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Abstract

Sex differences in neuromuscular functioning has been proposed as one of the factors behind an increased relative risk of non-contact anterior cruciate ligament (ACL) injury in females. No studies appear to have explored sex differences in electromechnical delay (EMD) of the hamstring muscles during eccentric muscle actions and during a range of movement velocities. This study recruited 110 participants (55 males, 55 females) and electromyography of the semitendionosis, semimembranosus and biceps femerois was determined during eccentric actions at 60, 120 and 240°/s. No significant sex differences were observed irrespective of muscle examined or movement velocity. Irrespective of sex EMD significantly increased with increasing movement velocity ($P < 0.01$). There was no significant difference in the EMD of the 3 muscles examined. Our findings suggest that during eccentric actions of the hamstrings that there are no sex differences, irrespective of movement velocity. This would suggest that other factors are probably responsible for the increased relative risk of non-contact ACL injury in females compared to males.

Introduction

Dynamic muscular control of knee joint alignment, specifically differences in muscle recruitment, firing patterns and strength, may be partly responsible for the sex differences in the incidence of ACL injury (Myer et al., 2010). It has been postulated that the hamstrings reaction time is one of the most important primary risk factor associated with ACL tears (Shultz and Perrin, 1999). Considering the time lapse and the need to develop sufficient muscle tension rapidly enough to provide dynamic knee stability, electromechanical delay (EMD) should be considered when evaluating muscular responses to an imposed perturbation or injurious stress (Yavuz et al., 2010). Specifically, longer hamstrings reaction times may negatively influence the muscle´s ability to quickly stabilize the knee against the large external loads generated during sporting tasks and subsequently might increase the risk of tear (Besier et al., 2003; Blackburn et al., 2009; McLean et al., 2010). Feedback or reactive motor control strategies alter muscle activation in response to situations that load the knee (Shultz and Perrin, 1999). EMD is defined as the time between the onset of muscle activity and the onset of force generation by that muscles contraction (Zhou et al., 1995). It is related to the rate of muscle force production and is also considered an indirect measure of muscle-tendon unit stiffness (Blackburn et al., 2009).

Winter and Brookes, (1991) have reported that the EMD of the soleus muscle during plantar flexion and elastic charge time were shorter in men than in women whereas for total reaction time, pre-motor time and force time no significant sex differences were observed. Zhou *et al.* (1995) found significantly longer EMD values in females compared to males from as young as 8 years-old. More recently Inglis et al. (2013) and Kim et al. (2011) reported significantly longer EMD in females compared to males during isometric actions. One study has also demonstrated significantly longer EMD in females compared to males after passive stretching, albeit the absolute difference was only 4% (Costa et al., 2012). Longer EMD in females may be as a result of differences in muscle composition; however, current limited evidence suggests that differences in muscle composition are not sufficient to account for the sex differences (Blackburn et al., 2009, Zhou et al., 1995, Grosset et al., 2009). Therefore differences in muscle activation, such as excitation-contraction coupling and muscle fibre conduction velocity have been implicated in the longer EMD for females. A number of adult studies have also suggested that males shorter EMD compared to females may be attributed to greater musculotendinous stiffness in males (Blackburn et al., 2009, Zhou et al., 1995, Grosset et al., 2009).

Although a number of adult studies have demonstrated a significantly longer EMD in women than men conflicting data are available showing no sex differences in EMD (Hannah et al., 2015; Conchola et al., 2015; Johnson et al., 2012; Linford et al., 2006). Conflicting findings may be due to the differing techniques used to determine EMD (voluntary vs evoked) and the type of muscle action used, as all have been during isometric or concentric muscle actions. Only three studies appear to have explored sex differences in EMD of the knee extensors and flexors during eccentric muscle actions (Ayala et al., 2014; Blackburn et al., 2009) and reported no significant sex difference. However, Ayala et al. (2014) indicated that women demonstrated consistently longer hamstrings total reaction time (23.5ms), pre-motor time (12.7ms) and motor time (7.5ms) values than men, but that this did not reach statistical significance. It is possible that this study did not reach statistical significance due to the relatively small sample size and further research is needed with larger sample sizes. These results suggest that neuromuscular hamstring function in females may limit dynamic knee joint stability, potentially contributing to the greater female ACL injury risk. Whether EMD contributes to the greater relative risk of non-contact ACL injury in females is unclear as further research is needed to explore the sex related changes in EMD, especially during eccentric actions of the hamstrings at a range of movement velocities.

