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**Ayala, Francisco, De Ste Croix, Mark B ORCID logoORCID:
<https://orcid.org/0000-0001-9911-4355>, Sainz de Baranda,
Pilar and Santonja, Fernando (2014) Inter-session reliability
and sex-related differences in hamstrings total reaction time,
pre-motor time and motor time during eccentric isokinetic
contractions in recreational athlete. *Journal of
Electromyography and Kinesiology*, 24 (2). pp. 200-206.
doi:10.1016/j.jelekin.2013.12.006**

Official URL: <http://dx.doi.org/10.1016/j.jelekin.2013.12.006>

DOI: <http://dx.doi.org/10.1016/j.jelekin.2013.12.006>

EPrint URI: <https://eprints.glos.ac.uk/id/eprint/3157>

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Ayala, Francisco and De Ste Croix, Mark B and Sainz de Baranda, Pilar and Santonja, Fernando (2014). *Inter-session reliability and sex-related differences in hamstrings total reaction time, pre-motor time and motor time during eccentric isokinetic contractions in recreational athlete*. Journal of Electromyography and Kinesiology, 24 (2), 200-206. ISSN 10506411

Published in Journal of Electromyography and Kinesiology, and available online at:

<http://dx.doi.org/10.1016/j.jelekin.2013.12.006>

We recommend you cite the published (post-print) version.

The URL for the published version is <http://dx.doi.org/10.1016/j.jelekin.2013.12.006>

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1 **Manuscript**

2 **1. Introduction**

3 Anterior cruciate ligament (ACL) injury is a common and potentially traumatic sports related
4 injury, presenting with substantial short- and long-term morbidities (Griffin et al., 2006). ACL
5 tears tend to occur during activities including sudden acceleration and deceleration, rapid
6 changes of directions, jumping and landing tasks; where rapid and unanticipated movement
7 responses of the ACL and the medial and lateral hamstring muscles (act as synergistic to the
8 ACL) are necessary to stabilize the knee joint and successfully counteract the extreme load
9 forces generated (McLean et al., 2008; Smith et al., 2012).

10 It has therefore been postulated that the hamstrings' reaction time is one of the most important
11 primary risk factor associated with ACL tears (Hughes and Watkins, 2006). Specially, longer
12 hamstrings' reaction times may negatively influence the muscle's ability to quickly stabilize
13 the knee against the large external loads generated during sporting tasks and subsequently might
14 increase the risk of tear (Besier et al., 2003; Blackburn et al., 2009; McLean et al., 2010). This
15 reaction time is comprised of two critical phases: the pre-motor time and motor time (Botwinick
16 and Thompson, 1966). The pre-motor time, defined as the time between the presentation of a
17 stimulus and the first detectable muscle activity (Weiss, 1965), incorporating perception,
18 decision making and information processing and transfer. The motor time is also called
19 electromechanical delay and is defined as the time between the onset of muscle activity and the
20 onset of force generation by that muscle's contraction (Zhou et al., 1995). It is related to the
21 rate of muscle force production and is also considered an indirect measure of muscle-tendon
22 unit stiffness (Blackburn et al., 2009).

23 Consequently, some authors have stated that ACL injury screening and prevention efforts may
24 ultimate benefit form the inclusion of hamstrings reaction times assessments (Hewett et al.,
25 2005, 2006; McLean et al., 2010; Ristanis et al., 2009). However, before the hamstrings total

1 reaction time, pre-motor time and motor time can be used for legitimate research and to
2 establish progress from training and/or rehabilitation practices, the inter-session reliability of
3 these measurements must be determined. Surprisingly, when the body of literature is carefully
4 scrutinised, it is shown that no studies (to the authors' knowledge) have addressed the
5 examination of their inter-session reliability of the hamstrings' total reaction time, pre-motor
6 time and motor time.

