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Differences in neuromuscular activity of ankle stabilizing muscles during postural disturbances: a gender-specific analysis

Running title: Gender differences in lower leg muscles during postural disturbances

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ABSTRACT

The purpose was to examine gender differences in ankle stabilizing muscle activation during postural disturbances. Seventeen participants (9females: 27±2yrs., 1.69±0.1m, 63±7kg; 8males: 29±2yrs., 1.81±0.1m; 83±7kg) were included in the study. After familiarization on a split-belt-treadmill, participants walked (1m/s) while 15 right-sided perturbations were randomly applied 200ms after initial heel contact. Muscle activity of M. tibialis anterior (TA), peroneus longus (PL) and gastrocnemius medialis (GM) was recorded during unperturbed and perturbed walking. The root mean square (RMS;[%]) was analyzed within 200ms after perturbation. Co-activation was quantified as ratio of antagonist (GM)/agonist(TA) EMG-RMS during unperturbed and perturbed walking. Time to onset was calculated (ms). Data were analyzed descriptively (mean±SD) followed by three-way-ANOVA (gender/condition/muscle;α=0.05). Perturbed walking elicited higher EMG activity compared to normal walking for TA and PL in both genders (p<0.000). RMS amplitude gender comparisons revealed an interaction between gender and condition (F=4.6,p=0.049) and, a triple interaction among gender, condition and muscle (F=4.7,p=0.02). Women presented significantly higher EMG-RMS[%] PL amplitude than men during perturbed walking (mean difference=209.6%, 95% confidence interval=-367.0 to -52.2%,p<0.000). Co-activation showed significant lower values for perturbed compared to normal walking (p<0.000), without significant gender differences for both walking conditions. GM activated significantly earlier than TA and PL (p<0.01) without significant differences between the muscle activation onsets of men and women (p=0.7). The results reflect that activation strategies of the ankle encompassing muscles differ between genders. In provoked stumbling, higher PL EMG activity in women compared to men is present. Future studies should aim to elucidate if this specific behavior has any relationship with ankle injury occurrence between genders.

Key Words: lower extremity, EMG, perturbation, split-belt treadmill, ankle

HIGHLIGHTS

- Artificial stumbling provokes greater ankle muscle activity compared to walking.

- In provoked stumbling, higher PL EMG activity in women compared to men is present.

- No influence of gender as response of ankle muscle reaction time was found.

1. INTRODUCTION

Gender differences in the prevalence of lower leg injuries are frequently discussed [1-3]. The majority of papers have focused on knee injuries in athletes with females showing up to 8 times higher ACL injury prevalence rates compared to males [2,4,5]. Especially in landing, pivoting or cutting maneuvers, described as non-contact situations in soccer, handball or basketball, women are at greater risk of injury [2,6]. Anatomical, biomechanical, hormonal and neuromuscular factors have been discussed as risk factors [2,6]. Gender-specific differences in knee muscle activity during various athletic tasks have mainly been investigated to explain these factors influencing ACL injury prevalence [7,8]. An increased quadriceps and decreased hamstring activation during stance phase while running, side cutting and cross cutting, respectively, could be shown in females compared to males [7,8]. Consequently, it has been concluded that females´ functional joint stability is reduced, which might explain the higher injury risk [2,6]. Hence, an association of neuromuscular activity with knee injury risk has been established [9].

Gender-specific differences involved in the incidence of ankle sprains are the subject of controversy [10-12]. Beynnon et al. [10] reported no differences in the injury rate of ankle sprains between males and females. In contrast, Lindenfeld et al. [12] showed a threefold higher injury risk for males compared to females. Doherty et al. [1] reported as a major finding of their systematic review and meta-analysis an incidence of ankle sprains in females up to two times higher than that of males. In contrast, no differences were found regarding the prevalence (10.6% females; 11.0% males). Nevertheless, controversies of prevalence rates between studies could be based on type of sport, mechanism (contact vs. non-contact) of injury and confounders (e.g. playing surface, shoe type etc.) analyzed. Moreover, the differences in neuromuscular activity of ankle stabilizing muscles between genders remain unclear and evidence is scarce [3,11]. In one of the only few articles focusing on gender differences in neuromuscular activity of the ankle muscles, Baur et al. [11] reported gender-

specific higher pre-activation (before heel strike) amplitudes of the M. peroneus longus in women during running. In addition, Mengarelli et al. (2017) reported an overall higher occurrence of ankle-muscle co-contractions in females during walking that should be associated to a more complex muscular recruitment pattern. They conclude a need for a higher level of ankle-joint stabilization in females [13]. Indeed, these neuromuscular responses might be expected since women's inversion-eversion [14] and dorsiflexion [15,16] ankle stiffness is reduced compared to that of men.

