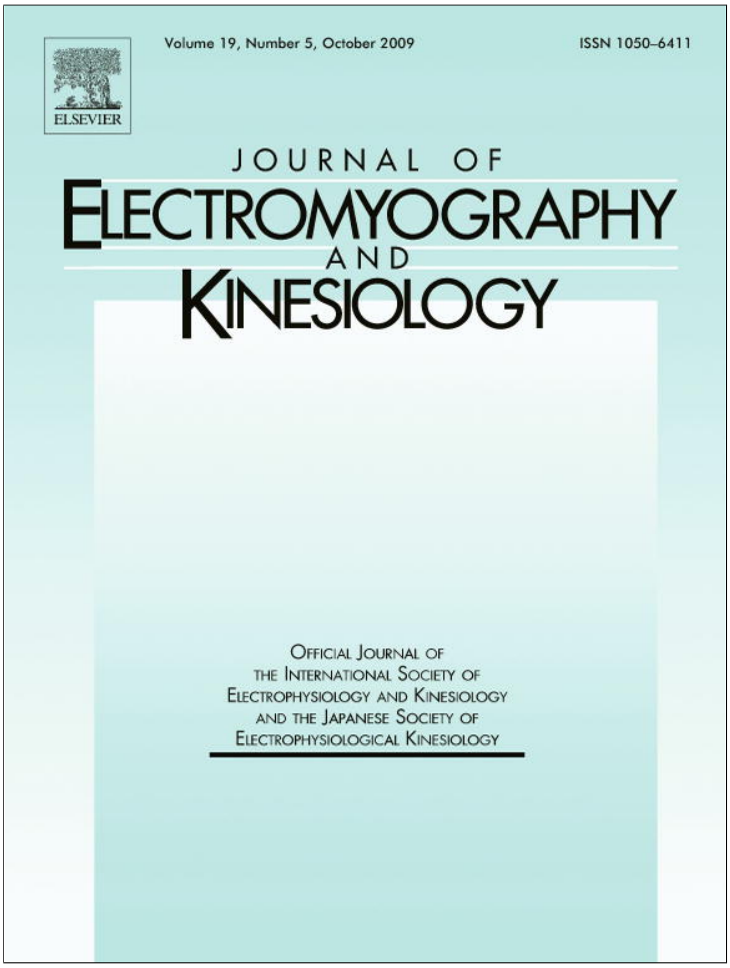
Volume 19, Number 5, October 2009
ISSN 1050-6411
ELSEVIER
JOURNAL OF
ELECTROMYOGRAPHY
AND
KINESIOLOGY
OFFICIAL JOURNAL OF
THE INTERNATIONAL SOCIETY OF
ELECTROPHYSIOLOGY AND KINESIOLOGY
AND THE JAPANESE SOCIETY OF
ELECTROPHYSIOLOGICAL KINESIOLOGY

Available online at www.sciencedirect.com
ScienceDirect
Journal of Electromyography and Kinesiology 19 (2009) e362–e369
JOURNAL OF ELECTROMYOGRAPHY AND KINESIOLOGY
www.elsevier.com/locate/jelekin

Comparison of hamstring neuromechanical properties between healthy males and females and the influence of musculotendinous stiffness

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Received 21 April 2008; received in revised form 11 August 2008; accepted 12 August 2008