Previous studies have also demonstrated decreased medial to lateral quadriceps muscle recruitment (Hewett et al., 2005) and disproportionate firing of lateral hamstrings during landing (Rozzi et al., 99) in female participants. These 2 factors combined compress the lateral joint, opening the medial joint and subsequently increasing anterior shear force and increasing load on the ACL. Others have also identified that females move from a distal to proximal firing pattern during sudden forward movements and during internal/external rotation (Shultz et al., 2000). However, few studies have explored the sex related differences between lateral and medial hamstrings and proximal to distal firing during eccentric muscle actions over a range of movement velocities.

Given the essential eccentric role that the hamstrings play in stabalizing the knee it is important for functional relevance to examine the EMD of the hamstring during eccentric muscle actions. Therefore the purpose of this study was to examine the sex associated difference in EMD during eccentric actions of the hamstrings over a range of movement velocities.

Methods

One hundred and ten healthy participants consisting of 55 males (age 29±5y, stature 1.82±0.07m, body mass 82±7kg) and 55 females (age 27±6y, stature 1.61±0.08m, body mass 69±9kg) were recruited from the university population. All participants in the study were aged between 18-35 y, without previous injury to their dominant leg and regularly involved in self reported moderate intensity exercise (at least three times per week). The University's Research Ethics Committee approved all procedures and written informed consent was obtained from all participants. Participants visited the laboratory one week prior to testing to familiarise themselves with the laboratory and the experimental procedures. For female participants, all testing was conducted during the luteal phase of the menstrual cycle (post ovulation phase, average start and end days 15 to 26) which was self reported by the participant. All participants were instructed not to: 1) participate in strenuous physical activities in the 48 h prior to testing; 2) drink or eat anything other than water in the final 3 h before each visit; 3) drink alcohol in the final 24 h before each visit or drink caffeine 12 h before the test.

The assessments of EMD of the dominant limb were performed using a Biodex System-3 isokinetic dynamometer (Biodex Corp., Shirley, NY, USA) and a wireless 8-channel Delsys electromyography telemetry system (Delsys Myomonitor III, Delsys Inc., Boston, MA, USA). The dynamometer and EMG data were interfaced by feeding the analogue data directly from the dynamometer in to the Universal Input Unit via a trigger box and were displayed online on a computer using dedicated software (Delsys, Boston, MA). This system allowed for the dynamometer data to be converted to a digital signal in parallel with the EMG signal; consequently both data sets were collected in synchrony before processing by the EMG software (EMG Works 2, Delsys, Boston, MA). Therefore, this method allowed that the data from the EMG and dynamometer were completely time aligned making it possible to determine the onset of surface EMG activity in relation to the onset of torque production. Before and after the testing procedure commenced, the dynamometer and the EMG device were calibrated according to their respective manufacturer's instructions to assure that no change occurred in the sensitivity.

Participants were secured in a prone position on the dynamometer with the hip passively flexed at 10-20º. The prone position (10-20º hip flexion) was selected instead of a seated position (80-110º hip flexion) for two main reasons: (a) the prone position is more representative of the hip position during running/sprinting in contrast with a seated position; and (b) a prone position replicates the knee flexor and extensor muscle length-tension relationships which occurs in the late phase and the early contact phase of sprinting, and when landing or pivoting, which is when the ACL experiences its greatest rate of loading (Worrell et al., 1989, 1990).