Regardless of gender, various studies could proof high injury rates for inadequate landings, cutting and stopping movements, especially in sports with a high proportion of running loads [5,17]. Besides, these non-contact situations typically occur unexpectedly, suddenly and quickly and therefore require a strong as well as rapid response of the muscles to adequately compensate the injury mechanism. Therefore, the use of a suddenly as well as rapidly applied stumbling perturbation allows assessing the capability of the ankle muscles to respond rapidly. In addition, the compensation of unexpected lower limb perturbations is an essential mechanism for achieving the postural control needed in high-performance sports. Therefore, the analysis of the neuromuscular response of the ankle muscles methodologically requires rapidly applied loading situations. In order to present experimentally real non-contact situations, these experiments initiate a disturbance stimulus that requires a direct involuntary compensation of additive rapidly applied loads. This elucidates the differences to previously used tilt platforms or more dynamic landing or cutting manoeuvres and therefore the need for continuous movement situations. In this respect, stumbling while walking seems to be a suitable functional testing situation in assessment of ankle muscle activity. The unique technique (split-belt treadmill perturbation [18]) allows the simulation of an automated movement task combined with a high intensity perturbation. Therefore the analysis of repetitive, continuous gait cycles is possible compared to previous studies investigating landing, pivoting, or cutting manoeuvres using single trials only [18].

To investigate ankle encompassing muscle activity in non-contact situations, relevant experimental tests with unanticipated high loading are necessary. However, provoked stumbling during gait remains poorly investigated so far. Consequently, the purpose of our study was to investigate gender differences in ankle muscle activation after provoked stumbling during gait. Due to the previously documented observations regarding women's ankle stiffness [14-16] and their different neuromuscular patterns during walking [13] and running [11]. It was hypothesized that females show higher EMG amplitudes in response to the walking perturbations, especially for the main ankle stabilizer muscles such as tibialis anterior and peroneus longus.

2. METHODS

Seventeen participants (9 females (f): 27±2 yrs., 1.69±0.07m, 63±7kg; 8 males (m): 29±2 yrs., 1.81±0.07m; 83±7kg), free of acute and chronic pain as well as recent injury at the lower extremities (e.g., no acute ankle trauma in the last 7 days and/or ACL rupture), were included in the study. All participants were physically active at least 2 to 3 times a week for a minimum of 60 minutes each session. In addition, no chronic pain at the lower extremities (e.g. chronic ankle instability), no surgery in the last 12-month at the lower extremities as well as no ligament rupture in the last 12-month were required for study participation. Moreover, acute and chronic infection, as well as limited suitability for walking, running and jumping movements as well as not being an elite athlete was set as exclusion criteria. The principal investigator assessed inclusion as well as exclusion criteria orally before inclusion into the study. Afterwards, the principal investigator informed all participants about the background, purpose and methods of the study. All participants signed their written informed consent before voluntary participation. The University´s Ethical Committee gave ethical approval.

A cross-sectional study design was used to evaluate gender differences in ankle muscle activity in response to provoked perturbations, using a novel split-belt device that has been recently validated and tested for reliability [18]. The measurement protocol started with the assessment of anthropometrics. Next, participants were prepared for electromyographic (EMG) recordings. Three pairs of surface EMG-electrodes were positioned on the M. tibialis anterior (TA), M. peroneus longus (PL) and M. gastrocnemius medialis (GM) of the right leg [19]. In addition, all participants wore a customized standard (running) shoe to ensure that all participants had comparable walking conditions. Subject preparation was followed by a 5-minute walking familiarization and warm-up on the treadmill at 1m/s (Split-belt treadmill, Woodway, Weil am Rhein, Germany). During this warm-up phase, no perturbations were applied. After a 3-minute resting period, the stumbling protocol began. Each subject walked

for about 8.5 minutes at a baseline velocity of 1m/s on the split-belt treadmill. While walking, 15 right- and 15 left-sided stumbling stimuli were randomly applied 200ms after initial heel contact triggered by a plantar pressure insole (Pedar X, Novel, Munich, D). This ensures that participants are perturbed in the early phase of the gait cycle (weight acceptance) and single support phase bearing already full load of body weight on the foot [20]. For each perturbation, one treadmill belt was decelerated by $40m/s^2$ within 50ms (Fig. 1) [21]. A minimum of 10s in-between two perturbations were fixed to ensure that participants regained their normal walking pattern after each perturbation. For safety reasons, participants were provided with a waist belt connected to an emergency stop release. However, no subject needed the help of the waist belt and no falls were registered.