Abstract

The hamstrings limit anterior cruciate ligament (ACL) loading, and neuromuscular control of these muscles is crucial for dynamic knee joint stability. Sex differences in electromechanical delay (EMD) and rate of force production (RFP) have been reported previously, and attributed to differences in musculotendinous stiffness (MTS). These characteristics define the neuromechanical response to joint perturbation, and sex differences in these characteristics may contribute to the greater female ACL injury risk. However, it is unclear if these differences exist in the hamstrings, and the relationship between MTS and neuromechanical function has not been assessed directly. Hamstring MTS, EMD, the time required to produce 50% peak force (Time50%), and RFP were assessed in 20 males and 20 females with no history of ACL injury. EMD did not differ significantly across sex ($p = 0.788$). However, MTS ($p < 0.001$) and RFP ($p = 0.003$) were greater in males, Time50% ($p = 0.013$) was shorter in males, and Time50% was negatively correlated with MTS ($r = -0.332$, $p = 0.039$). These results suggest that neuromechanical hamstring function in females may limit dynamic knee joint stability, potentially contributing to the greater female ACL injury risk. However, future research is necessary to determine the direct influences of MTS and neuromechanical function on dynamic knee joint stability and ACL injury risk.

Keywords: Electromechanical delay; Rate of force production; Gender

1. Introduction

Anterior cruciate ligament (ACL) injury affects as many as 250,000 individuals in the United States annually (Griffin et al., 2000, 2006), and females demonstrate a substantially greater injury risk compared to males (Arendt and Dick, 1995; Arendt et al., 1999; Gwinn et al., 2000). Joint stability is derived from a variety of factors, including capsulo-ligamentous integrity, articular forces, and musculotendinous forces. In the knee joint, stability provided by forces generated between the articulating surfaces is limited due to a relative lack of congruency. As such, dynamic neuromuscular control of the surrounding musculature is crucial in an effort to limit the stress imparted to capsuloligamentous structures (e.g. the ACL).

The ACL is loaded and potentially injured via anterior tibial translation (DeMorat et al., 2004; Sakane et al., 1999). The hamstrings are synergistic to the ACL, providing posterior tibial shear force which limits ACL loading attributable to anterior tibial translation. Hamstring contraction reduces anterior tibial shear force (MacWilliams et al., 1999) and translation (Li et al., 1999; MacWilliams et al., 1999), and ACL strain (Withrow et al., 2006) and loading (Markolf et al., 2004; Li et al., 1999) in the cadaveric knee, as well as *in vivo* ACL strain (Beynnon et al., 1995). Non-contact ACL injury typically occurs during landing and gait activities (Kirkendall and Garrett, 2000; Griffin et al., 2006) which incur rapid changes in the

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1050-6411/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jelekin.2008.08.005

forces applied to the knee joint. As such, timely dynamic neuromuscular control of the hamstrings appears to be essential for the provision of knee joint stability and limiting the load imparted to the ACL.

Skeletal muscle contraction is associated with a series of neuromechanical events which define the transmission of contractile force to the bony insertion. Electromechanical delay (EMD) has been used classically as a characterization of neuromechanical function, and is defined as the time interval between the onsets of electromyographic (EMG) activity and either force production or joint motion (Granata et al., 2000; Kubo et al., 2001; Isabelle et al., 2003; Moore et al., 2002). A substantial portion of this time interval is attributed to the removal of inherent series elastic "slack" (Viitasalo and Komi, 1981; Cavanagh and Komi, 1979). EMD is related to the rate of muscle force production (RFP), with longer EMD being associated with a lesser RFP (van Dieen et al., 1991; Vint et al., 2001; Bell and Jacobs, 1986). For a given mechanical system (e.g. the knee joint), a critical level of force is necessary to maintain equilibrium (i.e. stability) (McGill and Cholewicki, 2001). With a longer EMD and lesser RFP, this critical stabilizing force level would be attained later in response to joint perturbation, potentially delaying the stability provided via active force production (i.e. dynamic joint stability).

Shorter EMD has been noted in males compared to females (Bell and Jacobs, 1986), following isometric strength training (Kubo et al., 2001), in eccentric versus concentric contractions (Norman and Komi, 1979; Cavanagh and Komi, 1979), and in spastic cerebral palsy patients compared to healthy subjects (Granata et al., 2000). In each of these investigations, differences in musculotendinous stiffness (MTS) were suggested as potential contributors to the observed EMD differences. MTS refers to the ratio of change in force to change in length that the musculotendinous unit displays in response to tensile loading (Δforce/Δlength). Accordingly, greater MTS is hypothesized to correspond with shorter EMD by allowing greater tension to be developed per unit of length change, thus eliminating series elastic slack at a greater rate.

