

From the Departments of Surgical Sciences and Woman and Child Health, Karolinska Institutet and the University College of Physical Education and Sports, Stockholm, Sweden.

# Neuromuscular performance and balance during the menstrual cycle and the influence of premenstrual symptoms

Cecilia Fridén



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# ABSTRACT

Several studies have demonstrated a higher incidence of sports related knee injuries among female athletes compared to their male counterparts regardless of exposure time and activity level. Different intrinsic and extrinsic factors have been proposed as risk factors in women. The hormonal variation during the menstrual cycle has been suggested as one of the intrinsic factors and an association between sport injuries in female athletes and the menstrual cycle has been reported. An increased injury rate has been observed during the premenstrual and menstrual phase, whereas one author reported a higher incidence during the ovulatory phase. It has been suggested that impaired neuromuscular function due to the specific hormonal influence of the menstrual cycle contributes to the association between sport injuries and different phases of the menstrual cycle. Furthermore, women with premenstrual symptoms (PMS) have been shown to be at greater risk of injury compared to women without PMS.

The overall aim of this work was to explore if and how neuromuscular performance and balance vary across three well defined and hormonally confirmed phases of the menstrual cycle in moderately active women and to study if PMS can affect performance.

Muscle strength and endurance, knee joint kinesthesia, neuromuscular coordination and postural control were measured in the early follicular phase (FP), the ovulatory phase (OP) and the mid luteal phase (LP) of the menstrual cycle. Menstrual cycle phases were determined by analysis of sex hormone levels in serum and ovulation was detected by the luteinising hormone surge in urine. A prospective rating of PMS was used to divide the subjects into two groups, one with PMS and one without.

No significant variations in muscle strength or muscle endurance during the menstrual cycle were detected. An impaired knee joint kinesthesia was detected in the LP and women with PMS showed an overall greater threshold for detection of passive movements than women without PMS. Neuromuscular coordination was significantly improved in the OP compared to the other phases. A significantly altered postural control was detected in the LP in the PMS group, but no differences were shown between phases in the non-PMS group. There was no significant difference in hormone levels between the PMS group and the non-PMS group and there were no correlations between hormone levels and the variables tested.

The results of this thesis demonstrate a significant variation in knee joint kinesthesia, neuromuscular coordination and postural control during the menstrual cycle, while no differences in muscle strength or endurance were observed. Impaired neuromuscular performance and balance may contribute to the increased incidence of sports injuries in female athletes. Further studies are needed to clarify the precise mechanisms for menstrual cycle related variation in neuromuscular performance and postural control.

Key words: estrogen, progesterone, muscle strength, kinesthesia, coordination, postural control

# LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals (studies I-IV):

- I** Fridén C, Lindén Hirschberg A, Saartok T. **Muscle strength and endurance do not significantly vary across 3 phases of the menstrual cycle in moderately active premenopausal women.** *Clinical Journal of Sport Medicine* 2003 Jul; 13(4): 238-41.
- II** Fridén C, Lindén Hirschberg A, Saartok T, Bäckström T, Leanderson J, Renström P. **The influence of premenstrual symptoms on postural balance and kinesthesia during the menstrual cycle.** *Gynecological Endocrinology* 2003 Dec; 17(6): 433-439.
- III** Fridén C, Lindén Hirschberg A, Saartok T, Renström P. **Performance of knee joint kinesthesia and neuromuscular coordination vary significantly across three different phases of the menstrual cycle in moderately active women.** Submitted
- IV** Fridén C, Ramsey DK, Bäckström T, Benoit DL, Saartok T, Lindén Hirschberg A. **Altered postural control during the luteal phase in women with premenstrual symptoms.** Submitted

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# LIST OF ABBREVIATIONS

<b>ACL</b>	Anterior cruciate ligament
<b>a-p</b>	anterior-posterior
<b>CI</b>	Confidence Interval
<b>CNS</b>	Central nervous system
<b>COP</b>	Center of Pressure
<b>E2</b>	Estradiol
<b>EC</b>	Eyes closed
<b>EO</b>	Eyes open
<b>FP</b>	Follicular phase
<b>Free T</b>	Free testosterone
<b>FSH</b>	Follicular stimulating hormone
<b>GABA</b>	$\gamma$ -aminobutyric acid
<b>GH</b>	Growth hormone
<b>GnRH</b>	Gonadotrophin releasing hormone
<b>ICC</b>	Intraclass correlation coefficient
<b>LH</b>	Luteinising hormone
<b>LP</b>	Luteal phase
<b>m-l</b>	medial-lateral
<b>OP</b>	Ovulatory phase
<b>P-4</b>	Progesterone
<b>PMDD</b>	Premenstrual dysphoric disorder
<b>PMS</b>	Premenstrual syndrome/symptoms
<b>SHBG</b>	Sex hormone-binding globulin
<b>T</b>	Testosterone

