIC of each muscle	No differences between CAI and CG were found during running. CAI group showed a lower TA/PL co-contraction index during the pre-landing phase, and a lower activation of the PL in the postlanding phase.
Moisan et al. (2019) [72]	32 CAI (M 11, F 21; age, 25.3; PA: active;) 31 CG (M 11, F 20; age 23.7; PA: active)	In accordance with the International Ankle Consortium statement: 1) one or more LAS that occurred more than 12 months prior to the study; 2) ankle “giving-way”; 3) FAAM-ADL <90%; 4) FAAM-S <80%; 5) absence of a history of a previous surgery to the lower extremity musculoskeletal structures; 6) absence of a history of a fracture that required surgical realignment; 7) absence of lower limbs injuries in the 3-month period prior to the study; 8) absence of pathologies affecting balance.	Unilateral side-jump and drop landing on different surfaces	PL LG VM GM TA MG VL	EMG amplitude was quantified as RMS with an algorithm with a 100 ms moving window. EMG data were quantified during the landing and the preactivation (from heel off to initial contact) phases and were normalised to 0 to 100% of each phase. Initial contact was determined with the force plate using a 10N threshold and the heel lift was determined with the footswitch under the heel	Average peak RMS activity during the five trials of each task	The CAI group showed: increased BF muscle activity during the preactivation and landing phases; decreased GM and PL muscles activity during the preactivation phase of the WEDGE task; decreased VL muscle activity during the preactivation phase of the DROP task; decreased BF muscle activity during the preactivation and landing phases and decreased GM muscle activity during the preactivation phase of the SIDE task.
Monteleone et al. (2014) [70]	12 FAI (M 7, F 5; age, 23.7; PA: -; height: 174.3 cm; body mass: 68.7 kg) 12 CG (M 7, F 5; age 23.8; PA: -; height: 175.1 cm; body mass: 70.7 kg)	1) 1 or more ankle sprains; 2) persistence of pain, swelling, instability, or recurrent injuries; 3) absence of ankle sprain within the last 3 months; 4) absence of history of ankle fracture; 5) absence of history of major injuries to knee, ankle, foot, or hip injuries that required prolonged rehabilitation or surgery; 6) absence known vestibular, visual, or neurological disease.	Medial/lateral hopping over an obstacle	PL SOL TA MG	A moving 10-ms window was used to determine the amplitude in terms of RMS throughout the lateral hop movement. Amplitude of each muscle was determined at take-off, mid-flight, contact, and mid-contact events.	Maximal amplitude of that each for each trial.	The FAI group revealed greater tibialis anterior muscle activity at contact moving in the lateral direction.
Rosen et al. (2013) [71]	20 FAI (M 10, F 10 ; age, 20.9; PA: -; height: 173 cm; body mass: 76.2 kg; CAIT: 20.4; sprains: n 4.45) 20 CG (M 10, F 10 ; age 20.6; PA: -; height: 173.9 cm; body mass: 75.6 kg)	1) CAIT < 24; 2) absence of ankle injuries within 1 year from the study.	Lateral 2-legged lateral jump for height onto a platform	PL LG TA	The EMG area was calculated as the integral of the normalized EMG data. The initial ground contact was identified. Data were then extracted between 2 periods of interest: 150 milliseconds before ground contact and 250 milliseconds after ground contact. The EMG data were averaged over 3 trials across the 4 jumping conditions (eyes open at 35 cm and 50 cm and eyes closed at 35 cm and 50 cm).	The EMG data were normalized to the ensemble peak of the 50-cm eyes-open trial	FAI group had a lower preparatory TA and PL EMG area and TA and PL peak EMG in comparison with CG and independently from eyes-open or eyes-closed condition.

