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2015

17β-Estradiol Induced Effects on Anterior Cruciate Ligament Laxness and Neuromuscular Activation Patterns in Female Runners

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Recommended Citation

Khowailed, I. A., Petrofsky, J. S., Lohman, E., Daher, N., & Mohamed, O. (2015). 17β-estradiol induced effects on anterior cruciate ligament laxness and neuromuscular activation patterns in female runners. Journal of Women's Health, 24(8), 670-680.

17β-Estradiol Induced Effects on ACL Laxness and Neuromuscular

Activation Patterns in Female Runners

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Abstract

Background: To investigate the effects of 17β-Estradiol across phases of menstrual cycle on the laxness of the anterior cruciate ligament (ACL) and the neuromuscular control around the knee joint in female runners**.**

Methods: Twelve healthy female runners, who reported normal menstrual cycles for the previous 6 months were tested twice across one complete menstrual cycle for serum levels of 17β Estradiol (E), and knee joint laxity (KJL). Electromyographic (EMG) activity of the quadriceps and hamstrings muscles was also recorded during running on a treadmill. The changes in the EMG activity, KJL, and hormonal concentrations were recorded for each subject during the follicular and the ovulatory phases across the menstrual cycle.

Results: An observed increased in KJL in response to peak E during the ovulatory phase, was associated with increased preactivity of the hamstring muscle before foot impact ($p < 0.001$). A consistent pattern was also observed in the firing of the quadriceps muscle recruitment pattern throughout the follicular phase associated with decreased hamstring recruitment pattern during weight acceptance phase of running ($p = 0.02$). Additionally, low ratio of medial to lateral quadriceps recruitment was associated with a significant reduction of the quadriceps to hamstring cocontraction ratio during the follicular phase.

Conclusions: Changes in KJL during the menstrual cycle in response to 17β-Estradiol fluctuations changes the neuromuscular control around the knee during running. Female runners utilize different neuromuscular control strategies during different phases of the menstrual cycle which may contribute to increase ACL injury risk.

Keywords: 17β-Estradiol, ACL injury; knee joint laxity (KJL); EMG; neuromuscular control.

Abbreviation

E	17β-Estradiol
ACL	Anterior cruciate ligament
CNS	Central nervous system
EMG	Electromyography
H/Q	Hamstring quadriceps ratio
IEMG	Integrated Electromyography
KJL	Knee joint laxity
LH	Lateral Hamstring
MVC	Maximum voluntary contraction
MH	Medial Hamstring
ML	Medial to lateral
OC	Oral contraceptive
PA	Preactivation
PO	Push off
QH	Quadriceps hamstring
VL	Vastus lateralis
VM	Vastus medialis
WA	Weight acceptance

17β-Estradiol Induced Effects on ACL Laxness and Neuromuscular Activation Patterns in Female Runners

Introduction

A female athlete's increased risk for non-contact anterior cruciate ligament (ACL) injury has been well documented [1,2]. Women are two to eight times more likely to injure their ACL when compared to men in comparable sporting activities [3]. The discrepancy in ACL injury risk between sexes has been attributed to multiple factors including differences in anatomical, hormonal, biomechanical, and neuromuscular characteristics [2].

1.1. Hormone effects on injury risk

The normal menstrual cycle produces low serum levels of estrogen and progesterone in the early follicular phase (day 1–6), estrogen is elevated in the late follicular phase (day 7–14), and progesterone is elevated during the luteal phase (day 15–28) while estrogen remains elevated and slowly returns to baseline levels [4,5]. The link between ACL injury and fluctuations of the ovarian sex hormones during the female menstrual cycle is controversial. Some investigators have reported an increase in ACL injuries in the late follicular phase [3,6,7]. Other investigators have reported similar phenomena during the luteal phase [6,8] and during the early follicular phase [9]. These contradictory results fail to explain the role of sex hormones in ACL injury risk to one phase of the menstrual cycle.