# INTRODUCTION

Although women have been engaged in hard manual labour for centuries, sports activities have been considered to be deleterious for their health [49]. The first Olympics in Greece were strictly for men. Women were not even allowed to watch the games. In the beginning of the twentieth century, the only accepted sports for women were “social games” like tennis, badminton and golf [71]. The first time women were allowed to compete in the Olympic arena was in Paris 1900, but only in tennis, golf and sailing [22]. However, women participated without the official consent from the International Olympic Committee [22]. The greatest increase in women’s participation in competitive as well as recreational sports occurred during the 1970s and 1980s [71]. Currently women represent 44 percent of the members of the Swedish Sports Federation [71] and in Sydney year 2000, 37 percent of the members of the Swedish Olympic team were women [91].

## Sports related injuries in female athletes

Today we know that sports activities in general have many beneficial effects on women’s health. However, strenuous exercise has been shown to have certain negative health effects and women seem to be more vulnerable than men in this respect. Hard physical training in combination with inadequate nutrition may cause amenorrhea. Athletic amenorrhea is furthermore associated with eating disorders and osteoporosis. These medical disorders are related and known as the “Female athlete triad” [37, 83]. The triad is considered as one of the most serious medical problems in female elite sport of today. Menstrual disturbances in athletes have also been related to an increased risk of musculoskeletal injuries [6].

Several studies have reported an increased risk of sports injuries in female athletes compared to male athletes, especially injuries of the knee such as of the anterior cruciate ligaments (ACL) [4, 9, 40, 65, 66]. These studies have shown a two to eight time higher incidence of ACL injuries in women than men participating in the same sport [40]. High-risk sports are basketball, soccer [4] and handball [65]. Various intrinsic as well as extrinsic factors seem to be involved in the increased risk of female injuries. Gender differences in anatomy, body composition, muscle strength, muscular activation pattern, knee stiffness and jumping and landing characteristics have been suggested to be of importance for the increased incidence of knee injuries in women compared to men [35, 40, 109, 112]. Hormonal influences on tendons and ligaments have also been suggested [27, 53, 54, 56, 115]. However, the exact mechanisms are still not elucidated.



## Gender differences of importance for physical performance

Before puberty there are only minor differences between boys and girls in body size, body composition, aerobic/anaerobic capacity and muscle strength [22, 28, 107]. During puberty physiological gender differences start to develop when endocrine changes begin at the mean age of 10 for girls and a mean age of 12 for boys [93]. In boys, the testosterone increase accounts for an increased bone formation, muscle mass and an increased hemoglobin content in blood. Growth hormone (GH) and sex steroids are important for the pubertal growth spurt in both boys and girls. The rise of estrogen in girls causes breast development, increasing fat deposition, broadening of the pelvis and ossification of the epiphysial growth plate.

When full maturation is reached, there are major gender differences in body height, body mass, muscle mass, blood volume and cardiac size. Women are on the average 13 cm shorter and weigh about 14-18 kg less than men [107]. Furthermore, women have twice as much body fat as men, whereas men have approximately 1½ times the lean body mass, skeletal mass and muscle mass of women. These gender differences obviously lead to differences in physical performance.

The upper body muscle strength is 40-60 percent less in females compared to their male counterparts and even when related to fat free mass (FFM) a gender difference remains [107]. However, in the lower extremities, women are only 25-30 percent weaker than men, but when expressed relative to FFM this difference disappears [107]. Also in respiratory and cardiovascular capacities there are gender differences, mainly as a result of the women's smaller body size and greater fat mass. Relative  $VO_{2max}$  is 20-30 percent greater in male but when calculated for FFM, the difference is only 10-15 percent [28]. There are also anatomical differences between men and women of importance for physical performance. Women have narrower shoulders, a wider pelvis, an increased knee valgus and Q-angle, and a narrower intracondylar notch in the knee [22]. Some of these differences have been related to the increased risk of female athletic injuries.