Muscular activity was assessed by means of a 3-channel surface EMG during both unperturbed and perturbed walking. Muscular activity was recorded using a bipolar surface EMG system (band-pass filter: 5 Hz to 500 Hz, gain: 5.0, overall gain: 2500, sampling frequency: 4000 Hz; RFTD- 32, myon AG, Baar, Switzerland). The placement of electrodes was carefully determined according to Winter & Yack [22]. Therefore, all three muscles were palpated by the same experienced investigator during all measurements, in all subjects. Before electrodes (AMBU Medicotest, Denmark, Type N-00-S, inter- electrode distance: 2 cm) were applied, the skin was shaved and slightly exfoliated to remove surface epithelial layers. In addition, skin resistance was measured and kept below 5 kΩ. The longitudinal axes of the electrodes were in line with the presumed direction of the underlying muscle fibers. Preparation with electrodes was followed by validation to reduce cross-talk (e.g., btw. PL and TA); therefore subjects had to activate all muscles separately and the principal investigator visually controlled the activity level (raw signal on a screen) of all three muscles during activation of each muscle. In case of an invalid activation, the electrodes were removed and repositioned until observing a discrete activation corresponding to the performed movement pattern for each muscle.

For data analysis, only right-sided perturbations were analyzed due to direct triggering of the heel contact by the plantar pressure insole of the right foot. Left-sided perturbations were applied to ensure that participants would not adapt their normal walking pattern to only right-sided perturbations. EMG signals were full-wave rectified and low-pass filtered (10Hz, second order, zero lag Butterworth). The mean amplitude (root mean square, RMS) for each muscle was calculated out of the first 5 unperturbed and the first 15 perturbed strides. Normal walking is described as a stable and automated human movement pattern; therefore a lower number of repetitions is necessary to be analyzed compared to the stumbling [23,24]. As main outcome measure, to account for mono- and polysynaptic reflex activity, the RMS ([%]) was calculated for unperturbed and perturbed walking, in the same time window of 200ms. Therefore, unperturbed steps were analyzed 200ms after heel strike while perturbed steps were analyzed immediately after the perturbation, since perturbations were provoked 200ms after heel strike (Fig. 2). Perturbed and unperturbed steps were normalized to the RMS of the entire stride during unperturbed walking (formula unperturbed step: (RMS 200ms to 400ms after heel strike / RMS of entire unperturbed stride) * 100; formula perturbed step: (RMS 200ms after perturbation / RMS of entire unperturbed stride) * 100) [21]. Moreover, co-activation was calculated for both, normal and perturbed walking (formula: GM EMG-RMS: TA EMG-RMS x 100) [25].

Onsets of muscle activity [ms] in response to the perturbations were also measured. A semi-automated detection method (IMAGO process master, LabView®-based, pfitec, biomedical systems, Endingen, Germany) was used to define muscle activity onset [26]. Within this detection method, an increase in the averaged EMG signal (ensemble average; filter: 4th order moving average) of more than 3 standard deviations from baseline level was defined for automatic onset detection. All automatic detections were controlled through visual inspection. If automatic detection failed (e.g. due to movement artifact), the investigator applied manual correction. Two independent investigators (J.M and E.M-V) checked the consistency in the

detection of the onsets of muscle activation. Prior statistical comparisons, the results of the two independent reviewers were averaged as presented previously [26].

All non-digital data were documented in a paper-and-pencil-based case report form (CRF) and transferred to a database (JMP Statistical Software Package 9, SAS Institute®). Implausible and extreme values (range check; body height: $1.5m < x < 2.1m$; EMG latencies: $20ms < x < 200ms$; EMG-RMS: $0 < x < 800$ %MVC) were recalculated or revised in correlation with the handwritten CRF.

Before comparisons, all variables were tested for normality using the Shapiro–Wilk test. The Assumption of sphericity was checked by the Mauchly test, and, if violated, the Greenhouse–Geisser correction was made to the degrees of freedom. The effects of perturbed and unperturbed walking on RMS amplitude data were analyzed with a 3-way repeated measures analysis of variance (ANOVA) with factors of gender (male, female), condition (perturbed, unperturbed) and muscle (GM, TA and PL). The effects of the perturbations on the onsets of muscle activity, and co-activations were analyzed with 2-way repeated measures ANOVA, with factors of gender (male, female) and muscle (GM, TA and PL). Pairwise comparisons were made with Bonferroni corrected t-tests when ANOVA was significant. Finally, the intra-class correlation coefficient ($ICC_{2,1}$) and the standard error of the measurement ($SEM = SD\sqrt{1 - ICC}$) were used to check the inter-rater reliability of muscle activation onsets. Accordingly, ICC scores between 0.8-1 were interpreted as "excellent", 0.6-0.8 "good" and <0.6 "poor" [27]. Any result with an ICC below 0.6 was not analyzed further, since final results could be influenced by difficulties in the identification of onsets of muscle activation. Statistical significance was set at $p < 0.05$.