The longer EMD previously noted in females may indicate an inherent limitation to dynamic stability via a delay in protective musculotendinous force in response to joint perturbation. Interestingly, hamstring MTS is significantly greater in males than in females (Blackburn et al., 2004; Granata et al., 2002), potentially contributing to longer EMD and a lesser RFP. In combination, these sex discrepancies may contribute to the greater rate of ACL injury in females. However, the aforementioned sex differences in EMD and RFP were reported for the biceps brachii (Bell and Jacobs, 1986), and it is unclear if these differences are present in the hamstrings. Furthermore, while purported in the literature, the relationships between MTS and EMD and RFP have yet to be established experimentally. Therefore, the purposes of this investigation were to compare a series of hamstring neuromechanical properties between healthy males and females, and to evaluate the relationships between hamstring MTS and these neuromechanical variables.

2. Methods

2.1. Subjects

Forty healthy individuals (20 males, 20 females) volunteered for participation in this investigation. Subjects were required to have no history of: (1) neurological disorder, (2) lower extremity surgery, (3) ACL injury, or (4) lower extremity injury within six months prior to data collection, and (5) to be physically active, participating in a minimum of 20 min of physical activity three times per week. All subjects read and signed an informed consent document prior to participation which was approved by the University's Office of Human Research Ethics Institutional Review Board.

2.2. Experimental procedures

Hamstring MTS and neuromechanical function were assessed in a single testing session via a counterbalanced experimental design. Subjects performed five trials of each assessment with one minute of rest provided between trials to reduce the likelihood of fatigue. Prior to data collection, a preamplified/active surface EMG electrode configuration (DelSys, Inc., Boston, MA: interelectrode distance = 10 mm; amplification factor = 10,000 (20–450 Hz); CMMR @ 60 Hz > 80 dB; input impedance > 10^{15}//0.2 Ω//pF) was placed over the biceps femoris long head parallel to the direction of action potential propagation. Electrode locations were determined via palpation and identification of the area of greatest muscle bulk within the muscle belly. Proper electrode placement was verified via manual muscle testing (Hislop and Montgomery, 1995).

Active hamstring MTS was assessed by quantifying the damping effect imposed by the hamstrings on oscillatory knee flexion/extension induced by perturbation (Blackburn et al., 2004; Granata et al., 2002). Subjects were positioned prone on a padded table with the right thigh supported in 30° of flexion below horizontal, and a load representing 10% body weight was secured to the shank at the ankle joint. The investigator positioned the shank segment on horizontal, placing the knee in 30° of flexion, and subjects maintained this position via isometric hamstring contraction (Fig. 1). At random time intervals within 5 s of hamstring contraction, the investigator applied a brief downward manual perturbation approximately at the calcaneous. This perturbation forced the knee into extension from the original position (<5°), lengthening the hamstrings, and initiating oscillatory knee flexion/extension. An accelerometer (PCB Piezotronics, Depew, NY) was secured to a rigid splint placed on the distal shank and foot to measure tangential shank acceleration. With knowledge of the time instances of the first two oscillatory peaks in the tangential shank segment acceleration profile (t_1 and t_2),

