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Sex Hormones and Neuromuscular Control System

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1. Introduction

There is evidence that females sustain more exercise-related musculoskeletal injuries than males. Sex differences in injury rates are apparent in some connective tissues such as ligaments. Although girls and boys have an equal chance of ligament injuries prior to adolescence, girls have a higher rate immediately after maturation (Tursz et al., 1986). Female athletes participating in cutting, jumping and pivoting sports have a 4-6 times greater chance of ACL tearing than their male counterparts (Arendt et al., 1999).

Ligament, tendon, bone and endometrium contain estrogen receptors responsive to female sex hormones. Estrogen has direct effect on soft tissue strength, muscle function and collagen metabolism and behavior. It is demonstrated the influence of female endocrinology on knee joint behavior, as male and females differ substantially in the type, level and periodic exposure of circulating sex hormones after puberty. While hormone levels remain fairly constant in males, females are exposed to rhythmic fluctuations in endogenous hormones during the course of menstrual cycle. The absolute levels of estrogen and progesterone varying considerably during the course of a female's menstrual cycle and there are some variations in the hormonal levels. At the beginning of the menstrual cycle, estrogen (E) and progesterone (P) remain close to their minimum levels. Toward the middle of the cycle, estrogen level rises and in the middle of the luteal phase both E and P levels increase.

Also, estrogen indirectly influences the female neuromuscular system. Neuromuscular patterns in males and females differ during maturation. Males demonstrate power, strength and coordination increasing correlate with their age and maturational stage, whereas girls show little change throughout maturation (Kellis et al., 1999; Beunen et al., 1988). In females, quadriceps strength increases and you know it can increase the tibia anterior translation and subsequently the rate of ACL injury. The reliance of males on a more hamstring dominant strategy may be more protective of the ACL because the hamstring and ACL are agonist to prevent anterior displacement of the tibia on the femur. Also, females have shown more impaired proprioception assessing knee motion into extension than males (Rozzi et al., 1999). Significant slowing of muscle relaxation also occurs during the ovulation (estrogen surge) of the menstrual cycle. Serum estrogen concentrations fluctuate radically throughout the cycle and estrogen has measurable effects on muscle function and tendon and ligament strength.

Moreover Estrogen has effects on the central nervous system. It has demonstrated differences in skill performance in females during different phases of the menstrual cycle, also a decrease in motor skills in the premenstrual phase at the late luteal. These data

indicate that estrogen can be effective on neuromuscular function which may facilitate the potential for neuromuscular imbalances to develop in female athletes. There is also evidence that estrogen influences electrical activity of neurons both centrally and peripherally (Lee et al., 2002; Papka et al., 2001; Rozzi et al., 1999). Furthermore, postural control impairments have been demonstrated in females with premenstrual symptoms in the mid-luteal phase (Friden et al., 2003). These findings support the hypothesis that lower extremity neuromuscular performance may be influenced by circulating sex hormones.

Because of existing estrogen and progesterone receptors on the human ACL fibroblasts, females' sex hormones may directly influence the structure and composition of the ACL. Some researchers have indicated different cycle phases for increased incidence of ACL injury. Some of them found that females have a greater risk of ACL injury during the ovulatory phase of the menstrual cycle in which estrogen surge is present (Wojtys et al., 1998). However, the others implicated a significant greater number of ACL injuries occurred onset of the cycle (days 1 and 2) (Slauterbeck et al., 2002). They believed cyclic changes in estrogen and progesterone may change expression of genes encoding tissue-remodeling enzymes and proteins, which in turn could favor either net tissue degradation or repair at specific times during the menstrual cycle.

In some studies, researchers have suggested that the large number of non-contact ACL injuries in female athletes may be related to knee laxity, which might be influenced by hormones. In normal menstruating females, significant increases in knee laxity have been noted in the pre-ovulatory and mid luteal phases of the menstrual cycle compared to menses. This is believed to coincide with elevated levels of estrogen, and estrogen and progesterone respectively. Although hormonal changes might result in increased injury risk for female athletes, the effects of hormonal level on occurrence of injury in these athletes are not fully understood.