Simpson et al. (2019) [13]	15 CAI (- ; age, 21.3; PA: active; height: 171 cm; body mass: 73.4 kg; CAIT: 18.9; sprains: n 6) 15 CG (- ; age 21.5; PA: active; height: 170 cm; body mass: 75.5 kg)	1) at least two lateral ankle sprains with one of those lateral ankle sprains occurring within the previous 12 months; 2) sustained a lateral ankle sprain that required non-weight bearing activity and/or immobilization for ≥ 24 h; 3) ankle “giving way” or “feelings of instability”; 4) CAIT score of 24; 5) absence of history of ankle fracture; 6) absence of history of major injuries to knee, ankle, foot, or hip injuries that required prolonged rehabilitation or surgery; 7) absence known vestibular, visual, or neurological disease.	Single-leg drop landing expected and unexpected 20° tilting surface	PL PB TA MG	EMG amplitude was calculated for 200 ms pre-landing and 200 ms post-landing. Cocontraction index (CCI) was also calculated for pre- and post-landing TA and MG, and PL and PB and the TA. Latency of the PL and PB was determined from the rectified EMG signal as the time in ms from when the vertical component of the ground reaction force exceeded 15 N, which coincided with the initiation of the inversion perturbation, to the point where muscle activity exceeded 5 standard deviations (SD) above the averaged 200 ms pre-landing muscle activity.	MVIC of each muscle	When compare to CG, CAI showed a longer PL latency, a longer PB latency, a lower TA activation and frontal plane CCI during the post-landing phase during the unexpected perturbation.
Son et al. (2017) [30]	22 CAI (M 12, F 8 ; age, 22.7; PA: active; height: 174.6 cm; body mass: 73.4 kg; FAAM-ADL: 81.9%; FAAM-S: 60.9%; MAAI: 3.4; sprains: n 4.1) 22 COPERS (M 12, F 8 ; age, 22.1 ; PA: active; height: 174 cm; body mass: 72 kg; FAAM-ADL: 100; FAAM-S: 100; MAAI: 0; sprains: n 2) 22 CG (M 12, F 8 ; age 21.8; PA: active; height: 173.3 cm; body mass: 79.2 kg)	In accordance with the International Ankle Consortium: 1) a history of at least two repeated unilateral ankle sprains with the most recent ankle sprain that occurred more than 3 months prior; 2) FAAM-ADL score <90%; 4) no “yes” answer on questions four to eight on the MAII; 5) no previous formal rehabilitation for the test ankle; 6) a history of physical activity at least 3 days x week for a total of 90 min in the past 3 months. COPER: 1) at least one severe ankle sprain that occurred more than 12 months prior the study; 2) return to moderate levels of weight-bearing physical activity without repeated ankle injury in the past 12 months; 3) FAAM-ADL and FAAM-S 100%; 4) no “yes” answer on questions four to eight on the MAII; 5) no previous formal rehabilitation for the test ankle; 6) a history of physical activity at least 3 days x week for a total of 90 min in the past 3 months.	Jump landing with side-cutting 90°	PL TA MG VL MH GM Gmax	EMG RMS was calculated with a moving window of 125 ms and then data were normalized to the smoothed reference EMG data.	Reference EMG data were obtained during 3 s of an isometric double-leg squat position (e.g., 45-knee flexion and 30-hip flexion using a goniometer) .	CAI patients showed less TA activation during 41% to 100% of stance, less PL activation during 0% to 3% and 20% to 97% of stance, less MG activation during 27% to 65% of stance, less VL activation during 0% to 4% and 39% to 65% of stance, less Gmed activation during 0% to 2% and 35% to 74% of stance, and less Gmax activation during 0% to 3% and 28% to 72% of stance when compared to COPERS. CAI patients showed less TA activation during 36% to 100% of stance, less PL activation during 0% to 66% of stance, less MG activation during 23% to 65% of stance, more VL activation during 2% to 21% of stance and less VL activation during 44% to 60% of stance, more Gmed activation during 3% to 14% of stance and less Gmed activation during 35% to 45% of stance, and less Gmax activation during 24% to 71% of stance when compared to CG. None or small activation differences (e.g., PL, MG, VL, MH, Gmed, and Gmax) were observed between copers and controls.
Suda et al. (2009) [67]	21 FAI (- ; age, 20; PA: active; height: 189 cm; body mass: 79.4 kg) 19 CG (- ; age - ; PA: active; height: 191 cm; body mass: 80.9 kg)	1) at least one sprain needing reduction of activity more than three months before the study; 2) ankle “giving-way” during sports activities; 3) difficulties in walking and running on irregular surfaces; 4) difficulty to jump and change directions; 5) sprain recurrence.	Landing after blocking movement	PL TA LG	Amplitude of the EMG signal (RMS) was analysed in a period corresponding to a movement cycle: an interval of 200 ms before the time of impact and 200 ms after the time of impact. The muscular onset activity was expressed as the difference between the landing instant, i.e., instant in time in which the distance d between the integrated normalized EMG signal inclination and the reference line was the highest and was depicted in relation to the landing instant, and the instant of muscular activation.	Maximum voluntary contractions against manual resistance.	The FAI group showed a lower PL activation in the pre-landing and a higher activation in the post-landing. The CG had a simultaneous activation of PL and GL, with TA presenting a later activation. In FAI group all the three muscles activated simultaneously. There were no significant differences between groups for co-activation index.

Terada et al. (2014) [88]	19 CAI (M 10, F 9; age, 20.1; PA: active; height: 174 cm; body mass: 72 kg; FADI: 83.15%; FADI-S: 65.7 %) 19 CG (M 10, F 9; age 21.3; PA: active; height: 171.2 cm; body mass: 71.2 kg)	1) at least 1 acute unilateral ankle sprain that caused swelling, pain, and temporary loss of function; 2) a history of at least 2 self-reported episodes of “giving way” in the last 3 months; 3) absence of history of any musculoskeletal and neurovascular injury in the lower extremity in the last 2 years; 4) no previous history of low back pain in the last 6 months; 5) no previous fractures or surgery in the lower extremity in the last 2 years.	Double-legged vertical stop-jump	VM VL MH LH	The integrated EMG (IEMG) was calculated for each muscle during the prelanding, i.e., the 100 ms immediately before ground impact. IEMG was also calculated over the time period from the initial ground impact (the point at which the vertical ground reaction forces exceeded 10 N) to the point of 100 ms post-ground impact during the stop jump.	IEMG variables were normalized for each muscle with the ensemble peak (ie, the highest-amplitude) EMG values that were recorded during each landing phase of the stop jump trials and calculated from the average of the 5 trials.	CAI group showed a higher VM activity during prelanding in comparison with CG.
Watson et al. (2020) [74]	16 CAI (M 9, F 8; age, 24.3; PA: -; height: 174.2 cm; body mass: 73.7 kg; idFAI: 16.41) 16 CG (M 7, F 9; age 23.4; PA: -; height: 174.2 cm; body mass: 73.7 kg)	In accordance with the International Ankle Consortium: 1) ≥ 1 ankle sprain more than 1 year ago; 2) IdFAI score ≥ 11 ; 3) absence of lower-extremity injury for 3-months prior to testing; 4) absence of vestibular or cognitive deficits.	Hops-to-stabilization with or without cognitive load*	PL TA LG	Mean muscle activation was extracted in three time periods: 250-ms prior to landing (PRE), 0-250-ms after force plate contact (POST-1), and 250-500-ms after force plate contact (POST-2).	Activation was normalized to the ensemble peak across all hops.	PL and LG activation was lowest in SVN across all subjects. Lateral gastrocnemius activation was greatest in SDM.
Webster et al. (2016) [73]	16 CAI (M 8, F 8; age, 20.5; PA: active; height: 172.2 cm; body mass: 69.12 kg; FADI: 82%; FADI-S: 64.19%) 16 CG (M 8, F 9; age 22; PA: active; height: 170.5 cm; body mass: 69.63 kg)	1) History of ankle sprains; 2) 2 or more self-reported episodes of the ankle giving way in the 6 months before the study; 3) FADI score $< 90\%$; 4) FADI-Sport28 score $< 80\%$; 5) absence of previous lower extremity fracture or surgery; 6) absence of vestibular impairments.	Lateral hop with or without fatigue	PL TA GM Gmax	The EMG signals were collected for the periods of prelanding (200 milliseconds pre-IC to IC) and postlanding (IC until 200 milliseconds post-IC).	The prefatigue and postfatigue data were normalized to the mean peak EMG amplitude of the corresponding prefatigue and postfatigue hopping trials for each participant.	CAI group showed a higher PL and Gmax activation just before landing the lateral hop in comparison with CG. TA showed postfatigue activation higher than prefatigue activation. GM activation was no different between the two groups.
Yalfani et al. (2016) [65]	25 CAI (14 M, 11 F; age, 19.5; PA: active-competitive; height: 171 cm; body mass: 70.6 kg; FADI: 84.8%; FADI-S: 77.4%) 25 CG (14 M, 11 F; age 19.6; PA: active-competitive; height: 170 cm; body mass: 63 kg)	1) History of more than one ankle sprain; 2) ankle “giving way” within the last six months; 3) FADI score $\leq 90\%$; 4) FADI-S score $\leq 80\%$; 5) absence of major injuries or surgeries to lower limbs; 6) absence of pregnancy; 7) absence of pain; 8) absence of injuries in the last 6 months.	Drop-landing on an ankle supinator device	PL TA LG SOL GM Gmax	Amplitude of EMG data was calculated for the 200 millisecond (ms) pre and 200 ms after the contact time of a landing on an ankle-supinator device in condition of awareness and unawareness.	MVIC of each muscle.	LG, PL and SOL had higher activation in CAI group in pre CT. GM had higher activation in CAI group in post CT.