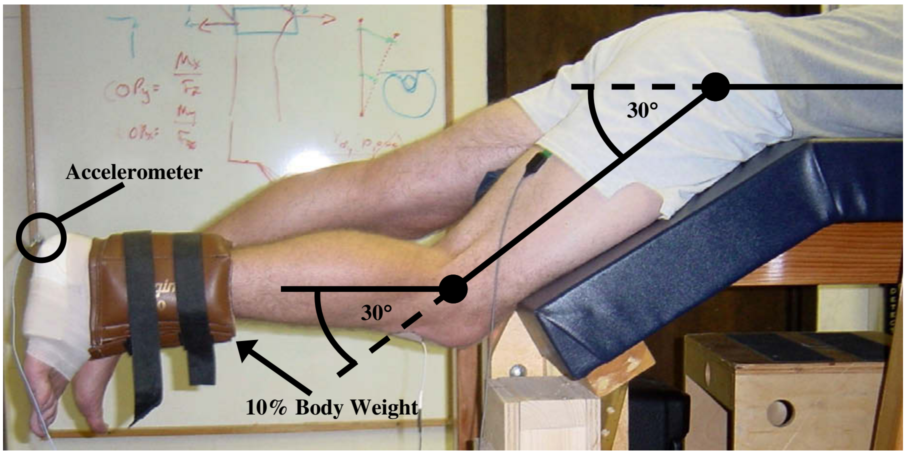

Fig. 1. Subject positioning during hamstring musculotendinous stiffness assessments.

the damped frequency of oscillation was calculated as $\frac{1}{t_2-t_1}$. Linear stiffness was then calculated by substituting this value into the equation $k = 4\pi^2 m f^2$, where k is linear stiffness, m is the summed mass of the shank and foot segment (Winter, 1990) and the applied load, and f is the damped frequency of oscillation. We demonstrated previously that this method produces moderate-to-high intra-session reliability ($ICC_{2,1} = 0.70$; $SEM = 1.63\ N\ cm^{-1}$) across five repeated measurements (Blackburn et al., 2004).

A series of hamstring neuromechanical properties were assessed during isometric hamstring contraction, including (1) electromechanical delay (EMD), (2) the time required to produce 50% peak isometric force (Time50%), and (3) the rate of force production (RFP). Subjects were positioned identically as in hamstring MTS assessments with the exception that the foot was secured to a loading apparatus (Fig. 2). The loading apparatus was interfaced with a compression load cell (Honeywell Sensotec, Columbus OH) positioned at the calcaneous such that hamstring (knee flexion) force could be captured. Hamstring neuromechanical properties were assessed by having subjects contract the hamstrings isometrically in response to a visual (light) stimulus. Subjects were instructed to remain in a passive/relaxed state until the stimulus was presented, at which time they were to contract the hamstrings maximally and as quickly as possible. The stimulus was presented at random time intervals to reduce the likelihood of anticipation.

2.3. Data sampling and reduction

EMG, accelerometer, and load cell data were sampled at 1000 Hz using The Motion Monitor motion capture software (Innovative Sports Training, Chicago, IL). Load cell and accelerometer data were lowpass filtered at 10 Hz (4th order, zero-phase-lag, Butterworth), while EMG data were corrected for DC bias, bandpass (20–350 Hz) and notch (59.5–60.5 Hz) filtered (4th order, zero-phase-lag, Butterworth), and smoothed using a 20 ms root-mean-square sliding window function to facilitate onset identification.

Hamstring MTS and neuromechanical variables were derived using computer algorithms employed via custom software (LabVIEW, National Instruments, Austin, TX). EMD was calculated as the time interval between the onsets of hamstring EMG activity and force production at the load cell (Fig. 3). The threshold for EMG onset was defined as $2 \times$ the mean noise level over the 100 ms interval prior to presentation of the visual stimulus for a