1.2. Hormone effects on the ACL

Ovarian sex hormone fluctuations have been associated with tissue alterations and an increased incidence of noncontact ACL injuries [7,10]. Estrogen and progesterone receptors have been detected within the ACL [11]. Several studies have demonstrated a relationship between peaks in estrogen serum concentration and increased laxity in the ACL [6,9,12]. This associated change in tissue tolerance may predispose the ACL to failure at lower tensile loads and/or alter the protective muscle reflex actions associated with ACL tissue receptor stimulation [13].

1.3. Hormone effects on tissue

The muscular system serves a protective role in limiting the external forces and moments created through the knee joints motions that ultimately result in tension loading of the ACL. Estrogen alpha and beta receptors have been reported in skeletal muscle thereby providing a plausible tissue-based mechanism for altering neuromuscular control and myofascial force transmission pathways during the menstrual cycle [14-16]. In addition, research has not fully described the influence of sex hormone receptors in skeletal muscle on tissue mechanisms that can alter neuromuscular control. However, estrogen both directly and indirectly affects the female neuromuscular system [17]. Sarwar & colleagues reported quadriceps strength increases and a significant slowing of muscle relaxation occurs during the ovulatory phase of the menstrual cycle [18]. Serum estrogen concentrations fluctuate radically throughout the cycle and estrogen has measurable effects on muscle function and tendon and ligament strength [17,19]. These data indicate that estrogen may have effects on neuromuscular function which may facilitate the potential for neuromuscular imbalances to develop in female athletes.

1.4. Hormonal effect on the central nervous system

Estrogen also has effects on the central nervous system including the higher motor centers, where it binds to membrane-bound receptors and influences transmitter systems in the brain [20]. Hence, during the menstrual cycle as the endogenous levels of estrogen undergo dynamic regulation; it stands to reason that their effect (s) on the CNS and thus neuromuscular control will also change [21,22].

1.5 Hormonal effects on neuromuscular control

Female athletes display different neuromuscular strategies from male athletes [23]. These sex differences in muscle recruitment and timing of muscle activation may affect dynamic knee stability. Neuromuscular preplanning allows feed forward recruitment of the musculature that controls knee joint positioning during landing and pivoting maneuvers [24]. Imbalanced or ineffectively timed neuromuscular firing may lead to limb positioning during athletic maneuvers that puts the female ACL under increased strain and risk of injury [23]. In addition, fine motor activity and reaction time performance have been reported to fluctuate over the course of the menstrual cycle [25], with more consistent performance in women using oral contraceptives (OC). Fridén et al., discovered an increase in postural sway [26] during single limb stance and threshold for detection of passive knee motion [20] in the mid-luteal phase of the menstrual cycle. Improved neuromuscular coordination may occur in women taking OC with a reduced number of premenstrual symptoms [27].

1.6 Neuromuscular control differences between sexes

Men and women demonstrate similar neuromuscular control strategies during different athletic activities until puberty [28]. A link between hormonal fluctuations and changes in neuromuscular control may exist, since alterations in hormonal levels constitute a primary change in development during and after puberty. Neuromuscular control strategies incorporated

during athletic movement appear to change in females after puberty, where increased knee valgus alignment places the ACL at greater risk for injury [28]. Several theories have been proposed to define the mechanisms for gender differences in ACL injury rates. These theories include gender differences, decreased knee ligament strength due to female sex hormones and neuromuscular imbalances in females [23].

The increased incidence of serious knee injuries in female athletes is well established, however the underlying neuromuscular mechanisms related to the elevated ACL injury rate that occurs after the onset of puberty in females has not been delineated. Remarkably, an observed association between hormonal fluctuation and ACL injury risk indicates that there were effects of hormonal fluctuation (and potentially hormone stabilization) on either passive or dynamic knee stability [29]. The effects of the menstrual cycle may be on the active restraints (neuromuscular in nature) rather than the passive restraints (ligament) of knee stability, because the menstrual cycle has effects on motor control and muscle strength [18,25]. Some reports suggest that more emphasis should be placed on investigation of neuromuscular factors that may be related to increased ACL injuries in female athletes [30].