Table 4. Data extracted from each article for qualitative synthesis. CAI: chronic ankle instability; FAI: functional ankle instability; CG; control group; PA: physical activity level; MVIC: maximal voluntary isometric contraction; RMS: root mean square; EMG: electromyography; SL: single limb; SLJ: single limb jumping; SEBT: star excursion balance test; PL: Peroneus longus; TA: Tibialis anterior; PB: Peroneus brevis; SOL: Soleus; LG: Lateral gastrocnemius; MG: Medial gastrocnemius; MH: medial hamstrings; LH: lateral hamstrings; G-SOL: gastrocnemius-soleus; VL: Vastus lateralis; RF:

Rectus femoris VM: vastus medialis; ST: semitendinosus; BF: Biceps femoris; GM: Gluteus medium; Gmax: Gluteus maximum (Gmax); TP: Tibialis posterior; AJFAT: Ankle Joint Functional Assessment Tool; FAAM-ADL: Foot and Ankle Ability Measure - Activities of Daily Living score ; FAAM-S: Foot and Ankle Ability Measure - Sport scale; FADI: Foot and Ankle Disability Index; FADI-S: Foot and Ankle Disability Index - Sport; idFAI: Identification of Functional Instability scale; CAIT: Cumberland Ankle Instability Tool.

* Benton's judgment of line orientation (JLO), the symbol digit modalities test (SDM), or a serial seven task (SVN).

CUTTING AND TURNING							
Authors (year)	Participants	Criteria for CAI	Task	Muscles	EMG analysis	Normalization method	EMG Results
Fuerst et al. (2018) [24]	18 CAI (FI) (8 M, 10 F; age, 23.6; PA: active; height: 175.4 cm; body mass: 70.1 kg; CAIT: 20.3) 18 CAI (FMI) AI (8 M, 10 F; age, 23.6; PA: active; height: 173.7 cm; body mass: 69 kg; CAIT: 19.5) 18 CG (8 M, 10 F; age 24.2; PA: active; height: 171.4 cm; body mass: 66.6 kg)	CAI (FI) group = 1) CAIT < 24; 2) two or more sprains to the same ankle with the last within the past two years; 3) absence of mechanical instability. CAI (FMI) group = 1) CAIT < 24; 2) two or more sprains to the same ankle with the last within the past two years; 3) mechanical instability. CG = 1) no history of ankle sprains; 2) no mechanical instability; CAIT = 30.	45° sidestep-cutting; 25° crossover-cutting; 180° turning	PL TA SOL LG	EMG amplitude was calculated by means of RMS values in time intervals between 100 ms before foot strike and initial foot contact, and for time intervals of the first 100 ms of ground contact.	EMG values were normalized to EMG activity during straight running.	CAI group with functional instability only showed a higher PL activation before the foot strike when compared with CG and CAI group with functional-mechanical instability.
Kim et al. (2019) [57]	100 CAI (M 54, F 46; age, 22; PA: active; height: 174 cm; body mass: 72 kg; FAAM-ADL: 82.6; FAAM-S: 62.2; MAAI: 3.6; sprains: n 4.5) 100 CG (M 54, F 46; age 22; PA: active; height: 173 cm; body mass: 71 kg)	1) At least two sprains in the past 6 months; 2) FAAM- ADL <90%; 3) FAAM- S <80%; 4) at least two “yes” answers on questions 5-9 of the AII; 5) absence of lower- extremity musculoskeletal injuries in the previous 3 months; 7) no lower- extremity surgery and/or fracture in lifetime.	Single leg jump landing/cutting task	PL TA MG VL MH GM Gmax	A rigid link model (foot, shank, thigh, and pelvis segments) was created using the static calibration, and this model was assigned to all landing and jumping trials, in order to calculate ankle, knee, and hip joint kinematics. Ankle, knee, and hip joint angles were calculated using a Cardan rotation sequence.	EMG data collected from eight muscles during 3 seconds of an isometric squatting with 45° of knee flexion and 30° hip flexion.	CAI showed a higher activation of MG, PL, ADD, VL, GM, and Gmax during initial landing in comparison with CG.
Kim et al. (2020) [58]	11 CAI (- ; age, 22.4; PA: active; height: 168 cm; body mass: 69 kg; FADI: 90.3; FADI-S: 88.6; MAAI: 3.6; sprains: n 4.5) 11 CG (- ; age 22.6; PA: active; height: 174 cm; body mass: 66.8 kg)	The Ankle Instability Instrument, the FADI and FADI-S questionnaires were used to identify individuals with CAI.	1) double-leg forward jump with single-leg landing, 2) double-leg forward jump with single-leg landing and anticipated cutting, and 3) double-leg forward jumping with single-leg landing and unanticipated cutting.	PL TA SOL MG LG	The wavelet transformations analysis was performed. It allow for the simultaneous analysis of EMG signals in the time and frequency domains. The intensity of the EMG signal was calculated with a wavelet intensity analysis in which EMG data was transformed in the time-frequency domain with a set of 11 nonlinearly scaled Cauchy wavelets (w1-w11). The center frequencies of the wavelets were 6.90, 19.29, 37.71, 62.09, 92.36, 128.48, 170.39, 218.08, 271.50, 330.63, and 395.46 Hz to capture the full range of the EMG signal spectrum. The intensities were time-normalized to make 0–100 % of stance phase. The intensity from w1 was excluded for further analyses because it was considered to reflect	-	CAI group showed smaller PC1 scores than CG across all muscles and tasks. CAI group exhibited also smaller PC2 scores than CG during only anticipated cutting.