## The menstrual cycle

The menstrual cycle implies a basic difference in the biology of men and women. The monthly hormonal variations have often been a reason for excluding women as subjects in research studies. How the menstrual cycle affects physical performance is still only partly understood from the scientific literature.

The female hormonal cycle is regulated by the gonadotrophin-releasing hormone (GnRH) produced in the hypothalamus, the release of the luteinising hormone (LH) and the follicle-stimulating hormone (FSH) from the anterior pituitary and by the secretion of estradiol (E2) and progesterone (P-4) from the ovaries. E2 and P-4 exert both positive and negative feedback on the gonadotrophin secretion from the pituitary [22, 46, 93].

The ideal menstrual cycle comprises 28 days, but the range of a normal cycle length varies between 22 and 36 days [46]. The menstrual cycle can be divided into three different phases (Fig 1), i.e. the follicular phase (from the onset of menses to ovulation), the ovulatory phase (one day before the LH surge to the time of ovulation) and the luteal phase (after ovulation to the onset of next menstrual bleeding). In women with ovulatory cycles, the follicular phase is variable in duration and accounts for the range of a normal cycle length, whereas the luteal phase is relatively constant and averages 14 days in most women [46].

The first day of bleeding is referred as cycle day one and is the first day of the follicular phase. During the first days of the follicular phase the levels of female sex hormones are low. Circulating concentrations of FSH increase in the early follicular phase and attain maximum levels during the first half of the follicular phase. During this period, FSH stimulates the growth and development of a group of follicles. Usually, one of the follicles will be selected for full maturation, the preovulatory (Graafian) follicle, and finally rupture at the time of ovulation. By stimulation of FSH, the growing follicle starts to produce E2 leading to a significant increase in peripheral levels by cycle day 7, and peak levels the day before ovulation [22, 46, 80, 93]. E2 production is essential for normal follicular maturation.

The rise in E2 exerts a positive feedback influence on LH secretion and a negative feedback on FSH secretion from the pituitary. At midcycle there is a large peak in serum concentration of LH, the LH surge, which induces ovulation about 12 hours later. After the LH surge, E2 levels fall for several days. Androgens, like androstendione and testosterone are stimulated by LH and secreted from the ovary with peak levels at the time of the midcycle LH surge. Androgen production at this stage enhances atresia of nondominant follicles and stimulates libido [93].

After ovulation the empty follicle creates the corpus luteum. During the third phase, the luteal phase, LH stimulates the production of P-4 from the corpus luteum leading to peak levels in the middle of the luteal phase. Progesterone acts to suppress new follicular growth. Estradiol is also secreted from the corpus luteum with a secondary increase in E2 levels that peak in the mid luteal phase. If the egg cell does not become fertilized, the corpus luteum will degenerate within 10-12 days after formation and the production of female sex hormones will decline. The withdrawal of E2 and P4 initiates breakdown of the endometrium and this leads to the next menstruation [22, 80].