In addition to sex hormones receptors, there are mechanoreceptors in human ACL. These mechanoreceptors are referred to as Ruffini receptors, Pacini receptors, Golgy tendon organ-like receptors and free nerve endings that they may have a proprioceptive function (Adachi et al., 2002). Proprioception is an important part of neuromuscular performance, and can be defined as the individual's awareness of his or her extremities' position and motion in space. It involves sensory activities of the tendons, ligaments, capsules and muscles, and can be internal peripheral areas of the body that contribute to postural control, joint stability and conscious sensation of movement. Position sense at the knee joint is influenced by central and peripheral mechanisms, such as muscles, tendons, articulate, cutaneous and ACL receptors (Shultz et al., 2005). The ACL have two complementary functions: mechanical and sensory (proprioception) (Ekenros et al., 2010). It has been implicated that sensory information from the ACL assists in providing functional stability to the knee joint by contributing to neuromuscular control (Riemann et al., 2002).

Since the relationship between ACL injury and female hormone concentration and also, the relationship between ACL injury and neuromuscular control (proprioception) deficit is supported by recent studies. This chapter is going to investigate the relationship between female hormonal level and proprioception, by measuring the knee joint position sense (JPS) and serum estrogen and progesterone concentrations throughout the menstrual cycle.

2. Neuromuscular control and proprioception

Neuromuscular control of the knee is defined as the unconscious response to an afferent signal concerning dynamic knee joint stability. These afferent signals are produced by

Proprioception which refers specially to conscious and unconscious appreciation of joint position, kinesthesia (the sensation of joint motion or acceleration) and the perception of force (an ability to estimate joint loads). These signals are transmitted to the spinal cord via afferent (sensory) pathways. Conscious awareness of joint motion, position and force is essential for motor learning and the anticipation of movements in sport, while unconscious proprioception modulates muscle function and initiates reflex joint stabilization. The efferent (motor) response to sensory information is termed neuromuscular control (Prentice 2011). The deficit of neuromuscular control of the knee joint may be responsible for the high rate of knee injury in female athletes (Hewett et al., 1996; Huston et al., 1996). The central nervous system (CNS) is the primary mediator for the perception and execution of musculoskeletal control and movement. Perception and sensation of joint movement are monitored by tree main subsystems: 1) the somatosensory system, 2) the vestibular system, and 3) the visual system. The somatosensory system often referred to as proprioception, receives input from peripheral articular and musculotendinous receptors concerning changes in muscle length and tension, in addition to information regarding joint position and motion. The vestibular system receives information from the vestibules and semicircular canals of the ear, which aid in keeping the body in balance, while the visual system provides the body with visual cues, contributing to balance by providing reference points for orientation. Information gathered by the somatosensory system, in addition to that from vestibular and visual systems, is processed at three distinct levels of motor control: the spinal level, the brain stem and the higher centers such as the motor cortex, basal ganglia, and cerebellum.

Fig. 1. Neuromuscular control pathway (reproduced from Lephart 1998)

Proprioception and neuromuscular control may be assessed clinically through evaluation of the afferent and efferent neuromuscular pathways. The afferent pathway is qualified through the examination of joint kinesthesia and joint position sensibility, which provide the researcher with a measurement of conscious appreciation of joint motion and position sensibility. Joint kinesthesia is determined clinically by establishing the threshold to detection of passive motion, an assessment of the ability to detect relatively slow passive joint motion. Testing joint position sense, another method of assessing the afferent pathway, determines the ability of the subject to comprehend a presented joint angle and then once

removed; actively or passively reproduce the joint angle. Assessment of the efferent pathway is conducted through measurements of balance and muscle activity, which provide a direct determination of the efferent response to afferent stimulation. (Lephart 2000)

3. Hormonal effects on ACL

Sex hormone fluctuations have been associated with tissue alterations and an increased incidence of non-contact ACL injuries among female athletes (Wojtys et al., 2002). Estrogen and progesterone receptors have been detected within the ACL (Liu et al., 1996). Several researchers have suggested the relationship between estrogen peak levels and increased ACL laxity (Shultz et al., 2004; Slauterbeck et al., 2001). 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 (Raunest et al., 1996).