					movement artifacts. The total intensities of each wavelet (w2-w11) were compiled into a matrix. The matrix had 915 rows representing (183 trials $\times$ 5 muscles) and 10 columns representing total intensity from w2-w11. A principal component analysis was applied to the matrix to find the principal components (PC) that accounted for 90 % of the total variance (VAF).		
Koshino et al. (2015) [39]	10 CAI (M 9, F 1; age, 21; PA: active; height: 174 cm; body mass: 65.9 kg; CAIT: ≤ 25) 10 CG (M 9, F 1; age 20.8; PA: active; height: 174 cm; body mass: 66.5 kg)	Partially in accordance with the recommendations of the International Ankle Consortium: 1) at least one sprain requiring protected weight bearing and/or immobilization; 2) a history of two or more lateral sprains to the same ankle; 3) ankle “giving-way”; 4) CAIT score ≤ 25 ; 5) absence of lower limbs fractures or surgical interventions; 6) absence of swelling and inflammation; 7) absence of any kind of injury in the last 3 months.	Walking; walking side-turning; running side-cutting	PL TA MG ST RF GM Gmax	Initial contact and toe-off were identified as the instant the vertical GRF first exceeded 10 N, and the first time the vertical GRF fell below 10 N after IC, respectively. EMG amplitude was calculated during the 200 ms before IC and the stance phase, i.e. from IC to toe-off. Mean values of the normalized EMG data were calculated by averaging the pre-IC phase and every 10 % windows of stance phase, respectively.	The maximum amplitudes during the MVICs were used to normalize the EMG data during all movement trials.	No between-groups differences were found during walking. During the side-cutting task, CAI showed a higher MG activity during the early stance phase when compared with CG.
Simpson et al. (2020) [75]	15 CAI (- ; age, 21; PA: active; height: 171 cm; body mass: 73.4 kg; CAIT: 18) 15 CG (- ; age 22; PA: active; height: 179 cm; body mass: 75.5 kg)	1) two or more lateral ankle sprains with the last within the previous 12 months; 2) non-weight bearing or immobilization for ≥ 24 h after the injury; 3) ankle “gives way”, or “feelings of instability” on the affected ankle; 4) CAIT < 24 ; 5) injuries or surgery to lower limbs; 6) musculoskeletal injuries in the last 6 months; 7) ankle sprain within the last 3 months (CAI only).	SIDE-CUTTING 45°	PL PB TA MG VM ST	EMG signals were analysed with a 50-ms RMS moving window algorithm. EMG data was reduced to 101 data points to normalize each participant's data to 100% of the stance phase during the side-cutting task. The stance phase was analyzed from initial foot contact (0%) to toe-off (100%). The ground reaction force threshold was set at 20 N.	The EMG data were then normalized to each participant's greatest 100 ms average of a 3 s maximum voluntary isometric contraction (MVIC) against a manual resistance for each muscle, respectively.	CAI group showed a higher TA activation from 86–94% of the stance phase when compared with CG.

Son et al. (2017) [30]	22 CAI (M 12, F 8 ; age, 22.7; PA: active; height: 174.6 cm; body mass: 73.4 kg; FAAM-ADL: 81.9%; FAAM-S: 60.9%; MAAI: 3.4; sprains: n 4.1) 22 COPERS (M 12, F 8 ; age, 22.1 ; PA: active; height: 174 cm; body mass: 72 kg; FAAM-ADL: 100; FAAM-S: 100; MAAI: 0; sprains: n 2) 22 CG (M 12, F 8 ; age 21.8; PA: active; height: 173.3 cm; body mass: 79.2 kg)	In accordance with the International Ankle Consortium: 1) a history of at least two repeated unilateral ankle sprains with the most recent ankle sprain that occurred more than 3 months prior; 2) FAAM-ADL score <90%; 4) no “yes” answer on questions four to eight on the MAII; 5) no previous formal rehabilitation for the test ankle; 6) a history of physical activity at least 3 days x week for a total of 90 min in the past 3 months. COPER: 1) at least one severe ankle sprain that occurred more than 12 months prior the study; 2) return to moderate levels of weight-bearing physical activity without repeated ankle injury in the past 12 months; 3) FAAM-ADL and FAAM-S 100%; 4) no “yes” answer on questions four to eight on the MAII; 5) no previous formal rehabilitation for the test ankle; 6) a history of physical activity at least 3 days x week for a total of 90 min in the past 3 months.	Jump landing with side-cutting 90°	PL TA MG VL MH GM Gmax	EMG RMS was calculated with a moving window of 125 ms and then data were normalized to the smoothed reference EMG data.	Reference EMG data were obtained during 3 s of an isometric double-leg squat position (e.g., 45-knee flexion and 30-hip flexion using a goniometer) .	CAI patients showed less TA activation during 41% to 100% of stance, less PL activation during 0% to 3% and 20% to 97% of stance, less MG activation during 27% to 65% of stance, less VL activation during 0% to 4% and 39% to 65% of stance, less Gmed activation during 0% to 2% and 35% to 74% of stance, and less Gmax activation during 0% to 3% and 28% to 72% of stance when compared to COPERS. CAI patients showed less TA activation during 36% to 100% of stance, less PL activation during 0% to 66% of stance, less MG activation during 23% to 65% of stance, more VL activation during 2% to 21% of stance and less VL activation during 44% to 60% of stance, more Gmed activation during 3% to 14% of stance and less Gmed activation during 35% to 45% of stance, and less Gmax activation during 24% to 71% of stance when compared to CG. None or small activation differences (e.g., PL, MG, VL, MH, Gmed, and Gmax) were observed between copers and controls.
Suda et al. (2011) [76]	16 CAI (M 15, F 1 ; age, 20.5; PA: active; height: 189 cm; body mass: 79.6 kg) 18 CG (M 17, F 1 ; age 20.5 ; PA: active; height: 189 cm; body mass: 79.8 kg)	1) at least two sprains within the previous two years; 2) ankle “giving-way”; 3) absence of sprains in the last 6 months; 4) integrity of the talofibular and calcaneofibular ligaments; 5) no mechanical instability.	LATERAL SHUFFLE	PL TA LG	EMG amplitude was calculated by means of the calculation of the RMS for the 50 ms before ground contact. The onset time of muscle activity related to initial ground contact was calculated using the criteria proposed by Santello & McDonagh (1998) [93].	MIVC of each muscle	CAI group showed a lower PL activation in the 50 ms before impact, together with a lower PL and LG peak activation when compared to CG. The onset of muscle activation was similar between CAI and CG. However, in CG the first muscle activated was LG, followed by PL and TA, while in CAI group the three muscles were activated at the same time.