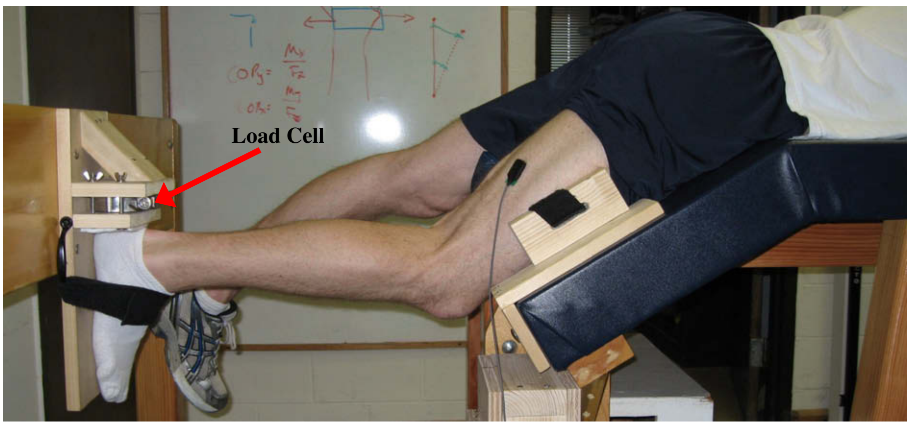

Fig. 2. Subject positioning during assessment of hamstring neuromechanical properties.

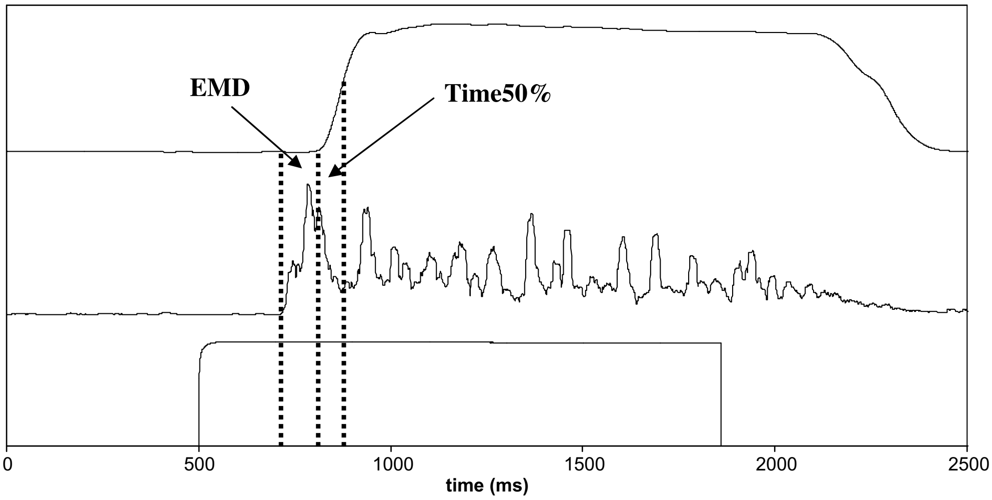

Fig. 3. Representative data for a single trial during assessment of hamstring neuromechanical properties. EMD refers to the interval between the onsets of EMG activity (1st vertical line) and force production (2nd vertical line). Time50% refers to the interval between the onset of force production and the production of 50% peak isometric force (3rd vertical line). The top, middle, and bottom traces represent load cell, EMG, and light stimulus data, respectively.

minimum of 50 ms to avoid erroneous identification. The threshold for the onset of force production was defined as 5% of the peak isometric hamstring force. Time50% was calculated as the time interval between the onset of force production and the instant at which 50% of the peak isometric hamstring force was attained (Fig. 3). The 50% peak force value was standardized to body mass to account for between-subject anthropometric discrepancies, and RFP was calculated as the ratio of this standardized force to the time required to produce that force (i.e. Time50%).

The computer algorithms accurately identified EMG onset in 193 of 200 trials (97%). For one female subject, however, multiple trials contained erroneous onsets, thus this subject was eliminated from statistical analyses. Descriptive statistics for the remaining subjects are detailed in Table 1. To account for the remaining trials with erroneous onsets, trials with the highest and lowest EMD values for each subject were discarded, and mean values for each neuromechanical variable were calculated across the remaining three trials. Mean values for MTS were calculated across all five trials. A series of statistical techniques were employed to assess normality of the dependent variables, including visual inspection of the distributions, assessments of skewness and kurtosis statistics, and Levene's test for equality of variances. MTS, EMD, Time50%, and RFP were compared across sex using two-tailed independent-samples t-tests. The respective relationships between MTS and EMD, Time50%, and RFP were evaluated via simple linear regression. Statistical analyses were conducted using commercially available software (SPSS, Inc., Chicago, IL) with significance established *a priori* as $\alpha \leqslant 0.05$.