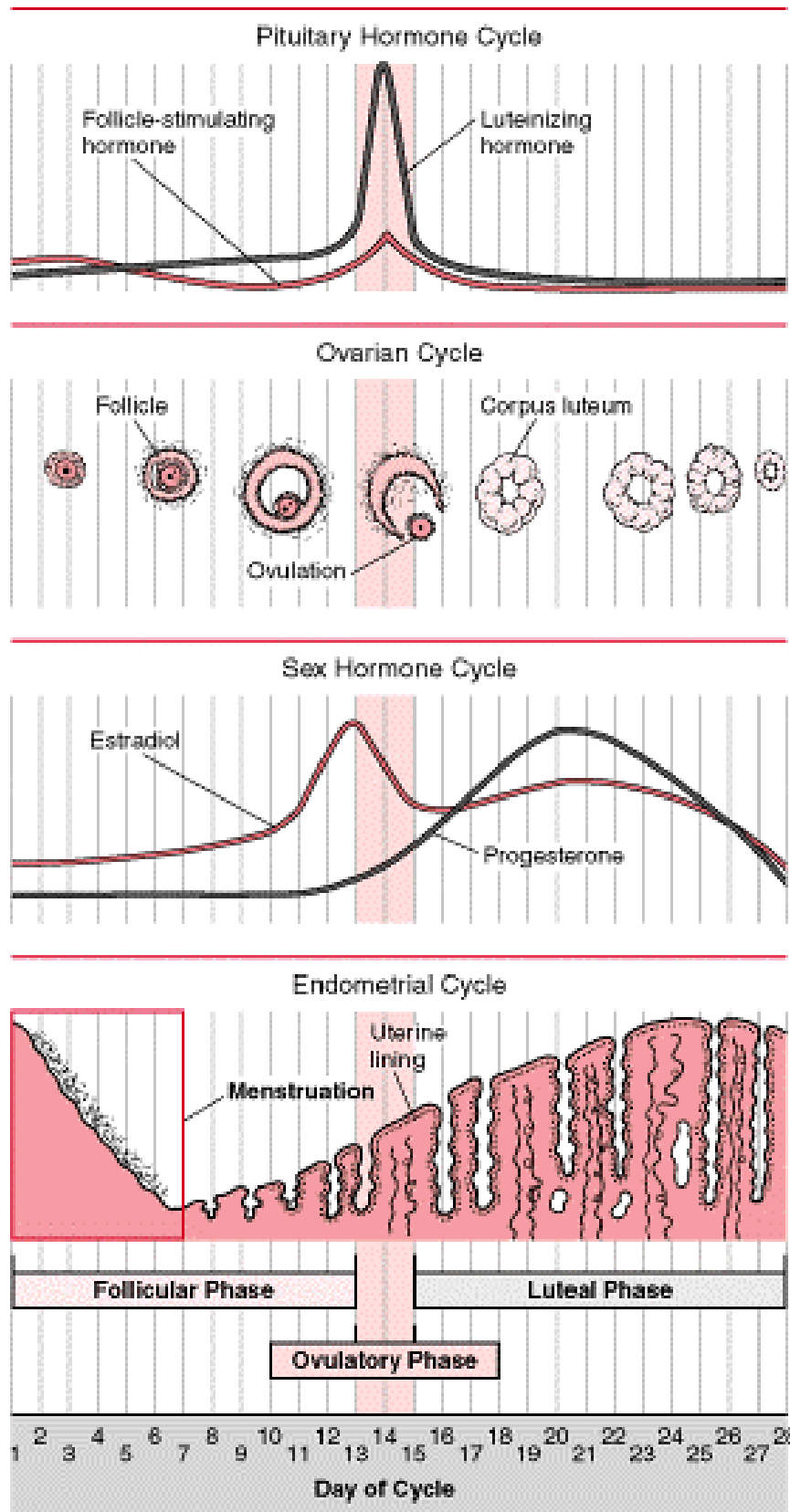


Fig 1. The menstrual cycle divided into three different phases: the follicular phase (from the onset of menses to ovulation), the ovulatory phase (one day before the LH surge to the time of ovulation) and the luteal phase (after ovulation to the onset of next menstrual bleeding). (From The Merck Manual of Medical Information- Second Home Edition, p. 1347, edited by Mark H. Beers. Copyright 2003 by Merck & Co., Inc., Whitehouse Station, NJ.)

Menstrual cycle studies imply methodological problems for several reasons. Firstly, the length of ovulatory cycles varies greatly between individuals [46]. Furthermore, anovulatory cycles may occur despite regular periods. Metcalf *et al* [61] reported that only 62% of women aged 20-25 years and 88% of women aged 25-29 years, ovulated in all cycles tested. Due to these great variations, it is not accurate to determine different phases of the menstrual cycle without hormone analyses or gynaecological evaluation [84]. It is also important to standardize the protocol for the sampling of blood for hormone analyses since several factors such as diurnal variation, stress and physical activity might affect hormone levels [49].

## Premenstrual syndrome

Premenstrual syndrome (PMS) is characterized by cyclical physical and mood disturbances during the luteal phase [95]. The severity of the symptoms gradually increases during the luteal phase and disappears a few days after onset of menses. Most women (75-90 percent) of fertile age experience cyclical changes during the menstrual cycle, but only 6-10 percent seek medical help for their symptoms [98]. The most common symptoms reported are irritability, fatigue, bloating and breast tenderness [3, 104]. According to The International Statistical Classification of Diseases and Related Health Problems, 10<sup>th</sup> revision (ICD-10) only one of the following symptoms is needed to fulfil the diagnosis of PMS:

- Mild psychological discomfort
- Feeling of bloating /weight gain
- Breast tenderness
- Swelling of hands and feet
- Various aches and pains
- Poor concentration
- Sleep disturbances
- Change in appetite