Table 5. Data extracted from each article for qualitative synthesis. CAI: chronic ankle instability; FAI: functional ankle instability; CG; control group; PA: physical activity level; MVIC: maximal voluntary isometric contraction; RMS: root mean square; EMG: electromyography; SL: single limb; SLJ: single limb jumping; SEBT: star excursion balance test; PL: Peroneus longus; TA: Tibialis anterior; PB: Peroneus brevis; SOL: Soleus; LG: Lateral gastrocnemius; MG: Medial gastrocnemius; VL: Vastus lateralis; RF: Rectus femoris VM: vastus medialis; ST: semitendinosus; BF: Biceps femoris; GM: Gluteus medium; Gmax: Gluteus maximum (Gmax); TP: Tibialis posterior; FAAM-ADL: Foot and Ankle Ability Measure - Activities of Daily Living score ; FAAM-S: Foot and Ankle Ability Measure - Sport scale; CAIT Cumberland Ankle Instability Tool; FAAQ: Foot and Ankle Ability Questionnaire; MAII: Modified Ankle Instability Index.

Journal Pre-proof

SINGLE-LIMB STANCE AND TRANSITIONS BETWEEN SINGLE- AND DOUBLE-LIMB STANCE							
Authors (year)	Participants	Criteria for CAI	Task	Muscles	EMG analysis	Normalization method	EMG Results
De Ridder et al. (2015)[77]	28 CAI (-; age, 20.3; PA: active; height: 171 cm; body mass: 73.4 kg; FADI: 90.6; FADI-S: 76.7; sprains: n 7.8; CAIT: 14.8) 28 CG (-; age 20.3; PA: active; height: 171 cm; body mass: 73.4 kg)	1) at least 1 severe ankle sprain resulting in limitations in participation for at least 3 weeks; 2) episodes of giving way; 3) repetitive ankle sprains; 4) absence of fractures, surgical interventions or complaints; 5) absence of balance impairments.	SL stance on uniaxial and multidirectional unstable surfaces	PL PB TA MG	EMG signals were analysed using a moving window of 100 ms calculating the RMS. The mean EMG value was determined over every 5 s balancing interval. Co-contraction between the invertor/evertor muscle activity, the mm. tibialis anterior/peroneus longus ratio (TA/PL ratio) was also calculated.	Highest maximal voluntary activation value of each trial.	No differences were found between CAI and CG.
Feger et al. (2014) a[69]	15 FAI (5 M, 10 F; age, 23; PA: active; height: 173. cm; body mass: 72.4 kg; FAAM-ADL: 87.2; FAAM-S: 68.5; sprains: n 4.5) 15 CG (5 M, 10 F; age 22.9; PA: active; height: 173 cm; body mass: 70.08 kg)	1) more than 1 ankle sprain with the initial sprain occurring more than 1 year before the study onset; 2) self-reported impairments in function and symptoms; 3) absence of ankle sprains within the 6 weeks before the study; 4) absence of lower extremity injury or surgery; 5) absence of balance disorders, neuropathies, diabetes; 6) absence of other conditions affecting balance.	SL stance eyes closed; SEBT; forward lunges; lateral hops	PL TA LG RF BF GM	<u>Forward Lunge.</u> Pre-IC and pot-IC EMG amplitude were calculated as the RMS of a 50-millisecond epoch immediately before IC and a 100-ms epoch immediately after IC, respectively. <u>Single-limb Eyes-closed Balance.</u> A 3-s epoch during the middle of the single-limb eyes-closed balance trial was analysed. <u>SEBT.</u> A 500-millisecond epoch before maximum excursion was averaged over 3 trials for each of the 3 directions. <u>Lateral Hops.</u> Pre-IC and pot-IC EMG amplitude were calculated as the RMS of a 50-millisecond epoch immediately before IC and a 100-ms epoch immediately after IC, respectively. Data were analysed also as distal lower limb (TA, PL, LG), proximal lower limb (RF, BF, GM) and entire lower limb (all the muscles).	Amplitudes were normalized to respective MVIC epochs	<u>Forward Lunge.</u> CAI showed: a lower proximal muscle activation before IC, a lower TA activation during post-IC, a lower distal and total post-IC activation when compared with CG. <u>Single-limb Eyes-closed Balance.</u> CAI showed a lower activation of TA and RF, and a lower activation of the entire lower limb in comparison with CG. <u>SEBT.</u> CAI showed a lower activation of the TA during the anterior and posteromedial directions. <u>Lateral Hops.</u> CAI showed a lower activation of the distal and the entire lower limb during pre-IC in comparison with CG.
Kwon et al. (2018)[32]	20 CAI (-; age, 22; PA: -; height: 169 cm; body mass: 73 kg; CAIT: 19.2; FAAM: 85.2) 20 COPERS (-; age, 23; PA: -; height: 170 cm; body mass: 72 kg; CAIT: 27.9; FAAM: 98.7) 20 CG (-; age 22; PA: -; height: 168	CAI group: 1) at least one significant ankle sprain; 2) ankle 'giving way'; 3) CAIT < 23; 4) FAAM - ADL < 90%; 5) absence of cerebral concussion, vestibular disorder, and lower limb injuries for the previous 6 months; 6) No rehabilitation. COPERS group: 1) at least one significant ankle sprain; 2) Returned to all pre-injury activities without recurrent injury, episodes of 'giving way' and/or 'feeling of instability' for at least 1 year prior to testing; 3) CAIT > 28; 4) absence of cerebral concussion, vestibular disorder, and lower limb injuries for the previous 6 months; 4) No rehabilitation.	Single leg stance with eyes open and eyes closed	PL TA MG	Subjects were asked to stand in the position for 20 s with their heads up and eyes forward. The means of three trials was used to calculate the normalized mean amplitude (NMA) for the three muscles were calculated.	Maximum voluntary isometric contraction (MVIC)	Muscle activation was higher in CAI compared to CG and COPERS for all the muscles and conditions. In all groups, TA had a lower activation in comparison to PL and MG with eyes-open.