4. Hormonal effects on tissue

Estrogen receptors alpha and beta have been reported in skeletal muscle thereby providing a plausible tissue-based mechanism for influencing neuromuscular control and force transmission pathways (Huijing et al 2005; Wilk et al., 2005). In addition, research has not completely described the influence of sex hormone receptors in skeletal muscles on tissue mechanisms that can alter neuromuscular control (Dedrick et al., 2008).

5. Neuromuscular control mechanisms between sexes

Similar neuromuscular control strategies have been reported between Men and women during landing up until puberty. There is a link between hormonal fluctuations and changes in neuromuscular control, since alterations in hormonal levels constitute a primary change in development after puberty. Neuromuscular control strategies incorporated during a landing sequence appear to change in adult females, where increased knee valgus alignment places the ACL at greater risk of injury (Hewett et al., 2004).

6. Hormonal effects on neuromuscular control

Neuromuscular control patterns, such as fine motor activity and reaction time performance have been reported to fluctuate over the course of the menstrual cycle (Posthuma et al., 1987), with more consistent performance in women using oral contraceptive (OC). It was discovered that there is an increase in postural sway during single limb stance and threshold for detection of passive knee motion in the mid-luteal phase of the cycle (Friden et al., 2003, 2005). Improved neuromuscular coordination may occur in women taking OC with a reduced number of premenstrual symptoms. However, the relationship between fluctuations in ovarian sex hormone levels and neuromuscular strategies has not been fully described (Dedrick et al., 2006).

Neuromuscular patterns in males and females change substantially during maturation. Males demonstrate improve in power, straight and coordination with age that correlate with their maturational stage, whereas, girls show little change throughout maturation.

For example, in study by kelis et al vertical jump height demonstrated by boys increased steadily during maturation, but in girls it did not (Kellis et al., 1999; Beunen et al., 1988).

Neuromuscular control of the knee can be defined as the unconscious to an afferent signal concerning dynamic knee joint stability. The absents of neuromuscular control of the knee joint may be responsible for the increased rate of knee injury in females, but it is not normally measured with the 3 dimensional kinematic system described below and were recorded during the same laboratory evaluation (Lephart et al., 2000).

Changes in neuromuscular control of the knee in adolescent athletes are documented. Several studies have documented a substantial increase in neuromuscular strength and coordination following the growth spurt in adolescent boys but not in the average adolescent girls.

7. Normal menstrual cycle

Normal menstrual cycle in women includes fluctuation of sex hormones' levels in a regular duration that usually takes 21-35 days. Ovarian cycle of the normal menstrual cycle has 2 phases, follicular and luteal. Follicular phase is started by menstruation with 2-4 days bleeding.

Estrogen and progesterone levels are in the lowest level at menses but little by little estrogen increases and it goes up to its highest level just before the ovulation, at the middle of the cycle. Duration of the estrogen surge is so limit and it just takes 24 to 48 hours. After ovulation, both estrogen and progesterone levels go up slowly and they are in their highest level at the middle of luteal phas (Berek et al., 2002).

Fig. 2. Menstrual cycle phases (reproduced from Berek, 2002)

8. Procedure

Although, joint proprioception includes some parts, most of the studies have measured JPS as an important part of it. There are different methods for knee JPS evaluation. Some researchers have measured JPS with isokinetic dynamometer- Biodex in non weight bearing position (Aydog et al., 2005; Hertel et al., 2006). In the other studies, JPS was measured by reproduction of the target angle in standing position by using a system comprised of skin markers and digital photography (Stillman BC et al., 2001; Shultz et al., 2003; Shultz et al., 2005; Mir et al., 2008).