Premenstrual dysphoric disorder (PMDD) is a psychoneuroendocrine disorder, which is a more severe form of PMS and requires functional impairment [96]. The American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, 4<sup>th</sup> edition (DMS-IV) have defined the criteria for PMDD [2]. To fulfil the diagnostic criteria, a patient needs to present five or more distressing symptoms during the luteal phase and at least one of these symptoms have to be mood symptoms. The cardinal symptoms are irritability, depressed mood, affect lability and tension. The PMDD diagnosis must also be confirmed by prospective ratings in two consecutive menstrual cycles since the severity of the symptoms may change between cycles. About two to six percent of women in fertile age meet the criteria for PMDD [98].

Although ovulation and production of E2 and P-4 by the corpus luteum are necessary for the development of PMS, no differences in plasma levels of these hormones have been detected between PMS patients and asymptomatic women [11]. However, the severity of PMS symptoms has been positively correlated to the levels of plasma estradiol in the luteal phase [34, 86]. The ovarian steroids appear to be involved in a combination of genomic and nongenomic actions in the brain [60]. Estradiol and P-4 can be synthesised into neurosteroids in the brain and may act in the central nervous system (CNS) via membrane bound receptors to influence transmitter systems. There is scientific support that progesterone metabolites interact with the  $\gamma$ -aminobutyric acid (GABA)- and the serotonergic system, respectively, and both these systems seem to be involved in the pathophysiology of PMS and PMDD [95].

## GABA

The most important inhibiting system in the mammalian CNS, GABA, is affected by sex steroids [96]. Benzodiazepins and barbiturates bind to GABA receptors and the GABAergic neurotransmission could lead to fatigue, anxiety and depression which are common symptoms of PMS [96]. Two progesterone metabolites, allopregnanolone ( $3\alpha$ -OH- $5\alpha$ -pregnan-20-one) and pregnanolone ( $3\alpha$ -OH- $5\beta$ -pregnan-20-one) also bind to the GABA receptor and are the two most potent modulators on the GABA-A receptor [101]. The enzymes needed for formation of allopregnanolone from P-4,  $5\alpha$ -reductase and  $3\alpha$ -hydroxysteroid oxidoreductase, are present in the brain [96]. In women of fertile age, circulating concentrations of allopregnanolone are higher in the luteal phase compared to the follicular phase [96]. Rapkin *et al* [78] showed that women with PMS had lower levels of allopregnanolone in plasma in the late luteal phase compared to women without these symptoms. However, other studies have not been able to detect such differences [96]. In another study, it was shown that women with PMS have a decreased sensitivity to pregnanolone in CNS compared to controls [96]. Women with more severe symptoms of PMS are also less sensitive to pregnanolone compared to women with mild symptoms [96].

## Serotonin

The serotonergic system also seems to be involved in the pathogenesis of PMS [96]. Serotonin (5-hydroxytryptamine, 5HT) is part of several physiological, behavioural and emotional processes such as mood, memory, learning, sexual behaviour and aggression [7]. Other physiological functions which involve serotonin are anxiety, pain, sleep, and appetite [96]. Estradiol and P-4 bind to receptors in the cell nucleus and are believed to affect the synthesis, turnover and release of serotonin [96]. Evidence of a possible dysfunction in the serotonergic neurotransmission is the fact that serotonin reuptake inhibitors (SSRIs) has been shown to be an effective treatment of PMS and PMDD [73, 94, 96].

# Physical performance in relation to the menstrual cycle

During the last few decades women's participation in recreational as well as competitive sports activities has increased dramatically. However, there is still a lack of knowledge about physical performance in relation to the menstrual cycle. In most investigations [49 review] the effect of extensive training on reproduction and on the neuro-endocrine system have been studied. There are only a few reports on the influence of the normal menstrual cycle on physical performance and sports related injuries.

The earliest studies [49 review] about the influence of the menstrual cycle on physical performance were retrospective questionnaires. In these studies the majority of the female athletes estimated their performance in sport activities to be at the highest level during the middle and late follicular phase and worst level during the luteal phase and the first days of menses [49 review]. However this data is based on retrospective questionnaires which have several weaknesses, such as difficulties for the subjects recalling accurate data related to the menstrual cycle in the past [49 review].