	cm; body mass: 66 kg)						
Levin et al. (2012)[80]	20 CAI (M 8, F 12; age, 21.8; PA: active/competitive) 20 CG (M 12, F 8; age 21.2; PA: active/competitive)	1) at least 2 sprains in the 2 years before the study; 2) ankle “giving way” during daily activities; 3) no injuries for the 3 months before the study; 4) absence of previous fractures or surgery; 5) no rehabilitation.	Double to single limb stance transition	PL TA MG VM VL AD TF GM Gmax	Muscle onset times were expressed with respect to the onset of displacement of mediolateral COP.		In CG, during eyes open condition, TA was the first activated muscle, while the Gmax was the last. In the eyes closed condition, no significant differences in onset times between muscles were observed. In CAI, TF activated earlier than PL, TA and MG. For this three muscles, onset times during eyes-open condition were shorter than those observed during eyes-closed condition.
Van Deun et al. (2007)[78]	10 CAI (- ; age, 22.5; PA: -; height: 170 cm; body mass: 63 kg) 30 CG (- ; age 21.2; PA: -; height: 170 cm; body mass: 64.7 kg)	1) at least 2 ankle sprains in the past 2 years; 2) no injuries at the ankle; 3) no previous fractures or surgery at the lower limb or back; 4) no rehabilitation.	Transition from double- to single leg stance with or without vision	PL TA MG MH VM VL TF GM	The onset of muscle activity was calculated on the basis of the onset of displacement of the COP in the mediolateral plane (i.e., the beginning of the transition from the double leg stance position to the single-leg stance position). An increase of more than 2 standard deviations on top of the mean baseline activity was identified as the onset of muscle activity in reaction to the transition. Then, muscles were ranked according to their onset time to determine the muscle recruitment order. Finally, the recruitment order was compared between the groups and the EO and EC conditions.		In both groups, no differences were found between eyes open and eyes closed conditions. In CAI all muscles except VL and VM showed a significantly later onset in comparison with CG. In CG, muscle activations started before the beginning of the transition from double-leg stance to single-leg stance, except for the VM. In CAI group, muscle activations started after the beginning of the transition from double-leg stance to single-leg stance. In addition, CAI subjects tended to firstly activate muscles in the more proximal regions, while the ankle muscles were activated last in both conditions.
Van Deun et al. (2011)[79]	20 CAI (M 8, F 12; age, 21.8; PA: active/competitive; height: 164 cm; body mass: 68.4 kg) 20 CG (M 12, F 8; age 21.2; PA: active/competitive; height: 176 cm; body mass: 71.7 kg)	1) at least 2 ankle sprains in the past 2 years; 2) no injuries at the ankle; 3) no previous fractures or surgery at the lower limb or back; 4) no rehabilitation.	Transition from double- to single leg stance with or without vision and on a balance pad	PL TA MG MH VM VL TF GM	The onset of muscle activity was calculated on the basis of the onset of displacement of the COP in the mediolateral plane (i.e., the beginning of the transition from the double leg stance position to the single-leg stance position). An increase of more than 2 standard deviations on top of the mean baseline activity was identified as the onset of muscle activity in reaction to the transition. Then, muscles were ranked according to their onset time to determine the muscle recruitment order. Finally, the recruitment order was compared between the groups and the conditions.		There were no differences between the two groups and between the conditions for the onset of muscle activations. CAI had mainly a proximal muscle activations strategy, while in CG used a proximal strategy mainly in closed eyes condition and on the balance pad.

Table 6. Data extracted from each article for qualitative synthesis. CAI: chronic ankle instability; FAI: functional ankle instability; CG; control group; PA: physical activity level; MVIC: maximal voluntary isometric contraction; RMS: root mean square; EMG: electromyography; SL: single limb; SLJ: single limb jumping; SEBT: star excursion balance test; PL: Peroneus longus; TA: Tibialis anterior; PB: Peroneus brevis; SOL: Soleus; LG: Lateral gastrocnemius; MG: Medial gastrocnemius; VL: Vastus lateralis; RF: Rectus femoris VM: vastus medialis; ST: semitendinosus; BF: Biceps femoris;

GM: Gluteus medium; Gmax: Gluteus maximum (Gmax); TP: Tibialis posterior; FAAM-ADL: Foot and Ankle Ability Measure - Activities of Daily Living score ; FAAM-S: Foot and Ankle Ability Measure - Sport scale; FADI: Foot and Ankle Disability Index; FADI-S: Foot and Ankle Disability Index - Sport; CAIT: Cumberland Ankle Instability Tool.