In our study the JPS was measured 3 times a month with different level of sex hormones. It was evaluated by reproduction of the target angle (30° flexion) in standing position and absolute angular error (AAE) was considered as a dependent variable.

Testing procedure was completed in an isolated room; the participants were asked to wear loose-fitting shorts. Since previous studies had shown there is no significant difference between the bilateral knee joints' position sense (Herington 2005), we chose the right leg as the tested limb. In all tests, visual cues were eliminated by a blindfold. Each participant was asked to lie down in supine position on the treatment plinth and four circular markers (4 cm in diameter) were attached to their leg at three locations: (1) proximal to a quarter of the distance along a line joining the greater trochanter to the lateral knee joint line, (2) over the fibular neck, (3) over the proximal part of the lateral malleolus. Then each subject asked to sit down (with hip and knee 90° flex), and the fourth marker was attached over the iliotibial tract adjacent to the superior border of the patella. The choice of marker locations was based on previous studies (Deie et al., 2002; Lamoreux et al., 1996). The participant then stood with their feet-shoulder width apart. The left foot was lifted from the floor. The right hand was placed over the chest, and the subject was allowed to use minimum contact of the fingertips in the left hand for balance. One goniometer was adhered onto the wall, out of sight of subject, at angle of 30° as an indicator.

Fig. 3. Measuring the knee joint angle in weight bearing position

9. Joint position sense (JPS) measurement

The movement capture system comprised of digital photography, skin markers and AutoCAD software was utilized for measuring joint angles during the joint position sense test. A digital video camera was located 185cm away from the subject and elevated 65cm from the ground. Photos were taken during movement and holding moments of the leg, respectively. After completing the procedure, the test and replicated angles were measured using the AutoCAD software.

To measure JPS in the knee joint, participants moved their limbs into flexion. Before the tests, each subject watched a video tape showing the squatting movement demonstrated by a trained person performing the movement at a controlled velocity and the subjects were asked to squat as shown in the film. We tried to control velocity approximately, because the JPS accuracy could be influenced by high or low velocity (Stillman et al., 2001). The starting position was knee straightening (0°). The subject stood with eyes closed, and was instructed to: (1) lift the unexamined foot (left limb) from the floor; (2) slowly flex the weight bearing (WB) limb (right limb) until told to stop ($\sim 30^\circ$); (3) identify the knee position while isometrically holding the test position for about 5 seconds; (4) return to the erect bilateral WB stance (for 7 seconds); and (5) reproduce the previous unilateral flexed position, concentrating on the knee. The holding times used in this study were based on previous studies (Hopper et al., 2003; Marks et al., 1994; Marks et al., 1993). Measurement of knee JPS was repeated three times and the average of the three measurements were calculated in each phase.

In some previous studies JPS was measured in NWB position that is not functional. For example, Aydog et al (2005) evaluated knee JPS throughout the three different phases of the menstrual cycle but they used Biodex System 3 dynamometer to measure knee JPS in a semi-horizontal position. Their method also stimulated cutaneous mechanoreceptors which have an important role on the knee JPS that may change the test accuracy.

In our study, JPS of the knee joint was evaluated in WB position, as it potentially provides more functional information.

10. Blood sampling

At the end of each session, venous blood samples (5 ml) were taken from a superficial forearm vein. The blood sample was allowed to clot at room temperature and then the serum was separated and stored to be analyzed. Serum estrogen and progesterone concentrations were measured with Elisa method by Elisa Reader (SLT model) in Endocrine Research Center lab.

Data was collected throughout the 3 phases of menstrual cycle i.e. early follicular phase (onset of menses), mid-follicular phase (the 7th to 9th days of the cycle) and mid-luteal phase (20th to 23th days of the cycle).