Table 1
Subject demographics (mean ± sd)

	Height (m)	Mass (kg)	Age (yr)
Males ($n = 20$)	1.79 ± 0.09	75.93 ± 10.86	20.65 ± 1.63
Females ($n = 19$)	1.64 ± 0.06	61.98 ± 9.03	20.37 ± 1.57

3. Results

Hamstring MTS was significantly greater in males than in females ($p < 0.001$), thus comparison of hamstring neuromechanical properties was performed across two groups possessing high (males) and low (females) musculotendinous stiffness. EMD was not significantly different across sex ($p = 0.788$). However, Time50% was significantly longer in females ($p = 0.013$), and females demonstrated a lesser RFP ($p = 0.003$). Mean values for each dependent variable are detailed in Table 2. The relationships between MTS and EMD ($r = 0.073$, $p = 0.657$) and RFP ($r = 0.251$, $p = 0.123$) were non-significant. However, MTS was significantly and negatively correlated with Time50% ($r = -0.332$, $p = 0.039$; Fig. 4).

4. Discussion

The primary findings of this investigation were that while hamstring EMD did not differ between males and females, Time 50% was shorter in males while RFP was greater in males. These differences were noted between

Table 2
Hamstring neuromechanical characteristics (mean ± sd)

	Males ($n = 20$)	Females ($n = 19$)	p-value
Stiffness (N cm^{-1})	14.04 ± 3.06	10.15 ± 1.91	<0.001[a]
EMD (ms)	125.43 ± 21.51	127.49 ± 25.87	0.788
Time50% (ms)	102.95 ± 45.62	140.83 ± 45.06	0.013[a]
RFP (N kg^{-1} s^{-1})	13.72 ± 7.41	7.80 ± 3.42	0.003[a]

[a] Significantly different between Males and Females.

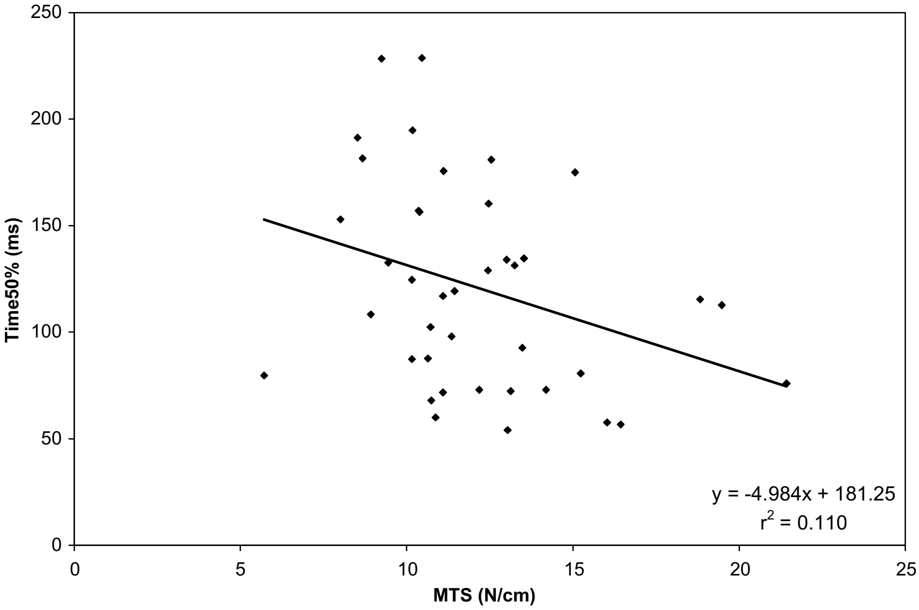

Fig. 4. Scatterplot detailing the relationship between musculotendinous stiffness (MTS) and the time required top produce 50% maximal isometric hamstring force (Time50%). Each data point (*x*, *y* coordinate) represents the average for the respective variables across trials for a single subject. The line is fit to the data in a least-squares sense.

samples demonstrating high (males) and low (females) MTS, and a significant relationship was noted between MTS and Time50%, indicating that individuals with higher stiffness are capable of reaching a specified relative force level in a shorter amount of time.