7 The incidence of non-contact ACL injury in women has been reported to be 6-8 times greater
8 than in men competing in the same sports (Griffin et al., 2006). Apart from the obvious
9 anatomical and hormonal differences between men and women, it is possible that sex specific
10 hamstrings' reaction times profile may play a role in the higher incidence of ACL injuries in
11 women. In some studies (Bell and Jacobs, 1986; Blackburn et al., 2004; Granata et al., 2002;
12 Winter and Brookes, 1991; Zhou et al., 1995), although not all (Blackburn et al., 2009; Moore
13 et al., 2002), the motor time (measured under isometric and concentric muscle actions) has been
14 found to be longer in women than men. However, among the aforementioned studies, only
15 Blackburn et al. (2009) reported data for the hamstrings during isometric muscle actions and
16 they showed that the motor time scores did not differ significantly across sex. In addition, to
17 the author's knowledge, no studies have examined sex-related differences in hamstrings total
18 reaction time and pre-motor time. This knowledge would provide insight into the understanding
19 of the factor associated with the sex-related differences in the ratio of ACL injuries.

20 Therefore, the aims of this investigation were twofold: (a) to ascertain the inter-session
21 reliability of hamstrings total reaction time, pre-motor time and motor time; and (b) to examine
22 sex-related differences in the hamstrings reaction times profile in recreational athletes.

23 **2. Methods**

24 **2.1 Participants**

1 Twenty-four men (age = 20.7 ± 2.8 y; stature = 174.3 ± 7.9 cm; body mass = 74.2 ± 9.7 kg) and
2 24 women (age = 20.1 ± 2.1 y; stature = 162.9 ± 7.9 cm; body mass = 61.1 ± 6.7 kg) who were
3 recreationally active adults (engaging in 2-5 hours of moderate physical activity 3-5 days per
4 week) completed this study.

5 Although all participants reported engaging in recreational sports performed at the intramural
6 or competitive university level, none of them were involved in a systematic and specific
7 strength-training programme. Participants were instructed to avoid their regular training
8 throughout the experimental period and refrain from vigorous physical activity 48 hours before
9 each testing day.

10 Other exclusion criteria were: (a) histories of orthopaedic problems, such as episodes of
11 hamstrings injury, fractures, surgery or pain in the knee or hamstring muscles over the past six
12 months; (b) missing one testing session during the data collection phase; and (c) presence of
13 delayed onset muscle soreness at any testing session to avoid the effects of muscle soreness on
14 muscle lengthening and force production. The women were not in the ovulation phase (self-
15 reported, days 10-14) of their menstrual cycle during testing as fluctuating concentrations of
16 oestrogen throughout the menstrual cycle affect musculotendinous stiffness (Bell et al., 2009;
17 Eiling et al., 2007) and joint laxity (Romani et al. 2003). The participants were verbally
18 informed about the study procedures before testing and provided written informed consent. This
19 study was approved by the University of Gloucestershire Research Ethics Committee (United
20 Kingdom).

21 **2.2 Research design**

22 A repeated measures design was carried out to determine the inter-session reliability of
23 hamstrings total reaction time, pre-motor time and motor time. A week before the testing
24 sessions commenced, the participants were familiarized with the testing procedure by
25 performing several sub-maximal and maximal isokinetic eccentric knee flexion movements

1 simulating the testing conditions that were carried out during the experimental sessions. In
2 addition, in order to ascertain the players' dominant limb, during this familiarization session all
3 the participants were required to undertake three tests: 1) jumping on one leg; 2) kicking a ball;
4 and 3) climbing onto a stool with one leg according to the methodology described by Wang et
5 al. (1993). The limb used to do at least 2 of the 3 tests was considered the dominant one.

6 After the familiarization protocol was completed, each participant undertook the testing
7 procedure on three different occasions, with a 72-96 hours rest interval between testing
8 sessions. The choice of a 72-96 hours interval period between testing sessions in our study is
9 based on the fact that a minimum of 36-48 hours rest interval appears to be necessary to restore
10 the optimal muscle function after a light bout of strength training (Bompa, 2000). Therefore,
11 we used a 72-96 hours interval period between testing sessions to ensure no carry over effects
12 between testing sessions.