11. Results

11.1 Hormones

Estrogen concentration was found 22.81 (16.75) pg/ml at menses, 125.65 (84.82) at mid-follicular and 179.5 (94.35) at mid-luteal phase. Fig. 4 depicts estrogen concentrations across the menstrual cycle phases. Serum estrogen concentration was significantly higher during the mid-follicular and mid-luteal phases as compared with the early-follicular (menses) phase ($P=0.0001$). There was no significant difference between peak estrogen concentrations in the mid-follicular versus the mid-luteal phase.

Fig. 4. Estrogen concentrations across three phases of the menstrual cycle

Serum progesterone concentration was found 0.58 (0.62) at menses, 0.51(0.71) at mid-follicular and 7.35(5.87) at mid-luteal phase. These results show that serum progesterone concentration was significantly higher during the mid-luteal phase as compared with the menses and mid-follicular phases ($P=0.0001$). However there was no significant difference between peak progesterone concentrations in the menses versus mid-follicular phase. Fig. 5 depicts progesterone concentrations across the menstrual cycle phases.

Fig. 5. Progesterone concentrations across the menstrual cycle

12. Joint Position Sense (absolute error of repositioning)

We found females' knee JPS accuracy changes in different phases of menstrual cycle. The greatest amount of mean (SD) value of absolute error was at menses and the least amount of it, was at mid-luteal phase. This finding is also in agreement with previous study that indicated the active knee JPS was significantly reduced during menstruation (Aydog et al., 2005).

	menses	Mid-follicular	Mid-luteal
AAE means	4.18 (2.13)	3.65 (2.78)	2.51 (1.66)

Table 1. The value of absolute angular error across the menstrual cycle

Pearson correlation test showed a negative correlation between AAE and estrogen level with no significant relationship ($r= -0.275$, $p= .058$). But, spearman correlation test indicated a negative correlation between AAE and progesterone level with a significant relationship ($r= -0.370$, $p= 0.010$). Therefore, the highest level of JPS error was related to the lowest level of estrogen and progesterone. Also, the lowest level of JPS error was related to the highest level of sex hormones. While hormones' levels increase, the knee JPS error decreases. Because of the significant relationship between progesterone and AAE, it seems this hormone has the main effect on the knee JPS accuracy.

Fig. 6. The linear correlation between estrogen and absolute angular error

Fig. 7. The linear correlation between progesterone and absolute angular error

13. Discussion

Previous authors report different results regarding changes in knee joint proprioception across the menstrual cycle. The main observation of our study was that higher value of reposition error was found in the menses and the participants made less error in reproduction of joint position sense in two other phases and they were more accurate during the luteal phase. While sex hormones' levels increase, the knee JPS error decreases. The significant relationship was between progesterone and absolute angular error and it seems this hormone has the main effect on the knee JPS accuracy.

These results are due to measurable effects of estrogen on muscle, tendon and ligament strength and function (Sarwer et al., 1996). Estrogen also has influence on the central nervous system and females have different skill performances during different phases of the menstrual cycle (Lebrun et al., 1994). Some researchers demonstrated a decrease in motor skills around the menstruation and this showed that estrogen may influence on neuromuscular function (Posthuma et al., 1987). It is indicated that ACL injuries happened most frequently on day 1 and 2 of menses and it isn't random but occurs usually around the time of menses, when circulating sex- hormones levels decrease and after both estrogen and progesterone elevation (Slauterbeck et al., 2002). In addition, it is believed that proprioception can be influenced by emotional and environmental conditions and because of females' behavioral and emotional character changing in early menstruation increasing in error of knee JPS in menses can be described. Aydog et al (2005) also indicated the active knee JPS was significantly reduced in the menstruation compared to follicular and early luteal phases. They believed that changes in proprioception might be a consequence of changes in distal latency or excitability of the mechanoreceptors.

On the other hand, it is observed that knee laxity exists more in mid-follicular and luteal phases of the females' menstrual cycle. Females with increased knee laxity are less sensitive to joint displacement or loading, and are more reliant on active control of the gastrocnemius and biceps femoris muscles to potentially compensate for reduced passive instability (Shultz et al., 2004; Park et al., 2007).