3. RESULTS

3.1. EMG amplitude

Men and women showed similar EMG-RMS [%] amplitude during normal walking (interaction; gender*muscle: F=0.7, p=0.534). Perturbed walking elicited higher EMG activity compared to normal walking for TA and PL in both genders (p<0.000). Overall, the muscle that produced the highest activity following the perturbation was TA, followed by PL and GM. Figure 3 shows EMG-RMS [%] values for men and women during normal and perturbed walking. Regarding RMS amplitude gender comparisons, a three way ANOVA (gender*condition*muscle), with the last two factors treated as repeated measure, revealed effect of gender (F=7.6, p=0.01), condition (F=155.6, p<0.000), muscle (F=18.4, p<0.000), interaction between condition and muscle (F=28.8, p<0.000), interaction between gender and condition (F=4.6, p=0.049) and most importantly, a triple interaction among gender, condition and muscle (F=4.7, p=0.02). Follow–up tests (Bonferroni corrected t-tests) showed that women presented significantly higher EMG-RMS [%] PL amplitude than men during perturbed walking (mean difference= -209.6%, 95% confidence interval (CI) =-367.0 to -52.2%, p<0.000). However, both TA and GM changed similarly following the perturbations in both men and women (p=0.5 and p=0.4, respectively). Results of co-activation analysis are detailed in table 1 and revealed significant lower values for perturbed compared to normal walking (p<0.000), without significant gender differences for both walking conditions (Tab. 1).

3.2. EMG muscle activation onsets

Good to high levels of inter-rater reliability were obtained for the identification of muscle activation onsets in all muscles (TA: ICC=0.95, SEM=3.7ms; PL: ICC=0.78, SEM=6.3ms and GM: ICC=0.65, SEM=18.4ms). TA, PL and GM activated at different time points following the perturbation. Repeated measures ANOVA revealed a significant effect of

muscle (F=25.4, p<0.000). Thus, GM activated significantly earlier than both TA and PL (p<0.01), which were activated almost simultaneously after the perturbation (p>0.20). There were no significant differences between the muscle activation onsets of men and women (interaction; muscle*gender: F=0.35, p=0.7) (Fig. 4).

4. DISCUSSION

The purpose of the study was to investigate gender differences in ankle muscle response during provoked stumbling. Provoked stumbling led to increased EMG activity of the lower leg muscles compared to normal walking with statistically significant gender difference for PL muscle activity. However, no gender differences were found in onsets of muscle activation in response to perturbations.

An increased EMG activity of the lower leg muscles analyzed during stumbling compared to normal walking is supported by the literature [28-31]. Previous studies have shown that reflex activity of the lower leg muscles in compensation of lower leg perturbations is led by increased activity of ankle surrounding muscles, especially tibialis anterior [28,29]. Furthermore, high PL and TA activity during stumbling confirms their main stabilizing function during gait [22,30,32,33]. According to Winter & Yack [22] as well as Cappellini et al. [32], the main activity phase of TA during walking is directly before initial heel contact and the first 20% of the stride cycle (stance phase). Therefore, main response to applied gait perturbation could be expected within 200ms after perturbation, aiming to stiffen the ankle during midstance. The time frame of 200ms after perturbation includes the mono- and polysynaptic reflex activity. Therefore, gait perturbations can be compensated by increased neuromuscular reflex activity of the PL as well as TA muscles in both genders [30]. In contrast, the GM main activity phase is expected during the 20-60% markers of the stride cycle [22,32]. This might explain the only slightly higher RMS of GM during perturbed compared to normal walking in both genders. Even though TA and PL are already active before and during heel strike [22,32], the applied (posterior-anterior) perturbation results in additional higher activity of TA and PL during midstance [32]. This pattern basically demonstrates the reflex activity of these muscles in response to the applied perturbation. This is in accordance with the main task of the muscle responses immediately after the perturbation, preventing the occurrence of a sprain or, even worse, a fall.