Circumstantial evidence exists supporting a link between hormonal fluctuations during the menstrual cycle and altered neuromuscular control during selected athletic movements. To date, few studies have focused on changes in neuromuscular control mechanisms over the course of the menstrual cycle and its impact on running activity. Therefore, the purpose of the study was to investigate the effects of 17β-Estradiol across phases of menstrual cycle on lower extremity neuromuscular control patterns and ACL laxity during running. We hypothesized that lower extremity muscle activation patterns and laxness of the ACL will be altered during the periods of

high level of serum estrogen concentration compared to the early follicular phase, when estrogen

is lowest in young healthy females' runners.

1. **Methods**

Subjects

A total of 12 female runners mean (±SD) age was 25.6 (±3.7) years, with regular menstrual cycles volunteered to participate in the study. All subjects were currently running not more than 20 km per week. Inclusion criteria were no history of pregnancy, no use of oral contraceptives or other hormone-stimulating medications for 6 months, nonsmoking behavior, two healthy knees with no prior history of joint injury or surgery, no medical conditions affecting the connective tissue, and physical activity was limited to 7 hours or less per week to reduce the likelihood of irregular or an ovulatory menstrual cycles that can occur with high volume or high intensity training. All subjects were heel strikers free of any obvious mal alignment or injuries at the time of data collection. The demographic characteristics of the subjects are displayed in table 1. All subjects had regular menstrual cycles of a mean interval of 28 (±2) days. All participants gave their written informed consent prior to entering the study. All procedures and protocols were approved by Institutional Review Board of Loma Linda University.

Insert Table 1 here

1.1 Hormonal Assessment

All subjects came to the laboratory prior to data collection for a precollection session to familiarize them with the study protocol. Subjects reported for neuromuscular testing and blood assay during each of the follicular and ovulatory phase of the menstrual cycle during a month period. The first measurement (follicular phase) was taken during days 1 to 2 at the beginning of the menstrual cycle, when estrogen levels were expected to be low [8,31]. The second data collection coincided with ovulation and occurred 24 to 48 hours after the estrogen surge detected by an ovulation predictor kit (Clearblue, Procter & Gamble, OH,USA) with 99% accuracy[32].

The subjects were given an ovulation predictor kit for home use and were instructed when to employ the predictor kit based on their menstrual history. For example, for a subject with an average 28-day menstrual cycle tested on days 13 to 15 of her cycle, ovulation testing was done at the same time of day. The procedure involved holding a test stick in the urine stream for 5 seconds or collecting the urine in a paper cup and dipping the test stick into the cup for 20 seconds. When a positive result occurred, as indicated by a smiley face on the test stick, the subject contacted the primary investigator to schedule data collection within the subsequent 24 hours.

1.2 Estradiol Serum Concentration

Estradiol serum concentration was analyzed using a Cobas e-602 (Roche/Hitachi ,Tokyo, Japan). On each day of testing, 5-7 cc of venous blood were drawn to assay serum levels of estradiol. The blood sample was obtained from the antecubital vein with a 21 gauge needle to yield a minimum of 500ul of plasma which was centrifuged at 1500 g for 2 minutes and 3000 g for 4 minutes. The centrifugation took place within the Roche MPA module. Specimens were stored in 2-8 degrees Celsius. The methodology was competition principle and the total duration of assay was 18 minutes. The mean intraassay concentration was 100.0 and the mean percentage of coefficient of variation (CV %) ranged from 3.4% to 3.7%. The mean (SD) of interassay was 100.0 and the mean percentage of CV ranged from 3.8 % to 7.4%. Assay sensitivity for the estradiol was 5 pg/ml.