In another study, some researchers demonstrated increased knee laxity was observed during ovulation (after estrogen surge) but no significant changes in knee mechanics corresponding to menstrual phases were found. They also found knee laxity correlates positively with knee joint loads. They suggested that increased knee joint laxity during menstrual cycle leads to greater knee joint loads in selected high risk movements in healthy young females (Park et al., 2009).

Although, some researchers said knee joint laxity may not explain the higher incidence of females' ACL injury, they suggested that muscle strength and dynamic stability are more important (Bowerman et al., 2006). Some others measured knee JPS with isokinetic dynamometer in sitting position with high skin stimulations and suggested passive joint position sense and joint laxity don't change across the menstrual cycle (Hertel et al., 2006). It is also indicated that ligament laxity does not affect the proprioceptive function of the knee, and it may compensate with muscle contraction (Adachi et al., 2002). These findings are consistent with Johanson's Final Common Input Theory. Based on this theory, due to the joint-tendon-muscle relationship, muscle spindles act with joint afferent information and then send final common signal. As the highest knee joint laxity has been observed in the luteal phase, in the current study the most JPS accuracy can be described.

However, some other researchers didn't find the highest level of laxity in luteal phase, and suggested antagonistic role for progesterone and estrogen. But they found no significant difference of laxity between mid-follicular and luteal phases (Park et al., 2007).

Therefore, according to progesterone effect on knee laxity in the mentioned studies and our finding about its influence on JPS, we suggest progesterone and estrogen are synergies in knee JPS accuracy. Hence we suppose that elevation of both these hormones in luteal phase is the reason of knee joint absolute angular error decreasing and knee JPS accuracy increasing.

The findings of our study are strengthened by controlling many confounding variables such as age, menstrual regularity, previous injuries and length of daytime because of its influence on females' sex hormones (Speroff et al., 2005). We collected all our sampling in one season with almost the same day length during the study. Moreover, we started our study with every participant being in their follicular phase (the menses) and we used the same sequence for all the participants. None of our subjects wasn't pregnant and didn't use oral contraceptive in their 3 recent months because of its effects on their knee joint performance (Lebrun et al., 1994) and it could influence on their postural control (Ekenros et al., 2010). We used skin marker to reduce the skin stimulation and we also used AutoCAD software to measure the knee angles as it has high test-retest reliability. Therefore our JPS test can be considered highly accurate. In the current study, JPS of the knee joint was evaluated in WB position which potentially provides more functional information (Baker et al., 2002).

14. Conclusion

It is concluded that sex hormones, especially progesterone can influence on accuracy of the knee JPS in healthy female athletes. Their reduction at menstruation can reduce the knee JPS accuracy and increase the knee joint injury probability. Knee JPS accuracy decreases in menses, when circulating sex- hormones levels are low and after a time when both were elevated.

As such, Female athletes are at risk of ACL tearing during menstruation. Further studies are needed to investigate the effect of some devices or some ways to protect the knee joint during this high risk time.

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Sex Hormones not only regulate reproductive function, but they also play a prominent role in the biology and physiology of several organs/tissues and in the pathophysiology of several diseases. During the last two decades, the information on the mechanisms of action of sex hormones, such as estrogens and androgens, has rapidly evolved from the conventional nuclear receptor dependent mechanisms to include additional non-nuclear, non-genomic and receptor-independent mechanisms. This highlights the need to update the current knowledge on sex hormones and their mode of action. Increasing evidence that exogenous/epigenetic factors can influence sex hormone production and action highlights the need to update our knowledge on the mechanisms involved. This book provides a systematic and updated overview of the male/female sex-hormones and their impact in the biology and physiology of various organs. Additionally, the book discusses their positive and negative association with the pathophysiology of various diseases (e.g. osteoporosis, cardiovascular-disease, hypogonadism, reproduction, cancer) and their therapeutic potential.

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