13 An experienced clinician carried out each of the three testing sessions at the same time of the
14 day under the same environmental conditions (room temperature at 25 °C) to avoid inter-tester
15 variability and to reduce the influence of individual circadian rhythm on test data. The clinician
16 was blinded to the purpose of the study and test results from previous testing sessions.

17 Before hamstrings total reaction time, pre-motor time and motor time testing was carried out,
18 participants warmed-up by performing 5 minutes of general warm-up (cycle at 50 W and 60–
19 70 rpm), 4 sub-maximal (self-perceived 50% effort) and 2 maximal eccentric knee flexion
20 actions to reduce the variability and standard error of measurements by minimizing the effect
21 of different body temperature on muscle strength performance (Bishop, 2003). There was a 2-
22 minute rest between the warm-up and the hamstrings total reaction time, pre-motor time and
23 motor time testing.

24 **2.3 Testing procedure**

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

15 **2.4 Participant and dynamometer orientation**

16 Participants were secured in a prone position on the dynamometer with the hip passively flexed
17 at 10-20° and the head maintained erect. The prone position (10-20° hip flexion) was selected
18 instead of a seated position (80-110° hip flexion) for two main reasons: (a) the prone position
19 is more representative of the hip position during running/sprinting in contrast with a seated
20 position; and (b) a prone position replicates the knee flexor and extensor muscle length-tension
21 relationships which occurs in the late phase and the early contact phase of sprinting, and when
22 landing or pivoting, which is when the ACL is loaded (Worrell et al., 1989, 1990). Although
23 the standing position appears to be the most ecologic valid testing position, it was not used
24 because of technical issues (the bench of the dynamometer could not adopt this position).

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

9 **2.5 Surface electromyography**

10 Surface electromyography was obtained from medial and lateral hamstring muscles of the
11 dominant limb represented by semitendinosus and biceps femoris using bipolar and
12 preamplified electrodes with a fixed interelectrode spacing of 10 mm (DE-02, Delsys, Bagnoli-
13 8, Boston, MA). The electrodes were attached parallel to the muscle fibers and over the
14 dorsomedial muscle bulge at two thirds of the proximodistal thigh length for the
15 semitendinosus, and at the dorsolateral side of the thigh at one half of the proximodistal thigh
16 length for the biceps femoris (Merletti and Parker, 2004). The visually largest area of muscle
17 belly was selected using a contraction against manual resistance. The ground electrode was
18 placed on the lateral malleolus of the ankle. Each electrode placement was marked with semi-
19 permanent ink during the familiarisation session and re-marked at the end of each testing
20 session to ensure consistent placement on subsequent testing days. Electrodes and cables were
21 secured with surgical tape to avoid movement artifacts. Before the placement of the electrodes,
22 the hair of the area was shaved, and the skin was cleaned using alcohol swabs and abraded
23 lightly with sandpaper to reduce impedance below 5 kΩ.

24 **2.6 Data acquisition**

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

10 After this baseline calculation process, participants were instructed to resist as hard and quickly
11 as possible the knee extension movement generated by the arm of the dynamometer by eccentric
12 action of the hamstrings throughout the full range of motion immediately after receipt of a
13 simultaneous auditory (dynamometer) and visual (trigger box) signal. Both signals, which were
14 given randomly within 1-4 s, and defined the beginning of data acquisition.. Participants were
15 also instructed to relax and not exert force on the level arm prior to the auditory and visual
16 signals in order to avoid pre-action of the muscle.

17 Functional multi joint testing procedures seem to be more ecologically valid than a single joint
18 isokinetic testing procedure, but due to the necessity of qualified technicians, longer and more
19 practice sessions to accustom participants to the task required (learning effect) and highly
20 sophisticated instruments, the use of these complex lab-based techniques may be limited in
21 clinic and sports contexts. Therefore, in an attempt to be more realistic with the clinic and sports
22 contexts, we decided using a isokinetic knee flexion testing procedure.

23 Six maximal voluntary eccentric knee flexion muscle actions were performed with 30 s rest
24 between each contraction. After each eccentric muscle action, the clinician passively returned
25 the tested limb to the initial position.