Our findings of a sex difference in Time50% and the lack of a significant sex difference in EMD are in agreement with the work of Winter and Brookes (1991) in the triceps surae. When comparing these investigations, it is essential to note the differences in operational definitions of the various neuromechanical properties. Winter and Brookes defined EMD of the triceps surae as the time interval between the onsets of soleus EMG activity and plantarflexion motion. Conversely, we defined EMD as the interval between EMG onset and force production (Bell and Jacobs, 1986; Granata et al., 2000), an interval Winter and Brookes termed Force Time. In both investigations, the duration of this interval did not differ significantly across sex. Winter and Brookes further delineated the time course of muscle contraction into a third neuromechanical variable termed Elastic Charge Time, defined as the interval between the onsets of force production and joint motion. Given the mechanical constraints about a joint (e.g. masses of the respective segments and level of agonist/antagonist co-contraction), a critical level of force is necessary to disrupt static equilibrium and produce joint motion. While the magnitude of this critical level of force varies as a function of the mechanical constraints, it can be considered a "meaningful" level of force. In a similar manner, we arbitrarily chose 50% of the peak isometric hamstring force as a meaningful force level, and evaluated the time required to attain this level of force under isometric conditions. As such, our definition of Time50% is similar to the Elastic Charge Time reported by Winter and Brookes. In both investigations, this interval was significantly shorter in males, suggesting that males are capable of attaining a given relative level of musculotendinous force in a shorter period of time relative to their female counterparts. This notion is also supported by our finding of a significantly greater RFP in males, a result which is in agreement the findings of Bell and Jacobs (1986) in the biceps brachii. We suggest that these differences are likely attributable to greater MTS in males as evidenced by the negative correlation between MTS and Time50%, thus allowing a greater increase in muscle tension per unit of length change.

Reported values for EMD differ substantially across muscles and investigations due to differences in operational definitions (Winter and Brookes, 1991), characteristics of the muscles being tested (e.g. fiber type distribution and architectural arrangement) (Viitasalo and Komi, 1981), contraction type (e.g. eccentric, concentric, voluntary, reflexive) (Zhou et al., 1995; Norman and Komi, 1979), and data processing techniques (Corcos et al., 1992). To our knowledge, this is the first investigation which has evaluated the neuromechanical properties of the hamstrings. As such, direct comparison of our reported values to previous literature is not possible. However, Corcos et al. (1992) evaluated biceps brachii EMD under various experimental conditions, including differences in hardware sensitivity, characteristics of the subject–force sensor interface, and time-scale resolution, and reported significantly different EMD values as functions of the various experimental characteristics. These findings suggest that when comparing between groups within an investigation, the absolute value of EMD is irrelevant given that the experimental and data processing methods (e.g. data smoothing) are identical for all groups.

Winter and Brookes (1991) suggested that the sex difference in Elastic Charge Time is attributable to a sex difference in MTS. The series elastic component of skeletal muscle exhibits considerable elasticity, introducing "slack" to the muscle-tendon complex which must be removed to allow contractile force to be transmitted to the bony insertion. Accordingly, a stiffer muscle should display shorter delays in force production and joint motion compared to a more compliant muscle due to the fact that a given change in musculotendinous length would be associated with a greater increase in tension, and more efficient and timely removal of series elastic slack. Our data validate these notions in that males demonstrated greater MTS, a greater rate of isometric force production, and required less time to attain a specified relative level of contractile force. Additionally, Time50% was negatively correlated with MTS, indicating that those who possessed greater stiffness required less time to attain 50% peak isometric force.