The axis of rotation of the dynamometer lever arm was aligned with the lateral epicondyle of the knee. The force pad was placed approximately 3 cm superior to the medial malleolus with the foot in a relaxed position. Adjustable strapping across the pelvis, posterior thigh proximal to the knee and foot localised the action of the musculature involved. The range of movement was set from 90° knee flexion (initial position) to 0º (0º was determined as maximal voluntary knee extension for each participant). All settings, including seat height, seat length, dynamometer height and lever arm length, were noted during the practice session so that they were identical throughout experimental trials.

Surface EMG was obtained from medial / lateral hamstring and calf muscles of the dominant limb represented by semitendinosus, biceps femoris and gastrocnemious using bipolar and preamplified electrodes with a fixed interelectrode spacing of 10 mm (DE-02, Delsys, Bagnoli-8, Boston, MA). The electrodes were attached parallel to the muscle fibers and over the dorsomedial muscle bulge at two thirds of the proximodistal thigh length for the semitendinosus, and at the dorsolateral side of the thigh at one half of the proximodistal thigh length for the biceps femoris. The visually largest area of muscle belly was selected using an isometric action against a fixed lever arm. The ground electrode was placed on the lateral malleolus of the ankle. Each electrode placement was marked with permanent ink during the familiarisation session and re-marked at the end of each testing session to ensure consistent placement on subsequent testing days. Electrodes and cables were secured with surgical tape to avoid movement artifacts.

Before the assessment of EMD all participants performed a “zero offset” function to establish a zero baseline from each of the EMG channels during 10 s of stationary lying. The EMG and dynamometer data were acquired at a sampling rate of 1000 Hz. The dynamometer data were lowpass filtered at 10 Hz (4th order, zero phase lag, Butterworth), and the root-mean-square amplitude for each muscle activity was calculated as follows: the raw EMG signals were measured in a band of 20 to 450 Hz, full-wave rectified, high-pass filtered (4th order, zero phase lag, Butterworth) to remove movement artefacts with a cut-off frequency of 20 Hz, and smoothed with a 100-millisecond RMS algorithm.

After this baseline calculation process, participants were instructed to resist as hard and quickly as possible the knee extension movement generated by the arm of the dynamometer by eccentric action of the hamstrings throughout the full range of motion immediately after receipt of a visual (trigger box) signal. The visual signal, was given randomly within 1-4 s of the ‘ready’ command, and defined the beginning of data acquisition. Participants were instructed to relax and not exert force on the level arm prior to the visual signals in order to avoid pre-activation of the muscle. Visual inspection of the EMG signal was used to be confident that there was minimal EMG activity prior to movement of the lever arm. If the investigators could observe that pre-activation was taking place, identified by tensing of the lower limb muscles and EMG activity, they would remind the participant to relax before starting the lever arm.

Three maximal voluntary eccentric knee flexion muscle actions were performed at 60˚/s, 120˚/s and 180˚/s with 10 s rest between each action and 30 s rest between velocities. After each eccentric muscle action, the clinician passively returned the tested limb to the initial position. The EMD was defined as the time interval between the onset of EMG activity (increase of 15μV above the baseline value) and torque development (time taken [milliseconds] to generate 9.6 Nm torque) (Zhou et al., 1995). The mean of the 2 trials with the closest EMD values for each participant were subsequently used as reliability studies have reported better consistency of a measure when the mean value from several trials (two or more) rather than the single highest or lowest value is used (Sole et al., 2007). To investigate the true EMD in a contraction, the maximal electromechanical delay (EMD max) value was determined as the longer EMD of the three muscles. In this case, the signal recorded from the electrodes placed closer to the motor point was used in the comparison (Zhou et al., 1995).