1 The biceps femoris and semitendinosus total reaction time, pre-motor time and motor time were
2 calculated under eccentric muscle actions because it more appropriately simulates the pattern
3 of movements that usually occur during the mechanisms of noncontact ACL injury (Hughes
4 and Watkins, 2006). Six maximal eccentric knee flexion muscle actions were chosen because
5 in a pilot study with 10 participants of similar age and training status, they subjectively
6 indicated that 6 eccentric muscle actions were the maximum number of repetitions that they
7 were able to undertake without feeling musculoskeletal fatigue and hence, bias the results.
8 Therefore, to reduce the likelihood of fatigue throughout the testing procedure, the current study
9 used 6 eccentric knee flexion muscle actions.

10 The speed of the isokinetic level arm throughout each repetition was preset at 240°/s so that the
11 onset of torque was developed during the acceleration phase in order to replicate better the high
12 speeds underlying in the mechanism of non-contact knee injury (Boden et al., 2000). The 240°/s
13 angular velocity was chosen because in the pilot study, participants subjectively indicated that
14 240°/s was the maximum velocity that they were able to perform comfortably during the test.

15 The reaction time was defined as the time interval from the application of the auditory and
16 visual stimulus to the torque development (9.6 Nm torque) (Winter and Brookes, 1991). The
17 pre-motor time was determined as the time between the initial auditory and visual stimulus and
18 the associated muscle activation onset defined by the EMG activity (change from the EMG
19 mean baseline level to $\pm 15\mu\text{V}$ deviation) (Zhou et al., 1995). Visual inspection of the EMG
20 signal was also used to be confident that there was no EMG activity prior to movement of the
21 lever arm. The motor time was calculated as the time interval between the onset of EMG activity
22 and torque development (time taken [milliseconds] to generate 9.6 Nm torque) (Zhou et al.,
23 1995). The 2 trials with the highest and lowest total reaction time, pre-motor time and motor
24 time values for each participant were discarded, and mean values for each neuromechanical
25 variable were calculated across the remaining four trials. In addition, reliability studies have

1 reported better consistency of a measure when they used the mean value from several trials
2 (two or more) rather than the single highest or lowest value (Sole et al., 2007).

3 **2.7 Statistical analysis**

4 The distributions of raw data sets were checked using the Kolomogorov-Smirnov test and
5 demonstrated that all data had a normal distribution ($p > 0.05$). Descriptive statistics including
6 means and standard deviations were calculated for hamstrings total reaction time, pre-motor
7 time and motor time variables for both men and women separately. A paired t-test was
8 performed to compare hamstrings total reaction time, pre-motor time and motor time variables
9 (the average value among the three testing sessions) between men and women.

10 The inter-session reliability for each hamstrings reaction time measure was calculated to
11 average the reliability for the consecutive pairs of trials (2-1, 3-2) (Hopkins, 2000). In addition,
12 in line with current consensus regarding establishment of between day reproducibility in human
13 performance based studies (Atkinson and Nevill, 1998; Hopkins, 2000; Wier, 2005), the
14 following three aspects were explored:

15 1) Assessment of the bias between scores obtained in consecutive pairs of trials (2-1, 3-2).

16 Thus, 3 separate 3-way repeated measures analysis of variance (RMANOVAs; time [testing
17 session 1, 2 and 3] x muscle [biceps femoris and semitendinosus] x sex [men and women])
18 were used to identify the change in the mean (CM) (systematic bias), the 90% confidence
19 limits (CL) and the standard deviation of the difference between consecutive pairs of trial
20 for each variable (using Bonferroni post hoc test). Mauchly's test was used to check the
21 sphericity of the data. The differences are reported as percent changes between pairs of
22 consecutive trials and their respective 90% CL using logarithmic values (Hopkins, 2000).
23 The 90% CL calculations also utilized t-distribution values corresponding to the appropriate
24 degrees of freedom in each sample.