1.2 Electromyography

Electromygraphy (EMG) activity was measured from the vastus medialis (VM), vastus lateralis (VL), medial hamstring (MH), and lateral hamstring (biceps femoris) (LH) in the thigh, of the dominant leg. Prior to electrode placement, the skin was lightly abraded, and cleaned with

alcohol. Circular pre-gelled 20 mm bipolar Ag–AgCl surface electrodes (EL503; Biopac Systems, Inc., Goleta, CA) were placed in parallel on the belly of each muscle in alignment with the direction of the muscle fibers and the distal tendon of each muscle with an inter-electrode distance of 20mm (according to standards provided by Seniam.org). The EMG electrodes were attached approximately parallel to pennation of muscle fibers half way between muscle insertion tendon and muscle belly to the vastus medialis and vastus lateralis. Electrode placement for the vastus medialis bisected the muscle anteroposteriorly, and was at a point distal from the motor point of the muscle half way to the insertion of the quadriceps tendon. The VL electrode location was centrally in a mediolateral fashion and distal from the midpoint of the belly to the tendinous junction. The MH electrodes were placed over the muscle belly half way between the ischial tuberosity and the tibial insertion point, at least 5 cm proximal to the musculotendinous junction. The LH electrodes were placed over the biceps femoris muscle halfway between the ischial tuberosity and the fibular insertion site, and a minimum of 5 cm proximal to the musculotendinous junction. A reference electrode for the EMG system was placed over the tibia. All electrodes were placed by a single experimenter to insure consistency thorough the study. Electrodes and telemetry amplifiers were secured to the skin using medical tape to minimize movement artifacts and to prevent the electrodes from losing surface contact due to sweating. Maximum voluntary contraction test were conducted for each subject. The MVC test for the vastus lateralis and vastus medialis muscles were performed while the subjects was in a sitting position with the knee flexed at 90. The MVC test for the biceps femoris and medial hamstring muscles were performed while the subject was in a prone position with the knee flexed at 30. During the MVC tests, the subject was instructed to perform three 5 second maximum voluntary isometric contractions for each selected muscle against the resistance of the same tester and was

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given verbal encouragement whilst doing so. The middle two seconds of the MVCs of each contraction were analyzed. A 3 min rest period was allocated between each contraction. Surface EMG was recorded using Biopac Inc., Goleta, CA. Acknowledge 4.3.1. The electromyography was recorded using a sampling rate of 2000 Hz through a 24 bit A/D converter. The raw data were processed using a band-pass filter (15-150 Hz). The EMG was integrated then divided by the maxmuim voluntary contraction (MVC) to normalize the EMG activity of every participant. Muscle activities were analyzed by the method described by Besier et al [33] , in the following conditions: (A) the preactivation phase: 50 ms before foot landing till foot landing; (B) the weight acceptance and (C) the peak push-off phase (Fig.1). The EMG activity of the selected group of muscles were synchronized with High Frame Rate Camera (CAM-HFR-A) SVHS Sony video camera (Basler, Biopac Systems, Inc., Goleta, CA) to capture the running phases as series of videos at 100 FPS (640*480 resolution). The camera was mounted on a tripod placed 2 m from the treadmill and aligned so the plane of the camera was parallel to the treadmill. The camera was leveled using the bubble level attached to the tripod and set to the height of the subject knee during running.

Insert Fig 1. here

1.3 Assessment of knee laxity

To quantify knee joint laxity, we utilized the KT-2000 (MEDmetric1 Corporation, San Diego, CA) instrumented knee arthrometer to measure anterior tibial translation (ATT) during the application of 133 N (30-Ib) anterior displacement force. Subjects are tested in the supine position in 30 degrees of knee flexion with 15 to 25 degrees of external rotation while the femur and tibia are supported by leg holders. The device was then placed on the anterior aspect of the leg and secured in place with circumferential straps. A strain gauge bridge arranged in a load cell

was used to measure the force necessary to generate an anterior glide of the proximal end of the tibia on the femoral condyles. This generated a force versus displacement curve for the anterior cruciate ligament. The process was accomplished by supporting both limbs with a firm, comfortable platform placed proximal to the popliteal space to keep the subject's knee flexion angle constant for the duration of the test (fig.2). Along with this device, a foot support accessory supplied with the ARTHROMETER® positioned the feet symmetrically allowing the leg position to be optimal for the test while reducing external rotation. For the most comfortable position during the flexion angle test, knee flexion angle was initially at 25° and the only movement was the tibia in relation to the patella. A thigh strap controlled hip external rotation while offering support to help relax the subject. Force used for the experiment was applied at 30 lbs (133 N). The force displacement data were plotted on an $X–Y$ plotter. The vernier caliper was used to measure anterior tibial translation (ATT) on the graph. The reliability of the KT 2000 has been has been established by , Van Lunen et al., reported an intraclass correlation coefficient of r = 0.92 (p = 0.001) [34].