Data were analysed using SPSS for Windows (version 21.0, SPSS, Inc., Chicago, IL, USA). Firstly the distribution of raw data sets was checked for homogeneity and skewness using the Kolmogorov-Smirnov test. Descriptive statistics including means and standard deviations were calculated for each variable. For the independent variables of knee angular velocity (60, 120, and 240°·s^{-1}), hamstring muscle (BF, SM, ST), and sex (male and female) a mixed-factorial (3 x 3 x 2) analysis of variance (ANOVA) was used to determine the influence on the dependent variable of electromechanical delay (EMD). The three independent variables include two within-subjects factors: knee angular velocity, hamstring muscle, and a between-subject factor of sex. Significant main effects were further examined using Bonferroni-corrected post hoc *t*-tests. The significance level was set to $p \leq 0.05$.

Results

EMD by muscle group and sex can be seen in table 1 below.

Table 1 Here

For each of the individual hamstring muscles, the interaction effect of joint angular velocity and sex was not statistically significant (Figure 1). The main effect of joint angular velocity was significant, indicating an increase in EMD with increasing joint angular velocity. No significant differences were observed between males and females at each of the three angular velocities.

Figure 1 Here

For EMD, there was no statistically significant interaction between joint angular velocity and EMD of the hamstrings muscle (BF, SM and ST) for males or females. Additionally, no main effects for hamstrings muscles were observed for both males and females at all three joint angular velocities.

Discussion

EMD has previously been used to determine the efficacy of proprioceptive feedback mechanisms in stabilizing the joint under sudden loading conditions and sex differences in these mechanisms have been proposed as one of the factors behind the increased injury incidence in females. The current study demonstrated no significant sex difference in EMD of the hamstring and calf muscles during eccentric muscle actions, suggesting that neuromuscular activation or mechanics in response to proprioceptive feedback does not provide us with an explanation for the sex disparity in ACL injury rates. These findings are in agreement with some previous work examining voluntary EMD (Minshull et al. 2007; Blackburn et al. 2009), whilst other investigations have reported conflicting data demonstrating a shorter EMD in males compared with females (Bell & Jacobs, 1986; Zhou et al. 1995). As EMD is considered to reflect an important aspect of neuromuscular reaction time (Minshull et al. 2007) a short EMD might reflect a reduced delay in force transmission from the muscle tendon unit to bone, which could facilitate the performance of explosive movements, such as jumping. The reason why we may have found differences to studies that demonstrate a sex differences in EMD might be attributed to the fact that EMD is influenced by: 1) the type of muscle action; 2) joint angle; 3) the effort level; 4) fatigue; and 5) the age and sex of the participants (Shultz and Perrin 1999).

Seeing as well-timed activation of the hamstring muscles can protect the ACL from mechanical strain by stabilising the tibia and reducing anterior tibial translation, the speed of this activation is vital for the subsequent joint stability. Given that we found no sex differences in voluntary EMD suggests that there are no differences in the voluntary neural aspect of the delay between men and women. Electromechanical delay is also considered to be influenced by stiffness (e.g muscle–tendon mechanics) (Kubo et al. 2001) and fibre type distribution (Viitasalo & Komi, 1978) and the lack of a sex difference would suggest that both these parameters are not different in males and females. The reason why we have found differing results to other may be attributed to the assumption that EMD is related to tendon stiffness, however, Wilson et al (1994) suggested that there is no relationship between muscle-tendon unit stiffness and eccentric force production. The EMD values found in the current study at 60º/s are similar to those previously reported by Inglis et al (2013) during isometric actions for both males (26ms vs 25ms) and females (34ms vs 27ms). It is important to note that EMD is reflective of neuromuscular feedback mechanisms and whether sex differences are apparent in neuromuscular feedforward mechanisms remains to be identified. Indeed ? et al. suggest that short, medium and long latency reflexes are essential to help provide joint stability and control during landing and cutting movements. Further investigation into low-threshold receptors in the cruciate ligaments is important, as these may influence the sensitivity of the muscle spindle, and provide preparatory stiffening of the muscle before excessive loading (Shultz and Perrin 1999).