Gender analysis of muscle amplitudes revealed differences for PL with women presenting significantly higher EMG-RMS amplitude than men during perturbed walking. In strong support of the findings presented here, Baur et al. [11] also found differences in PL activity between genders during pre-activation (before heel strike) of normal running. This suggests that differences between genders might be observed towards the end of the swing phase (pre-activation phase) and during provoked posterior-anterior perturbations while in midstance. Increased activation of the PL following perturbations might be needed to compensate the increased inversion-eversion and dorsiflexion joint laxity (decreased stiffness) and decreased dynamic postural control that has been documented in women [14-16]. Indeed the PL muscle has an important role in maintaining medio-lateral stability of the ankle [34], therefore, it can be argued that higher passive tissue compliance of the ankle in women can be compensated by higher activation of the PL muscle.

Regarding muscle onset analysis, no gender differences could be found for all muscles analyzed as response to the applied walking perturbation. This is in contrast to Baur et al. [11], Who reported significant earlier muscle onset of PL in women compared to men during unperturbed running (pre-activation strategy, before heel strike). Even though, Baur et al. [11] interpreted the consequences of their findings of PL onset as questionable. The small absolute differences do not imply different neuromuscular control strategies in the time domain. They therefore recommend that it is not reasonable to misinterpret timing differences between genders.

However, as response to the applied perturbation, the investigated muscles (GA, PL, TA) show a specific pattern of recruitment (muscle onset) from posterior to anterior. GM activated significantly earlier followed by an almost synchronous but later onset of TA and PL. Additionally, the synchronously activation of PL and TA confirms their main stabilizing function during perturbed walking [6,33]. This is supported by the results of the co-activation analysis revealing significant increased TA activity in relation to GM activity for (posterior-

anterior) perturbed compared to normal walking, without significant gender differences [25]. Regardless of gender, it might be discussed that TA is mainly responsible for controlling the ankle joint for slipping perturbations (posterior-anterior direction) [25]. Interestingly, Oliveira et al. [31] demonstrated higher GM activity in relation to TA activity as response to an anterior-posterior perturbation.

Besides, some aspects have to be considered while interpreting the presented results. The used perturbation mainly involved the sagittal plane of the ankle. Nevertheless, the perturbations were unpredictably applied during walking, instead of predictable perturbations over a tilt platform as seen on previous studies. Even though there were significant gender differences for mass and body height, both factors did not correlate to EMG amplitude (e.g., mass/EMG-RMS PL perturbation; $p=0.12$, correlation -0.41) and therefore did not influence the presented gender results. In addition, all subjects had to be physically active, at least 2 to 3 times a week, to ensure a basic fitness level. Detailed information on type of activity and total amount per subject was not assessed. Therefore, one cannot rule out the possible influence of activity level on neuromuscular strategies of the ankle surrounding muscles as response to the applied perturbation. However, all participants were recruited out of a student population without any elite athletic background. During stumbling, all participants walked at the same baseline velocity, despite different anthropometrics (body height, body mass) and stride parameters (individual preferred walking velocity; individual stride length), even between genders. Moreover, the influence of walking speed on neuromuscular activity has been thoroughly investigated [35]. As a result, for a standardized and comparable test situation between all participants, a consistent velocity during the stumbling protocol was favored [36]. Besides, only right-sided perturbations were analysed. It cannot be ruled out that participants were stressed to different extents due to individual foot dominance. Nevertheless, the human gait is described as an automated and stable movement pattern with high intra-individual reproducibility [23,37]. Consequently, there is no need to expect asymmetries in participants

without pain, complaints and/or injuries at the lower limbs. This was ensured by the principal investigator before inclusion into the study.

5. CONCLUSION

To summarize, artificial stumbling provokes higher ankle muscle activity compared to normal walking regardless of gender. The results reflect that activation strategies of the ankle encompassing muscles are only partly gender-specific: peroneal muscle amplitude appears to be higher in females compared to male during perturbed walking. Hence, no influence of gender as response of ankle stabilizing muscle reaction time was found. Future studies should aim to elucidate if this specific behavior has any relationship with ankle injury occurrence between genders.

Conflict of Interest

There are no conflicts of interest.