These differences in neuromechanical function of the hamstrings may contribute to the greater incidence of ACL injury in females. For a given mechanical system such as the knee joint, a critical level of force is required to maintain stability. For example, if an anterior tibial shear force of 100 N is applied to the knee joint, a posterior shear force of at least 100 N must be provided to prevent ACL loading attributable to anterior tibial translation. A delay in posterior tibial shear force provided by hamstring contraction may increase the risk of ACL injury by allowing a greater amount of anterior tibial translation to occur before the hamstrings are able to produce the critical level of force necessary to stabilize the joint. While the magnitude of the critical force required for stability differs based on the external demands placed on the system and the anthropometric characteristics of the individual, our data suggest that females require a greater amount of time to reach a specified percentage of maximal force. Interestingly, the temporal delay preceding the smallest detectable level of force production (i.e. EMD) did not differ across sex. Given the relatively small magnitude of this force (i.e. 5% maximal isometric force), it is likely that this force level does not contribute substantially to dynamic joint stability. However, the time required to reach a substantial level of force production (i.e. 50% max) was shorter in males compared to females. Additionally, MTS increases as a function of background effort/pretension (Sinkjaer et al., 1988; McNair et al., 1992), and Granata et al. (2002) and Padua et al. (2005) demonstrated that the MTS increase per unit of background effort is greater in males than in females. As such, the sex differences Time50% and RFP may be magnified at higher levels of relative force production, as may the strength of the relationships between these variables and MTS.

4.1. Limitations and future research

Future research is necessary to determine the clinical/physiological implications of heightened hamstring MTS and the associated influences on neuromechanical function, as well as the ability to modify these neuromechanical properties via training and enhancement of MTS. It is unclear if the sex difference in Time50% of ~40 ms has an appreciable effect on dynamic joint stability and injury risk. Additionally, while MTS and Time50% were significantly correlated, the strength of this relationship was weak in statistical terms. However, it should be noted that anterior tibial shear forces which load the ACL have been reported in excess of 60% body weight during landing activities, and are greater in males than in females (Yu et al., 2006). As such, it seems the substantially larger RFP noted in males, even after controlling for differences in body size, could potentially influence injury risk. Furthermore, the sex discrepancy in MTS increases with the level of contractile effort (Granata et al., 2002; Padua et al., 2005), suggesting that the observed differences in Time50% and RFP and the relationships between MTS and these neuromechanical variables may be magnified at higher loading levels. Future research is necessary to evaluate these influences at greater loading levels. Additionally, hamstring neuromechanical properties were evaluated under isometric conditions in this investigation. While these contractions are ideal for neuromechanical assessment in the laboratory setting, they likely do not mimic the behavior of the hamstrings during dynamic activities in which ACL injury typically occurs. Additional research is necessary to determine the extent to which these results are applicable to dynamic muscle contraction. Lastly, EMG data were only sampled from the long head of the biceps femoris, thus neglecting contributions from the medial hamstrings (i.e. semimembranosus and semitendinousus). Given the functional coupling of these muscles during knee flexion, future research is necessary to determine if these same observations apply to the hamstrings group as a whole.

5. Conclusions

The results of this investigation indicate that males demonstrate greater stiffness and rate of force production, and require less time to attain a relative level of isometric force in the hamstrings. These characteristics determine, in part, the ability of the neuromuscular system to respond to joint perturbation. As such, the differences in neuromechanical function and musculotendinous stiffness may contribute to the greater incidence of ACL injury in females.

Acknowledgements

This study was supported, in part, by grants from the National Athletic Trainers' Association Research and Education Foundation and the Injury Prevention Research Center at The University of North Carolina at Chapel Hill.

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