1 2) The precision of measurement was determined. This was done using the typical percentage
2 error (within-subjects variation in %) (Hopkins, 2000). The typical percentage error (as a
3 coefficient of variation [CV_{TE}]) and its 90% CL was calculated using the log-transformed
4 data via the following formula: $100(e^s - 1)$, where s is the typical error (standard deviation
5 of the difference between consecutive pairs of trial / $\sqrt{2}$). Logarithmic transformations of
6 the data were performed and used to reduce the possible heteroscedasticity (when the
7 difference scores demonstrate a trend toward larger values for participants at one end of the
8 plot) of the raw data (Atkinson and Nevill, 1998). To interpret the CV_{TE} values, the current
9 study used the arbitrary value suggested by Vincent (Vincent, 1994) with an analytical goal
10 of $\approx 10\%$ or below.

11 3) Assessment of the relative reproducibility. This was achieved using intraclass correlations
12 (ICC) and was chosen for generalization purposes (Wier, 2005). ICCs for the sample were
13 calculated from the analysis of variance via the formula: $(F - 1) / (F + k - 1)$, where F is the
14 F-ratio for the subject term and k ($= 3$) is the number of testing sessions. Magnitudes of
15 correlations were assessed using the following scale of thresholds: <0.80 low, $0.80-0.90$
16 moderate, and >0.90 high (Hopkins, 2000).

17 Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, v.
18 16.0 for Windows; SPSS Inc, Chicago) and Microsoft Excel 2003. The level of significance
19 was set at $\alpha = .05$.

20 **3. Results**

21 Descriptive statistics (mean values for each testing session [$k = 3$] \pm standard deviation) for
22 each variable are displayed in table 1 and table 2 for men and women respectively. Paired t -test
23 analysis reported no significant differences between men and women for biceps femoris and
24 semitendinosus total reaction time ($p = 0.06$), pre-motor time ($p = 0.18$) and motor time ($p =$
25 0.09). Mauchly's test of sphericity was not significant ($p > 0.05$) for all variables. The

1 RMANOVA analysis indicated no significant interaction effect among trials for each variable
2 for both men (table 1) and women (table 2), demonstrating that no systematic bias were present.
3 Men and women reliability statistics (CM, CV_{TE} and ICC) for biceps femoris and
4 semitendinosus total reaction time, pre-motor time and motor time values are also presented in
5 table 1 and table 2 respectively. The CV_{TE} for all variables ranged from 4.3% (women
6 semitendinosus total reaction time) to 10.8% (women biceps femoris pre-motor time) with ICC
7 scores higher than 0.65. The total reaction time demonstrated the lowest inter-session variability
8 in both men ($CV_{TE} = 4.5\%$) and women ($CV_{TE} = 6.0\%$).

9 *****Table 1 and table 2 near here*****

10 **4. Discussion**

11 There is a growing body of literature that has proposed the use of preseason screening of the
12 dynamic knee stabilizers reaction time (mainly from the hamstring muscles) to identify athletes
13 at increased risk of incurring ACL injuries (Hewett et al., 2005, 2006; McLean et al. 2010) and
14 to assess the impact of therapeutic interventions or the effects of physical training (Hewett et
15 al., 1999). However, before these data can be used for clinical and training goals, values must
16 be defined so as to provide guidance in deciding whether an observed change on reassessment
17 is within the boundaries of assessment error or whether there has been a true change (Hopkins,
18 2000). Therefore, one of the purposes of the current study was to determine the inter-session
19 reliability of hamstrings total reaction time, pre-motor time and motor time in recreational
20 athletes.

21 In this regard, the first aspect of reproducibility explored was the assessment of systematic
22 error, the presence of which has been implicated to potentially occur in total reaction time, pre-
23 motor time and motor time testing to due to learning effects or insufficient recovery time
24 (Hopkins, 2000). It is clearly evident from the results obtained in the current investigation that
25 the utilized familiarization session was sufficient in eliminating possible systematic error. This

1 may be partly attributed the fact that the participants in the study were generally accustomed to
2 physical activity performance and because the testing procedure was simple enough that just a
3 familiarization session was necessary to successfully avoid learning effects.