Insert Fig 2. here

Procedures

Subjects were instructed to begin using an ovulation Predictor kit (Clearblue, Procter & Gamble, OH,USA) with 99% accuracy [32] on day 13 to 15 of their menstrual cycle, and were asked to report to the research study coordinator the day the test became positive. The day of ovulation was confirmed to insure an ovulatory menstrual cycle had occurred; provide a common reference point by which to counterbalance participants and to mark the beginning and ending of data collection; and to provide indirect confirmation that female subjects were not pregnant. Hormone assays, neuromuscular testing, and knee joint testing were performed at Loma Linda University.

Subjects were tested twice across one complete menstrual cycle, undergoing the same data collection procedures on each day of testing. Testing was performed in the morning (8:00 A.M.– 12:00 P.M.) to obtain the most stable concentrations [35] and to control for diurnal fluctuations in hormone levels. Within this window, every attempt was made to bring subjects in at the same time each day. However, some flexibility was needed to accommodate participant's class and work schedules given the daily data collection requirements. Subjects were counterbalanced to begin and end data collection either at ovulation (ovulation kit detecting the estrogen hormone surge), or the onset of menses (self-report of the first day of menstrual bleeding). Although each subjects was familiar with treadmill running, each had adequate time to become accustomed to treadmill running prior to the introduction of the experimental measurments. Subjects then were asked to complete a standardized 6 min running session on a Zebris FDM-T instrumented treadmill (zebris Medical GmbH, ISny Germany) with 0 inclination at 10Km/h with heel strike pattern. The treadmill had an embedded pressure mat containing more than 15,000 pressure sensors, from which data were integrated to produce the vertical ground reaction force to measure the ground reaction force. Once the runners demonstrated a stable running pattern, data were sampled at 200 Hz for 10 seconds. Lastly the ACL laxity was measured by the KT2000.

Data Analysis

A power analysis was conducted using an effect size of 0.75 a probability of type I error of 0.05, and a power of 0.80. This analysis indicated that a sample size of 12 subjects would provide a statistical power of 80 (G*Power v3.0.10 free software). The data was summarized using means and the standard deviations of the hormonal concentration, normalized EMG, and laxness of the ACL during each phase. Normality of the quantitative variables was confirmed using Kolmogorov–Smirnov test. A Paired T test was conducted to compare the changes in mean

estradiol serum concentration, ACL laxity and EMG activity between follicular and ovulatory phase. Statistical analysis was performed using SPSS for windows version 20. The level of significance was set at $\alpha \leq 0.05$.

3. Results

The data of one subject was discarded from the analyses after examining her hormonal assays because of a significant deviation from the normal expected hormonal profile for enumenorrhic women, with hormonal profile irregularities of low estrogen level $< 5Pg/ml$ [3].

3.1 Hormonal Profile

Descriptive data about the menstrual phases indicated typical values [3] including days between cycles (28.8±1.1) length of menstruation (6.3±1.2). Descriptive data for blood assay verified the menstrual cycle phases indicated that all subjects included in the statistical analysis were in the correct phase of the menstrual cycle. 17β estradiol serum concentration was significantly higher in the ovulatory compared with the follicular phase (P<0.001). The lowest estradiol concentration was found during menstruation (34.14 ± 15.47 pg/ml) and the highest estradiol concentration was found during ovulation (207.74 ± 53.42 pg/ml). Table 2 Fig.3a

Insert Table 2 here
Insert Fig 3a here

3.2 Anterior Cruciate Ligament Laxity

Laxity of the anterior knee ligament was measured by the anterior tibial translation (ATT). There was significant difference in the ATT between the follicular phases and the ovulatory phase of the menstrual cycle (p<0.01). The greatest ATT was found during ovulation (4.18 ± 0.27) and the least ATT was found (5.75 ± 0.47) during follicular phase (Table 2; Fig.3b)

Insert Fig 3 b here

3.3 **Neuromuscular Control variables**

The results of this study demonstrate differences in muscle activation strategies during different phases of menstrual cycle. A summary of the activation values are presented in table 3.