STAR EXCURSION BALANCE TASK							
Authors (year)	Participants	Criteria for CAI	Task	Muscles	EMG analysis	Normalization method	EMG Results
Ahn et al. (2011)[81]	10 FAI (M; age: 21.5; PA: active; height: 174.9 cm; body mass: 71.7 kg) 10 CG (M; age: 21.9; PA: active; height: 175.2 cm; body mass: 69.8 kg)	1) at least one ankle sprain episode requiring immobilization; 2) at least 2 ankle sprains and “giving way” symptoms in the year before to participate in the study; 3) absence of ankle sprains in the 6 weeks before the study; 4) absence of lower limbs’ fractures or surgery.	SEBT	PL TA TP SOL VL RF BF	Average RMS of 10 seconds of two trials in each direction of the SEBT	MVIC of each muscle	PL, TA and BF activation was lower in the anterior direction in CAI patients. TA and SOL were lower in the posterior direction. VL was lower in the lateral direction. PL and TA were lower in antero-medial and anterolateral direction. TP and SOL were lower in postero-medial and postero-lateral directions.
Feger et al. (2014)[69]	15 FAI (5 M, 10 F; age, 23; PA: active; height: 173. cm; body mass: 72.4 kg; FAAM-ADL: 87.2; FAAM-S: 68.5; sprains: n 4.5) 15 CG (5 M, 10 F; age 22.9; PA: active; height: 173 cm; body mass: 70.08 kg)	1) more than 1 ankle sprain with the initial sprain occurring more than 1 year before the study onset; 2) self-reported impairments in function and symptoms; 3) absence of ankle sprains within the 6 weeks before the study; 4) absence of lower extremity injury or surgery; 5) absence of balance disorders, neuropathies, diabetes; 6) absence of other conditions affecting balance.	SL stance eyes closed; SEBT; forward lunges; lateral hops	PL TA LG RF BF GM	<u>Forward Lunge.</u> Pre-IC and pot-IC EMG amplitude were calculated as the RMS of a 50-millisecond epoch immediately before IC and a 100-ms epoch immediately after IC, respectively. <u>Single-limb Eyes-closed Balance.</u> A 3-s epoch during the middle of the single-limb eyes-closed balance trial was analysed. <u>SEBT.</u> A 500-millisecond epoch before maximum excursion was averaged over 3 trials for each of the 3 directions. <u>Lateral Hops.</u> Pre-IC and pot-IC EMG amplitude were calculated as the RMS of a 50-millisecond epoch immediately before IC and a 100-ms epoch immediately after IC, respectively. Data were analysed also as distal lower limb (TA, PL, LG), proximal lower limb (RF, BF, GM) and entire lower limb (all the muscles).	Amplitudes were normalized to respective MVIC	<u>Forward Lunge.</u> CAI showed: a lower proximal muscle activation before IC, a lower TA activation during post-IC, a lower distal and total post-IC activation when compared with CG. <u>Single-limb Eyes-closed Balance.</u> CAI showed a lower activation of TA and RF, and a lower activation of the entire lower limb in comparison with CG. <u>SEBT.</u> CAI showed a lower activation of the TA during the anterior and posteromedial directions. <u>Lateral Hops.</u> CAI showed a lower activation of the distal and the entire lower limb during pre-IC in comparison with CG.

Jaber et al. (2018)[31]	16 CAI (7 M, 9 F; age, 29.6; PA: active; height: 170.2 cm; body mass: 72.6 kg; CAIT: 16.3) 16 COPERS (11 M, 5 F; age, 27.8; PA: active; height: 172.1 cm; body mass: 73.2 kg; CAIT: 28.1) 16 CG (5 M, 11 F; age 25.8; PA: active; height: 170.8 cm; body mass: 73.9 kg; CAIT: 29.4)	1) at least 1 ankle sprain leading to pain and loss of function (for CAI and coper groups); 2) the ankle sprain occurred not less than 12 months ago with no complaint of disability and/or giving way episodes since the injury (for copers); 3) ankle 'giving way' symptoms in the past 6 months (for CAI group); 4) never has ankle sprains (for the control group); 5) at least 90 minutes of sport practice each week; 6) absence of bilateral ankle instability; 7) absence of neuromusculoskeletal or vestibular disorders; 8) absence of previous surgeries to lower limbs; 9) absence of any kind of injury to the lower limbs for at least 3 months prior to the study; 10) absence of participation in supervised physical rehabilitation within 3 months from the study; 11) absence of drugs or alcohol within 24 hours prior to testing. If subjects scored 24 or less on CAIT, thus they were classified as having CAI. If they scored 28 or more they were defined as functionally stable ankles (copers or controls). Subjects who scored between 24 and 28 were excluded from the study.	SEBT	PL GM TA Gmax	The peak of the EMG amplitude was calculated as the average over a 500 ms time window, and then normalized. Muscle activation onsets were identified in correspondence with the lifting of the limb off the force plate. The onset of muscle activation was defined as the time at which EMG signal deviated by more than 2 standard deviations, for a minimum of 50 milliseconds (ms), above the baseline taken 100 ms prior to movement begin.	Maximal voluntary isometric contraction of each muscle during all SEBT.	There was a lower activation of the TA in the anterior direction and of the Gmax in the posterolateral direction in CAI with respect to CG and COPERS.
Pozzi et al. (2015)[29]	9 CAI (M 4, F 5; age, 26; PA: active; height: 170. cm; body mass: 76.7 kg; CAIT: 18) 9 COPERS (M 7, F 2; age, 26; PA: active; height: 173. cm; body mass: 79.3 kg; CAIT: 28) 12 CG (M 6, F 6; age 26; PA: active; height: 169. cm; body mass: 65.9 kg; CAIT: 29)	1) at least one lateral ankle sprain which resulted in pain, swelling, and loss of function and/or participation in activity (for CAI and coper groups); 2) never had a lateral ankle sprain (for the control group); 3) absence of any cardiovascular, pulmonary, neuromuscular and/or musculoskeletal diseases that might interfere with motor performance; 4) absence of any lower limb surgeries; 5) absence of any musculoskeletal injuries within the past 6 months; 6) consumed drugs and/or alcohol within 24 hours; 7) lack of poor plantar flexor muscle strength assessed via a standard single leg heel-rise-test.	SEBT	PL TA	The integral of the EMG signal between Toe-Off and Touch-Down was calculated for the tibialis anterior and peroneus longus muscles during the reach.	The maximal muscle activation of the TA and PL muscles during all SEBT trials was used to normalize the EMG signal between subjects.	PL and TA activation was higher in COPERS than CG. CAI group had 78% and 61% higher activation of the PL and TA muscles compared to CG (not significant). COPERS showed a 20% higher activation of PL and TA compared to CAI (not significant).