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Tables

Tab. 1 Co-activation (formula: GM EMG-RMS / TA EMG-RMS) for the lower leg muscles during normal and perturbed walking separated by gender

(TA: M. tibialis anterior; GM: M. gastrocnemius medialis)

|	Gender	
	Males	**Females**
Normal walking	2.96 ± 1.91	2.52 ± 1.61
Perturbed walking	0.43 ± 0.26	0.30 ± 0.07

Figure Legend:

Fig. 1   A. Customized Split belt treadmill with 2 separate selectable belts (Woodway)

   B. Treadmill perturbation characteristics (HC: initial heel contact)

Fig 2. Example of a typical muscular response pattern of perturbed walking for tibialis anterior (TA), peroneus longus (PL) and gastrocnemius medialis (GM) of the right leg separated by gender (ensemble average of 15 repeated right sided perturbations represented by mean ± SD)

Fig. 3 EMG-RMS for the lower leg muscles for (A) normal walking and perturbed walking during the subsequent 200ms after perturbation separated by gender

(TA: M. tibialis anterior; PL: M. peroneus longus; GM: M. gastrocnemius medialis)

Fig. 4 Time to onset [ms] for the ankle muscles separated by gender as response to perturbation

(TA: M. tibialis anterior; PL: M. peroneus longus; GM: M. gastrocnemius medialis)

Figures
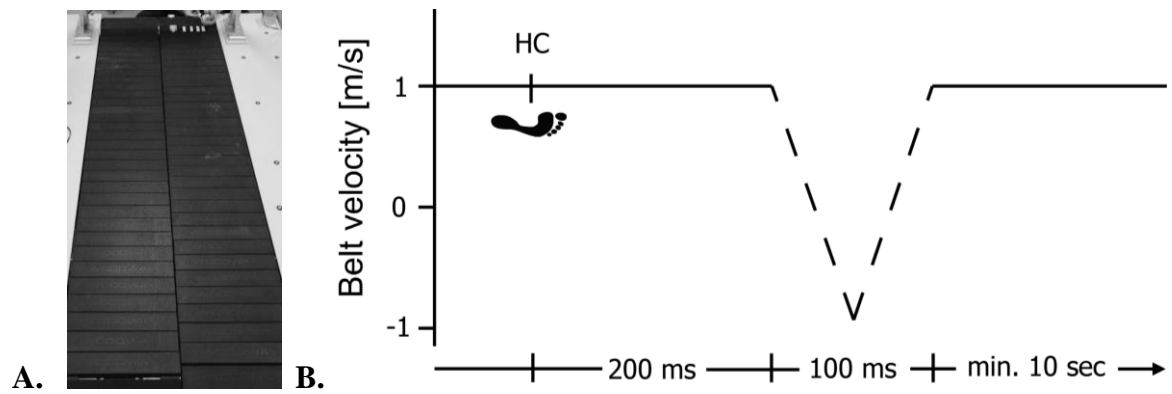
Fig. 1 A. Customized Split belt treadmill with 2 separate selectable belts (Woodway)

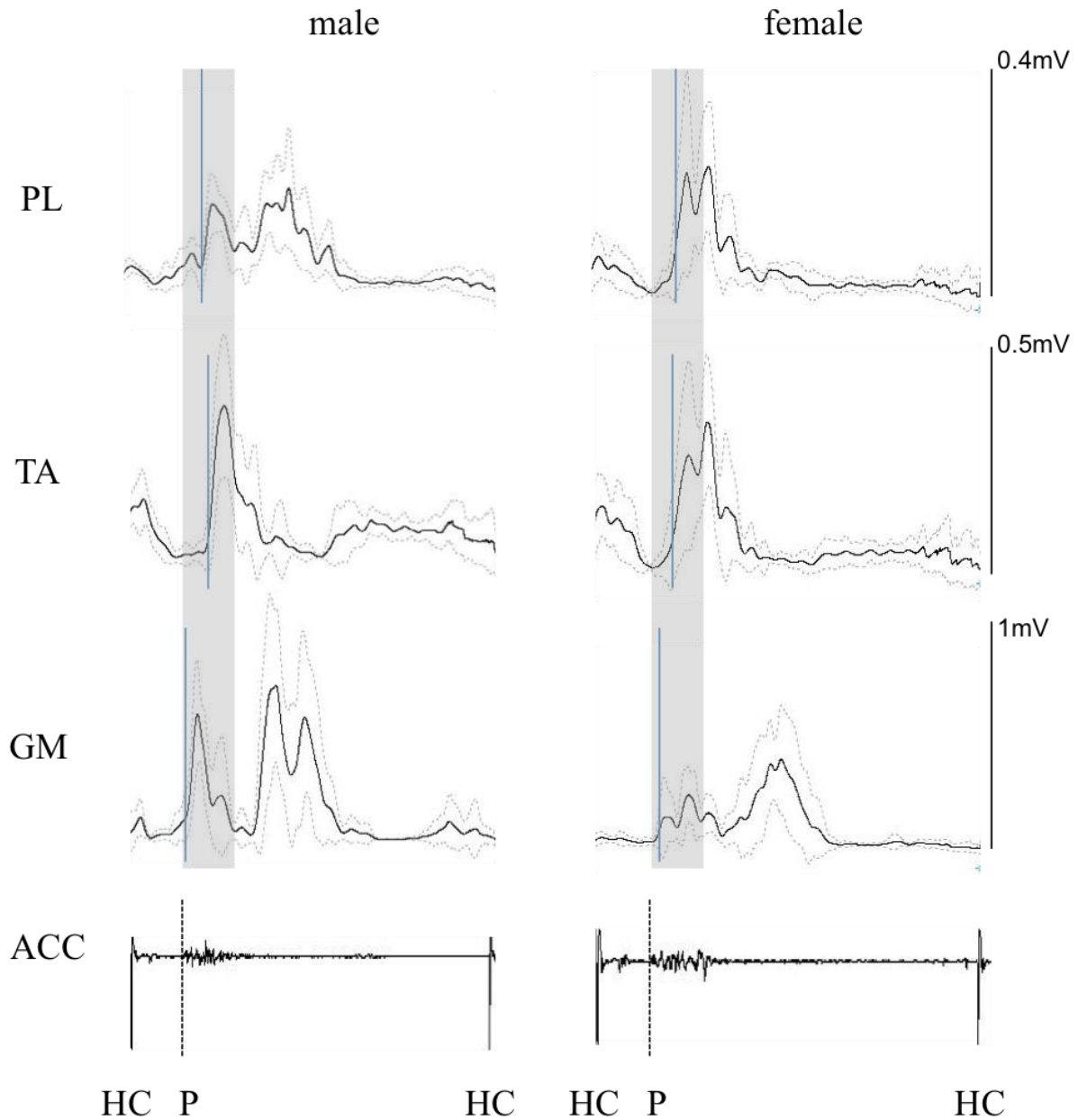**B. Treadmill perturbation characteristics (HC: initial heel contact)**