Quadriceps Muscle Activity

The quadriceps muscle exhibited increased activity during the early follicular phase compare to the ovulatory phase in the precontact and weight acceptance phase of running (p= 0.02, 0.04 respectively) (Table 3, Fig 4A). The lateral and medial quadriceps were analyzed separately. For the lateral quadriceps, a significant increase was observed during the follicular phase compared with ovulatory phase (p=0.014) (Table 3; Fig. 4B). Remarkably, females subjects demonstrated a significant decrease in medial to lateral quadriceps ratios during follicular phase compared to ovulatory phase ($p < 0.001$) during weight acceptance phase (Table 3; Fig. 5). Fig. 4a shows typical data of increased IEMG activity of the VL and VM muscles of a single subject during the follicular phase and fig 4b showed the decreased activity of the two vasti during the ovulatory phase. As shown in the figure, the raw EMG muscle activity was greater in the follicular phase than the ovulatory phase. Below each raw EMG is the integrated EMG showing the same phenomena.

Hamstring Muscle Activity

The ovulatory phase altered the hamstring muscle preactivity before impact. The average peak hamstring activity during the precontact and weight acceptance phase was significantly increased during ovulation compared with the early follicular phase (Fig. 5B; Table 3). Specifically the medial hamstring showed increased activity before impact during the ovulatory phase compared to the follicular phase (p <0.001) (Fig. 5A; Table 3). The increased activity of the hamstring was also observed during weigh acceptance with increased EMG amplitude ($p < 0.001$). Moreover

the quadriceps hamstring cocontraction was significantly higher compared to the follicular phase (p<0.001) (Fig. 5C; Table 3).

Insert Fig 4a here

Insert Fig 4 b here

Insert Fig 5A here

Insert Fig 5B here

Insert Fig 5 C here

Insert Table 3 here

Discussion

The physical disability and long rehabilitation process associated with anterior cruciate ligament (ACL) injury can be both psychologically and financially devastating to the individual, ultimately resulting in a decreased quality of life. Female athletes have a higher rate of ACL injury than do men, and many of these injuries require extensive surgical and rehabilitative interventions, with a financial burden to the American healthcare system estimated to approach $650 million annually [16]. Bearing that in mind, it is imperative to understand the mechanisms leading to such an injury in an effort to prevent its occurrence and its subsequent sequelae. Although both men and women are susceptible, the literature states that women have a 4 to 6 fold increased incidence of ACL injury [29,36].

While the increased incidence of serious knee injuries in female athletes is well established, the underlying neuromuscular mechanisms related to the elevated ACL injury rate has yet to be delineated. Maintenance of joint congruency is important in prevention of injury. Both the ligamentous structures and the muscular system contribute [37]. The role of the muscular system is particularly important when the static restraints are jeopardized and therefore not providing restraint to abnormal motion within the joint.

Our study supports the previous studies which have reported a greater knee laxity during ovulation when estrogen levels are high [12,38]. Conversely, women who experienced high plasma concentration of estrogen experienced a marked increase in joint laxity behavior following peak ovulatory levels. However other studies [34,39] found that knee ligament laxity doesn't differ by menstrual cycle day. Interestingly to note that in these studies, that did not identify changes in knee laxity across select days of the menstrual cycle. The average estradiol levels were near the upper limits of normal ranges at menses (56 and 73 pg/mL) and considerably below the normal ranges postovulation (137 and 120 pg/mL) using similar hormone assay.