Table 7. Data extracted from each article for qualitative synthesis. CAI: chronic ankle instability; FAI: functional ankle instability; CG; control group; PA: physical activity level; MVIC: maximal voluntary isometric contraction; RMS: root mean square; EMG: electromyography; SL: single limb; SLJ: single limb jumping; SEBT: star excursion balance test; PL: Peroneus longus; TA: Tibialis anterior; PB: Peroneus brevis; SOL: Soleus; LG: Lateral gastrocnemius; MG: Medial gastrocnemius; VL: Vastus lateralis; RF: Rectus femoris VM: vastus medialis; ST: semitendinosus; BF: Biceps femoris;

GM: Gluteus medium; Gmax: Gluteus maximum (Gmax); TP: Tibialis posterior; FAAM-ADL: Foot and Ankle Ability Measure - Activities of Daily Living score ; FAAM-S: Foot and Ankle Ability Measure - Sport scale; CAIT: Cumberland Ankle Instability Tool.

OTHER TASKS							
Authors (year)	Participants	Criteria for CAI	Task	Muscles	EMG analysis	Normalization method	EMG Results
Fatima et al. (2020)[83]	17 CAI (6 M, 11 F; age, 24.4; PA: -; height: 157.8 cm; body weight: 54.9) 17 CG (6 M, 11 F; age 24.6; PA: -; height: 159.7 cm; body weight: 57.7)	1) history of at least 2 ankle sprains for the past 2 years, which lead to protected weight bearing or immobilization; 2) “giving-way” of the ankle.	Y-balance test; single limb squat with and without a Swiss ball	GM Gmax	RMS of the EMG signal recorded from the beginning to the end of each functional task, and an average of 3 repetitions was calculated for each exercise.	MVIC of each muscle	CAI group showed a lower activation of GM and Gmax in all the tasks when compared to CG.
Feger et al. (2014)[69]	15 FAI (5 M, 10 F; age, 23; PA: active; height: 173. cm; body mass: 72.4 kg; FAAM-ADL: 87.2; FAAM-S: 68.5; sprains: n 4.5) 15 CG (5 M, 10 F; age 22.9; PA: active; height: 173 cm; body mass: 70.08 kg)	1) more than 1 ankle sprain with the initial sprain occurring more than 1 year before the study onset; 2) self-reported impairments in function and symptoms; 3) absence of ankle sprains within the 6 weeks before the study; 4) absence of lower extremity injury or surgery; 5) absence of balance disorders, neuropathies, diabetes; 6) absence of other conditions affecting balance.	SL stance eyes closed; SEBT; forward lunges; lateral hops	PL TA LG RF BF GM	<u>Forward Lunge.</u> Pre-IC and post-IC EMG amplitude were calculated as the RMS of a 50-millisecond epoch immediately before IC and a 100-ms epoch immediately after IC, respectively. <u>Single-limb Eyes-closed Balance.</u> A 3-s epoch during the middle of the single-limb eyes-closed balance trial was analysed. <u>SEBT.</u> A 500-millisecond epoch before maximum excursion was averaged over 3 trials for each of the 3 directions. <u>Lateral Hops.</u> Pre-IC and post-IC EMG amplitude were calculated as the RMS of a 50-millisecond epoch immediately before IC and a 100-ms epoch immediately after IC, respectively. Data were analysed also as distal lower limb (TA, PL, LG), proximal lower limb (RF, BF, GM) and entire lower limb (all the muscles).	Amplitudes were normalized to respective MVIC	<u>Forward Lunge.</u> CAI showed: a lower proximal muscle activation before IC, a lower TA activation during post-IC, a lower distal and total post-IC activation when compared with CG. <u>Single-limb Eyes-closed Balance.</u> CAI showed a lower activation of TA and RF, and a lower activation of the entire lower limb in comparison with CG. <u>SEBT.</u> CAI showed a lower activation of the TA during the anterior and posteromedial directions. <u>Lateral Hops.</u> CAI showed a lower activation of the distal and the entire lower limb during pre-IC in comparison with CG.

Rios et al. (2015)[82]	21 CAI (M 8, F 13; age, 25.; PA: active; CAIT:17) 21 CG (M 8, F 13; age 25; PA: active)	1) history of two or more sprains of the same ankle with at least one sprain in the last six months; 2) sensation of ankle “giving way”; 3) absence of acute inflammatory symptoms; 4) absence of fracture or surgery in either ankle; 3) other pathological conditions that could affect sensation or ankle control.	Ball-kicking on stable or unstable surfaces	PL TA MG LG SOL BF RF GM ES RA ADD	Anticipatory postural adjustments (APA), simultaneous postural adjustments (SPA) and compensatory postural adjustments (CPA) were calculated. To calculate the integrals of the EMG the mean data were demarcated around the onset, which was considered time zero (t0), encompassing time intervals of 200 ms: i.e., from 200 ms before t0 to t0 for APA; from t0 to 200 ms after t0 for SPA; from 200 ms to 400 ms after t0 for CPA1; and 400 ms to 600 ms after t0 for CPA2.	The normalization included obtaining the absolute maximum from the integral of EMG values of a particular muscle for each subject across all conditions and time intervals and dividing each integral of EMG index by that absolute maximum value individually.	CAI group showed o lower activation of the ankle muscles and an increased activation of the hip muscles when compared to CG. The PCA highlighted that CAI group primarily activating hip/spine muscles to compensate for ankle instability.
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Table 8. Data extracted from each article for qualitative synthesis. CAI: chronic ankle instability; CG; control group; PA: physical activity level; RMS: root mean square; EMG: electromyography; PL: Peroneus longus; TA: Tibialis anterior; PB: Peroneus brevis; SOL: Soleus; LG: Lateral gastrocnemius; MG: Medial gastrocnemius; VL: Vastus lateralis; RF: Rectus femoris VM: vastus medialis; ST: semitendinosus; BF: Biceps femoris; GM: Gluteus medium; Gmax: Gluteus maximum (Gmax); TP: Tibialis posterior; EDL: extensor digitorum longus; ADD: Thigh adductors muscles; ER: Erector spinalis (ER); RA: Rectus abdominis; CAIT: Cumberland Ankle Instability Tool; PCA: Principal Components Analysis; FAAM-ADL: Foot and Ankle Ability Measure - Activities of Daily Living score ; FAAM-S: Foot and Ankle Ability Measure - Sport scale.