Fig 2. Example of a typical muscular response pattern of perturbed walking for tibialis anterior (TA), peroneus longus (PL) and gastrocnemius medialis (GM) of the right leg separated by gender (ensemble average of 15 repeated right sided perturbations represented by mean ± SD)

Legend: solid black line (HC): heel contact; dashed black line (P): start point of perturbation; grey area: 200 ms window post perturbation; solid blue line: onset of muscle activation
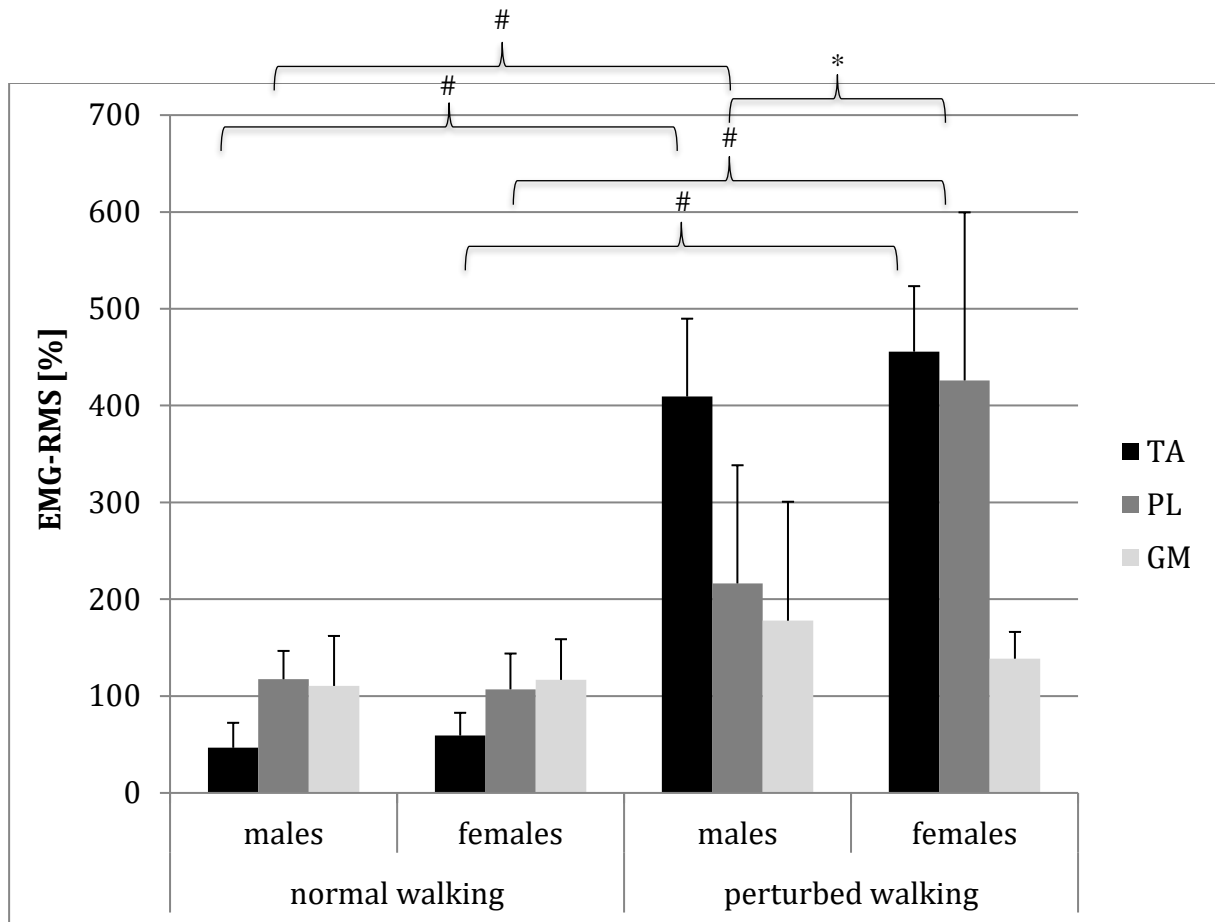
Fig. 3 EMG-RMS for the lower leg muscles for (A) normal walking and perturbed walking during the subsequent 200ms after perturbation separated by gender