The results of our study reveal differences in muscle activation strategies during different phases of the menstrual cycle. Our results showed that women place greater reliance on their quadriceps during the follicular phase to modulate the torsional joint stiffness about the knee joint during running. The increased quadriceps activity observed during the follicular phase was associated with decreased hamstrings activity. We speculate that the observed differences in neuromuscular recruitment strategies may have implications for the greater incidence of non-contact ACL injures observed in women.

A consequence of differences in neuromuscular activation patterns might be injury susceptibility. Markolf & colleagues, found that muscle activation about the knee increased valgus stability threefold, highlighting the influence of the muscular system on knee stability [40]. Previous investigations of neuromuscular control [41,42]; have not considered muscle activation patterns during running. The present study discovered that the quadriceps and hamstring cocontraction ratios decreased during the early follicular phase compared to ovulatory phase. This suggests a different co-contraction (onset timing of agonist/antagonist around a joint) mechanism between these muscles. This alteration in neuromuscular control may explain the

non-significant knee valgus variable since the quadriceps and hamstring work together to control torsional motions of the femur and tibia that may contribute to valgus alignment of the knee [43]. This co-contractive mechanism suggests a different neuromuscular control pattern when estrogen levels are low; however, more investigation is necessary.

While we are unable to find another published study that evaluated neuromuscular activation patterns in healthy females runners with non-pathological knee elasticity, our findings are surprisingly consistent with those demonstrated in ACL deficient individuals. Alkjaer & colleagues, reported a marked increase in hamstring coactivation towards more extended joint positions in ACL deficient subjects (Alkjaer et al., 2012). Notably, this progressive rise in coactivation may reflect a compensatory strategy to provide stability to the knee joint in the anterior–posterior plane during knee extension. In agreement, our investigation showed that participants with increased knee joint laxity during ovulation demonstrated increased levels of muscle preactivity in the hamstring muscles before impact as well as during weight acceptance phase. Coactivation of the hamstring muscles during dynamic knee extension may compensate for increased knee joint laxity in anterior cruciate ligament. Increased coactivation of the hamstring muscles has been suggested to provide a compensatory strategy to reduce Anterior tibial translation (ATT) in functional conditions that include knee extension [44] [45]. Several studies have shown that the hamstring muscles are active during submaximal and maximal quadriceps agonist contraction [46] and that coactivation of the antagonist hamstring muscles during knee extension effectively reduces the amount of ATT [45,47].

The primary purpose of the current study was to investigate whether estradiol fluctuation during the menstrual cycle has an influence on the neuromuscular control around knee joint

mechanics during running. Previous studies investigating the relationship between knee joint mechanics and the menstrual cycle found significant changes in biomechanical [1] or neuromuscular [12] characteristics corresponding to changes in hormonal levels during the menstrual cycle. The present study also found significant changes in the neuromuscular control around knee joint between the different phases of the menstrual cycle. As a result, ACL injury in female athletes may not be explained simply by the hormonal cycle but is likely influenced by a more complicated and indirect injury mechanism incorporating hormonal fluctuations and dynamic knee joint function that may be individual specific.

Although, we are currently unaware of any other study in the literature that investigated the neuromuscular control variables presented here in females' runners. Previous studies had evaluated gender differences in neuromuscular control. Our study showed that females' runners use a different neuromuscular strategy during different phases of menstrual cycle. Subjects demonstrated a decreased ratio of medial quadriceps to lateral quadriceps recruitment. A preactivation difference did exist for the lateral quadriceps between the follicular and ovulatory phase. The decreased ratio of the medial quadriceps musculature recruitment may be related to decreased control of coronal plane forces at the knee [48].