Previous studies have suggested that females disproportionately fire the lateral versus medial hamstring and that a distal to proximal firing pattern predominates (Rozzi et al., 1999; Shultz et al., 2000). However, the findings from the current study demonstrate no sex differences in medial and lateral hamstring EMD during eccentric muscle actions, and over a range of movement velocities. These dare are in agreement with our previous studies examining EMD of the hamstrings during eccentric muscle actions in both children (De Ste Croix et al., 2015) and adults (Ayala et al., 2014), were we found no significant muscle specific differences in EMD. These findings suggest that during eccentric hamstring actions there are no differences in the feedback timing between the medial and lateral hamstrings, and thus all of the active muscles are equally contributing to the stabalisation of the joint from a neuromuscular perspective. These data also agree with the previous findings of Georgoulis et al. (2005) who found no significant differences for the EMD for either the rectus femoris (RF) or the vastus medialis (VM) muscle during maximal isometric voluntary action. The current findings also do not support a distal to proximal firing pattern in both males and females. However, a limitation of the current study, is that the role of the hip abductors/adductors or gluteal muscles was not explored. Ethically it is difficult to explore the role of gluteals but future research is necessary in order to investigate the neuromuscular response of both the hip musculature and core muscles.

The present study demonstrated significant main effects for angular velocity, indicating an increase in the EMD in all hamstring muscles with increasing angular velocity, irrespective of sex or time. These results suggest that neuromuscular functioning of the hamstring muscles, with increasing angular velocity, is reduced and may limit dynamic knee joint stability, potentially contributing to the greater ACL injury risk. It has previously been suggested that EMD will vary substantially due to the characteristics of the muscles being tested (e.g. architectural arrangement and fibre type distribution) (Viitasalo and Komi, 1981); muscle action (e.g. eccentric, concentric, voluntary, reflexive) (Norman and Komi, 1979, Zhou et al., 1995); and data processing techniques (Corcos et al., 1992). However, a limited number of studies have investigated the influence of movement velocity on EMD, and only one during eccentric actions. This is surprising given the range of movement velocities produced during sporting performance and that non-contact ACL injury may be velocity dependent.

Given the many injury risk factors experienced by females, habituated exposure to scenarios where knee joint control may be under threat might condition the neuromuscular system of the healthy female athlete at functional joint angles. The subsequent formation of pre-programmed responses that provide fast compensatory reactions to joint perturbations (Latash, 1998) may quickly harness the SEC and account for the parity in EMDV performance observed between the sexes at baseline. Under conditions of muscle fatigue and sustained loading, however, this capability may be diminished due to a reduction of the effectiveness of the fastest most powerful motor units, impairing the temporal capability of the muscle to 'gather in' a more compliant SEC.

In conclusion, the current study is the first to identify no significant sex differences in EMD of the hamstrings during eccentric muscle actions. The current study's finding would suggest that neuromuscular hamstring function in females does not limit dynamic knee joint control, or contribute to the greater ACL injury risk in females. These data would suggest that other risk factors may play a greater role in the increased relative risk of injury in female athletes, and these factors should be targeted in both screening and prevention programmes.

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Conflict of Interest: There are no conflicts of Interest

Table 1 Pre-post fatigue EMD values (mean ± SD) of hamstring muscles at 60, 120 and 240°·s^{-1} obtained for males and females.

Variables	EMD values at 60°·s^{-1}		EMD values at 120°·s^{-1}		EMD values at 240°·s^{-1}	
	Male	Female	Male	Female	Males	Females
EMD of BF	24 ± 3.2	27 ± 4.1	40 ± 12	42 ± 13.1	52 ± 8.5	57 ± 9.5
EMD of SM	25 ± 3.6	27 ± 3.9	40 ± 11.9	42 ± 12.6	53 ± 10.8	58 ± 9.5
EMD of ST	25 ± 4	26 ± 4.5	40 ± 14.4	43 ± 12	53 ± 9.6	57 ± 8.6
Max of EMD	27 ± 3.1	29 ± 3.5	47 ± 11.2	51 ± 10.8	59 ± 7.2	63 ± 7.7