4 Another aspect of reliability explored is the precision of measurements. The typical percentage
5 error (CV_{TE}) and the 95% Limits of Agreement (95% LoA) are the most widely used reliability
6 statistics to determine the precision of a measurement or testing procedure. However, the
7 current study selected the CV_{TE} as an indicator of precision of measurement instead the 95%
8 LoA because it has been argued that the 95% LoA values may be too severe for assessing true
9 change in participant status (Hopkins, 2000). Thus, the findings of the present study indicated
10 that in both men and women, biceps femoris and semitendinosus total reaction time, pre-motor
11 time and motor time measures have a good precision (using the standard thresholds of precision
12 previously suggested) when measured during eccentric muscle actions of the hamstrings (CV_{TE}
13 $\approx 6\%$). Unfortunately, we are not able to compare the precision results for total reaction time,
14 pre-motor time and motor time obtained in the current research with other studies because to
15 our knowledge, no studies have examined the inter-session reliability of hamstrings total
16 reaction time, pre-motor time and motor time during either concentric or eccentric muscle
17 actions.

18 After calculation of the minimal amount of change necessary to reflect a real improvement
19 (typical percentage error), clinicians and practitioners should make a judgment on the
20 implication of the typical error on the real application of the measurement (Atkinson and Nevill,
21 1998; Hopkins, 2000). Admittedly, the clinical decision regarding the exact reference value is
22 challenging, especially since there are no clear guidelines for reference value establishment, as
23 well as the potential need to evaluate multiple factors (training status, sex, age) to reach a
24 knowledgeable decision. However, a possible way of arriving at an acceptable percentage of
25 error may be to decide whether the variability associated with a particular measurement is small

1 enough to allow the detection of the expected changes with training interventions. In this sense,
2 Minshull et al. (2009) using a repeated measures design and a testing procedure similar to that
3 reported in the current study, although using concentric muscle actions; observed that the
4 participant's biceps femoris and semitendinosus motor time was increased by 20% after
5 performing a standardised fatigue task in contrast with the control condition. Consequently, it
6 would be appear that the errors associated with biceps femoris (8%) and semitendinosus (9%)
7 motor time may be narrow enough to detect the expected changes after acute interventions.
8 However, it is unclear whether the precision of motor time is satisfactory to monitor changes
9 with neuromuscular training because to our knowledge no studies have examined the effects of
10 a long lasting training intervention on hamstring motor time. Furthermore, for the biceps
11 femoris and semitendinosus total reaction time and pre-motor time, it is unclear whether the
12 precision of measurements (5.5% and 5.6% respectively) is satisfactory, mainly because there
13 is a lack of information regarding changes in performance as a result of interventions (acute,
14 chronic and surgical).

15 The last aspect assessed concerns relative reproducibility, which was quantified using ICC. The
16 results of the current study reported moderate relative reliability scores for all the variables
17 assessed in both men and women (ICC ranged from 0.65 to 0.91).

18 The findings of the current study indicated that there were no significant differences between
19 hamstrings reaction times values of men and women. These results are in agreement with the
20 only other study (to the author's knowledge) that has compared hamstring motor time
21 (measured during isometric contractions) between men and women (Blackburn et al., 2009).
22 However, it should be highlighted that, although not significant, women reported consistently
23 longer total reaction time (22.7 ms and 24.2ms for biceps femoris and semitendinosus), pre-
24 motor time (10.4 ms and 15.1 ms for biceps femoris and semitendinosus) and motor time (8.9
25 ms and 6.1 ms biceps femoris and semitendinosus) than men. Furthermore, considering that