In addition to low ratio of medial to lateral quadriceps recruitment combined with increased lateral hamstring firing may compress the lateral joint, open the medial joint and increase and increase shear force, which directly loads ACL. This disproportional recruitment of the quadriceps musculature increases anterior shear force at the low knee flexion angles that occur during landing. The quadriceps, through the anterior pull of the patellar tendon on the tibia, contributes to ACL loading when knee flexion is less than 30 degrees [48]. Of interest is that our participants demonstrated increased activity of quadriceps muscles during the follicular phase

compared to the ovulatory phase, which is thought to maximize axial compression, joint congruency and frictional forces to effectively limit joint displacement. Muscular co-contraction compresses the joint, due in part to the concavity of the medial tibial plateau, which may protect the ACL against anterior drawer. However, Zazulak & colleagues reported greater peak quadriceps activity in female than male subjects [49]. Decreased balance in strength and recruitment of the flexor relative to the extensor musculature may put the ACL at greater risk [50]. Adequate cocontraction of the knee flexors is needed to balance contraction of the quadriceps, compress the joint, and control high knee extension and abduction torques [50]. Appropriate hamstrings recruitment may prevent the critical loading necessary to rupture the ACL during maneuvers that place the athlete at risk of an injury. Female subjects may display a longer latency period that is, electromechanical delay between preparatory and reactive muscle activation. Preparatory muscle activity can stiffen joints before unexpected perturbations. Neuromuscular training that reproduces loads similar to those encountered during competitive sports may assist in the development of both feed forward and reactive muscle activation strategies that protect the knee joint from excessive load [51-53].

Our findings support the previous studies which have reported a decreased neuromuscular response and/or control around the time of menstruation [12,54]. Despite these observations, our findings of decreased neuromuscular control around the knee which may be a potential mechanism for increased risk of injury at this stage of the cycle, there is no consensus as to whether injury risk is also elevated during menstruation [29]. These conflicting results could be due to the difficulty in performing a prospective study to assess injury risk, with the majority of protocols consisting of retrospective assessment [29,55]. These results can also be confounded by participation levels during each phase of the cycle, because women who do not take the oral

contraceptive pills are significantly less inclined to participate in physical activity during menstruation [56]. Taken together, these results suggest that during menstruation the performance of the neuromuscular system is compromised, which may limit both participation and intensity of activity in sporting events and therefor counterbalance the increased risk of injury due to an impaired motor control strategy.

Decreased neuromuscular control of the lower extremity during menstruation may increase the potential for valgus lower extremity position and increased ACL injury risk. Identification of these neuromuscular imbalances has potential for both screening of high risk athletes and targeting interventions to specific deficits. Dynamic neuromuscular training can increase active knee stabilization and decrease the incidence of ACL injury in the female athletic population [57,58]. Training may facilitate neuromuscular adaptations that provide increased joint stabilization and muscular preactivation and reactive patterns that protect the female athlete's ACL from increased loading. Neuromuscular training will help female athletes adopt muscular recruitment strategies that decrease joint motion and protect the athletes ACL from the high impulse loading while also improving their measures of performance. More investigation is necessary to determine if the neuromuscular control changes occur due to alterations in force transmission properties of passive tissues, levels of feedback from ligamentous and dynamic tissues, levels of feedback from ligamentous and dynamic tissues or centrally driven feed forward mechanism.

Utilization of a relatively simple task (running) may not adequately stress the neuromuscular system at the level of athletic population, thus limiting the generality of the results to athletes. Also, the current protocol didn't measure the muscle activation at high speeds which may limit the applicability of our findings to conditions of higher speed joint loading. In

addition, we examined only the quadriceps and hamstring and we didn't measure other lower extremity musculature that may influence knee joint mechanics. Also, the absence of randomized order of measurement is a threat to the validity of the study.

Conclusions

This study is the first to examine the influence of the menstrual cycle on knee joint laxity and neuromuscular control around the knee during constant velocity running. Our results suggested that decreased knee joint laxity during the menstrual cycle leads to decreased neuromuscular control during running. A consistent pattern was observed in the firing of the quadriceps muscle recruitment pattern throughout the menstrual cycles associated with unbalanced hamstring recruitment. In addition to low ratio of medial to lateral quadriceps recruitment combined with increased lateral hamstring firing which may compress the lateral joint, open the medial joint and increase shear force, which directly loads ACL. This disproportional recruitment of the vastus musculature increases anterior shear force at the low knee flexion angles which may increase the potential for valgus lower extremity position and possibly increased risk of ACL injury.

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