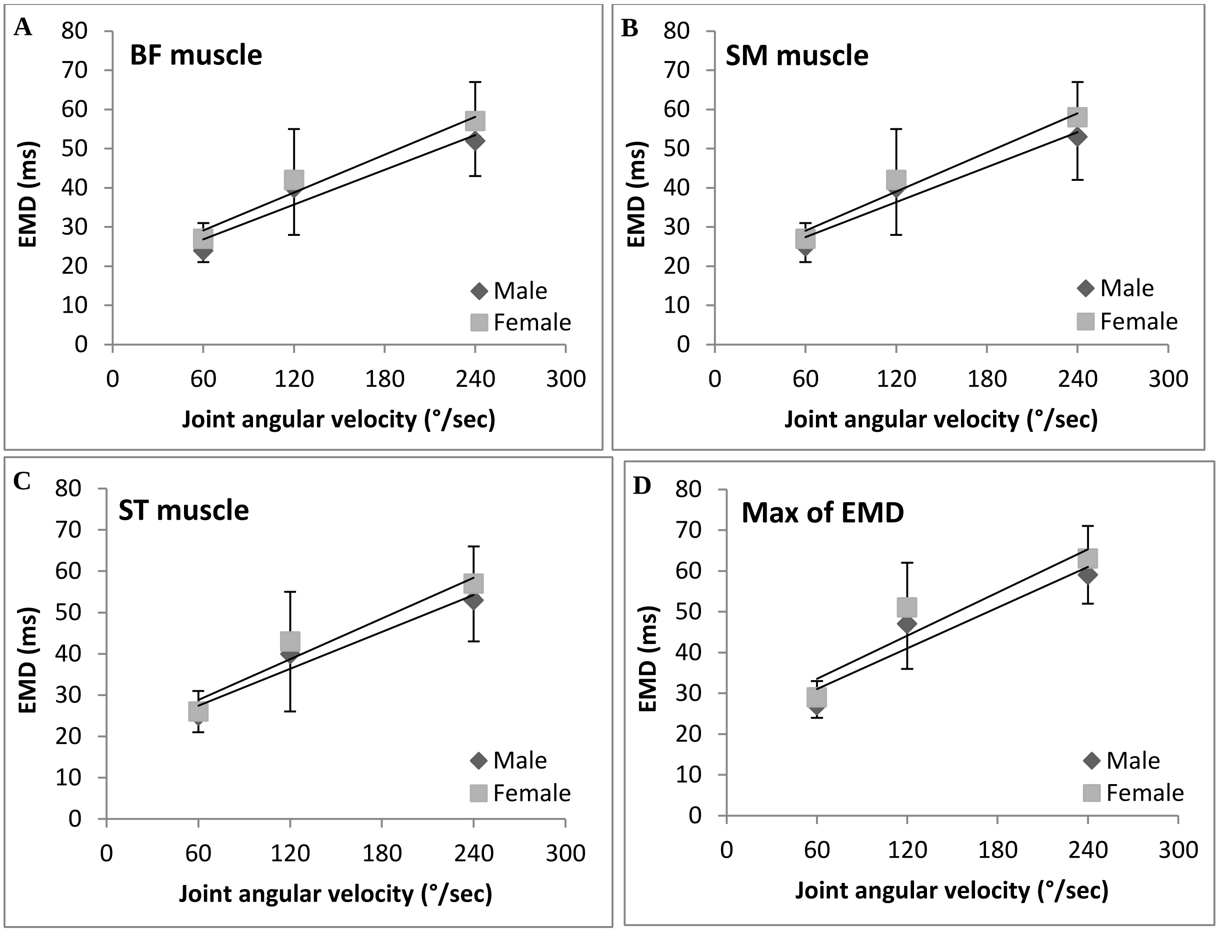

Figure 1: EMD for each muscle by sex and movement velocity. Panel a) BF = Biceps Femoris; b) SM = Semimembranosis; c) ST = Semitendonosis; d)