1 injury occurs within a very narrow time frame (e.g., 73 milliseconds for medial collateral
2 ligament injury [Pope et al., 1979]), the differences in neuromechanical function of the
3 hamstrings that we found in our study, although small may be clinically significant and
4 potentially large enough to play a role in ACL injury risk. Blackburn et al. (2009) have
5 theoretically suggested that a delay in posterior tibial shear force provided by hamstring
6 contraction may increase the risk of ACL injury by allowing a greater amount of anterior tibial
7 translation to occur before the hamstrings are able to produce the critical level of force
8 necessary to stabilize the joint. While the magnitude of the critical force required for stability
9 differs based on the external demands placed on the system and the anthropometric
10 characteristics of the individual (Blackburn et al. 2009), the data presented in the current study
11 suggest that women may require a greater amount of time to reach a specified percentage of
12 maximal force. However, future studies are necessary to determine whether this sex difference
13 in hamstring reaction times has a real appreciable effect on dynamic joint stability and injury
14 risk.

15 Interestingly, the results of this study did not found differences between biceps femoris and
16 semitendinosus reaction times values in both men and women. A possible explanation for this
17 result is that the biceps femoris works synergistically with the semitendinosus during knee
18 flexion to provide functional balance to the knee. Direct comparison of our reported raw values
19 to previous literature is not possible because, to the author's knowledge, this is the first
20 investigation which has evaluated the reaction times of the hamstrings during eccentric actions.
21 One of the potential limitations of the current study is the population used. The age distribution
22 of participants was relatively narrow and the generalizability to the broader population cannot
23 be ascertained. Similarly, whether the tests would be as reliable in a population of injured
24 participants must also be considered although pre-season screening is generally performed in
25 healthy, uninjured populations. In addition, the current study explored the reliability of the

1 hamstrings total reaction time, pre-motor time and motor time during eccentric isokinetic knee
2 flexion movements adopting a prone position because it may simulate better the mechanics of
3 ACL injury. Whether the reliability of these parameters during concentric isokinetic knee
4 flexion movements and/or in seated and/or standing position produces different reliability
5 results was not explored. Since the EMG and dynamometer data were treated differently,
6 different timing offsets might be present so future comparisons should be made with that in
7 mind. Finally, the non-ovulation phase of the menstrual cycle in each participant was not
8 verified with an ovulation test but was self reported. This may have potentially influenced our
9 findings as the menstrual cycle is not always consistent between and within women (Bell et al.,
10 2009).

11 It is recommended that physiotherapists undertaking pre-season screening of athletes consider
12 the use of the test methodologies described here, as standardised testing protocols enable
13 comparisons to be made across different clubs and sports. Future studies should examine
14 possible strategies designed to increase the reliability of hamstrings total reaction time, pre-
15 motor time and motor time such as different approaches to determine the onset of EMG activity
16 and force production (2 times the baseline standard deviation, 2 times the baseline noise level)
17 and with different devices (i.e. force plates and EMG). Furthermore, studies examining the
18 reliability in different populations are needed to ascertain the clinical application of hamstrings
19 total reaction time, pre-motor time and motor time.

20 **5. Conclusions**

21 The current study provides clinicians and practitioners with important information regarding
22 the reliability threshold for the hamstrings total reaction time, pre-motor time and motor time
23 and can serve as a guide for establishing whether an observer change between testing sessions
24 is real or simply a product of typical error. Thus, an observer change larger than 5%, 9%, 8.0%,
25 for total reaction time, pre-motor time and motor time respectively from baseline scores after

1 performing acute/chronic fatigue tasks or neuromuscular training would indicate that a real
2 change was likely.

3 In addition, the results of this investigation indicate that, although not significant, women
4 reported consistently longer hamstrings total reaction time (23.5 ms), pre-motor time (12.7 ms)
5 and motor time (7.5 ms) values than men. These differences may play a role in the greater
6 incidence of ACL injuries in women. However, future studies should address this issue.

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1 **FIGURE LEGEND**

2 Figure 1: A typical time plot of a single trial on the presentation of the stimulus (sound and
3 visual signals) to the onset of the electromyography (EMG) signal (biceps femoris) and force
4 generation (torque). The onset of torque development is defined as a 9.6 Nm deviation above
5 the baseline level and the onset of EMG signal as $\pm 15 \mu\text{V}$ deviation above the baseline.