(TA: M. tibialis anterior; PL: M. peroneus longus; GM: M. gastrocnemius medialis)

* significant gender differences (p<0.05)

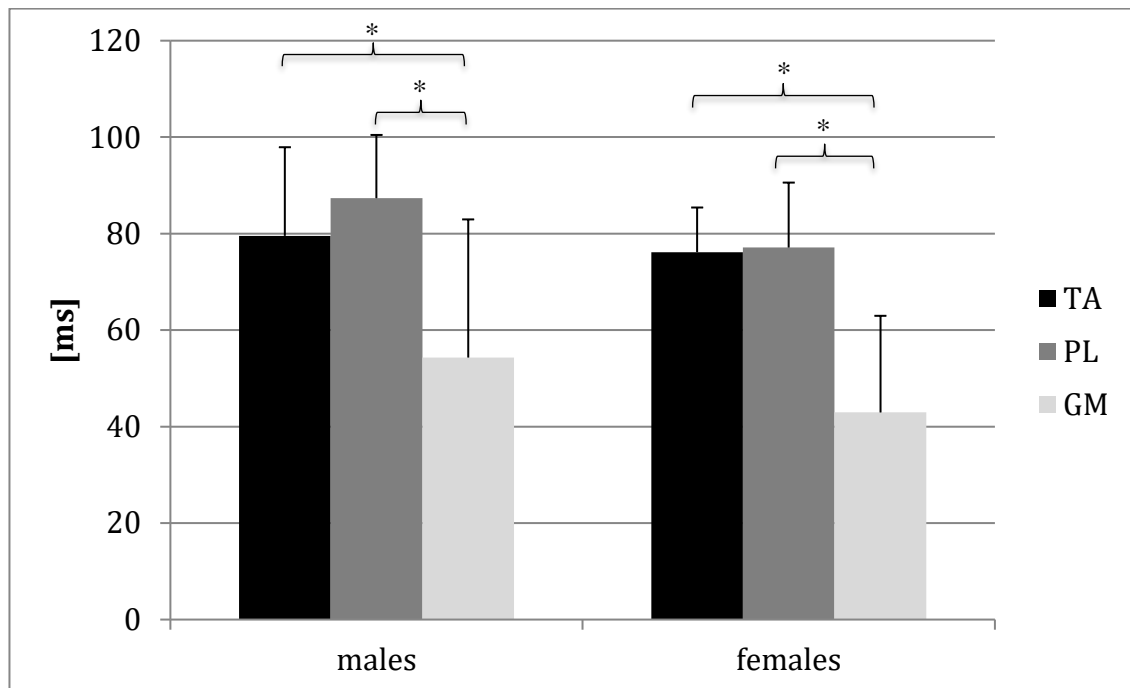# significant condition differences (p<0.05)

Fig. 4 Time to onset [ms] for the ankle muscles separated by gender as response to perturbation

(TA: M. tibialis anterior; PL: M. peroneus longus; GM: M. gastrocnemius medialis)

* significant muscle differences (p<0.05)