## Sport injuries

An association between sport injuries in female athletes during the different phases of the menstrual cycle has been reported. Higher injury rates have been observed during the premenstrual and menstrual phase [66, 67], whereas Wojtys *et al* [110, 111] reported higher incidence of ACL injuries during the ovulatory phase. Impaired physical capacity, muscle strength, neuromuscular coordination, proprioception and postural control, due to the specific hormonal influence of the various menstrual cycle phases, have been suggested to explain the higher incidence of injuries. Furthermore, Möller-Nielsen and Hammar [67] found women with PMS to be at greater risk for injury compared to women without these symptoms. However, the mechanism behind the association between athletic injuries and the menstrual cycle is still not clear.

## Aerobic and anaerobic performance

The influence of the menstrual cycle on  $VO_2$  max, ventilation and anaerobic performance have been studied with varying results [5, 20-22, 49, 50, 55, 59, 106]. Some studies were not able to detect any significant difference in  $VO_2$  max, ventilation, blood lactate or time to exhaustion [5, 20, 21], while other studies showed such differences [22, 59, 106]. Several studies have demonstrated an increased minute ventilation during the luteal phase compared to the follicular phase [22]. This has been explained by increased progesterone levels in the luteal phase affecting the chemoreceptors in medulla oblongata. Progesterone is also considered to affect central thermoregulation, but the exact effect on thermoregulation is still not fully understood [22, 49, 55]. More studies in this area are needed to make any definite conclusions about the aerobic and anaerobic performance during the menstrual cycle.

## Effects on soft tissue

During the last years, basic research on the effects of different hormones on tendons and ligaments has been initiated. Estrogen and progesterone receptors have been identified on fibroblasts in the human ACL [53, 54]. Collagen, which is produced by fibroblasts, has the main load bearing function in the ligament [53, 54] and has been divided into subgroups. Type 1 collagen imparts a great mechanical strength to the tissue and Type 3 collagen has been correlated with tissue elasticity [115]. A larger Type 1/Type 3 ratio gives a greater strength of the ligament while a lower ratio may lead to ligament laxity [115]. Estrogen has been shown to decrease the proliferation of fibroblasts and the synthesis of Type 1 procollagen [115]. In contrast, Ernst *et al* [27] and Majeska *et al* [56] have reported an estrogen dose dependent increase of Type 1 collagen mRNA. More research is needed to make any conclusions of the effects of estrogen on soft tissue. Even though P-4 receptors on ligaments have been detected [53], the effect of P-4 is not well understood.

The peptide hormone relaxin is produced by the corpus luteum and reaches the highest levels during pregnancy. Relaxin is also increased in the luteal phase of the menstrual cycle [35]. It is believed that relaxin contributes to increased ligament laxity during pregnancy and may therefore also affect ligaments during the luteal phase [44].

## Muscle strength

It has been suggested that estrogen has an anabolic effect on the muscle, while progesterone might have a catabolic effect [79] but studies on muscle strength in different phases of the menstrual cycle have shown contradictory results [18, 31, 32, 50, 75, 76, 84]. Davies *et al* [18] reported an increase in explosive muscle strength during the early follicular phase (cycle day 1-4) compared to the late follicular phase (cycle day 12-14) and the luteal phase (cycle day 19-21). The authors speculated that E2 and P-4 inhibit skeletal muscle performance, whereas low levels of these female sex steroids during the early follicular phase enhance muscle strength [18].

In contrast, other reports have suggested that muscle strength is increased by E2. Phillips *et al* [76] reported an increase in isometric muscle strength of the adductor pollicis muscle around ovulation (cycle day 14). Sawar *et al* [84] confirmed this effect of E2 in a study measuring maximum voluntary isometric force in quadriceps and handgrip during the menstrual cycle. They showed significantly higher values of these variables during ovulation (cycle day 12-18) compared to the other menstrual cycle phases. Furthermore, they showed a significantly slower muscle relaxation time and an increase in muscle fatigability during ovulation compared to the other phases [84]. In another study by Phillips *et al* [75], low levels of E2 were reported to decrease muscle strength in postmenopausal women and they suggested that this decrease may be prevented by hormone replacement therapy.

Lebrun *et al* [50] measured isokinetic muscle torque of knee flexion and extension during the early follicular phase (cycle day 3-8) and the luteal phase (4-9 days after ovulation) and found no significant variation in muscle performance between these phases. Recent studies of Gür *et al* [31, 32] could not detect any differences in either concentric or eccentric isokinetic muscle torque of the knee flexors and extensors during the menstrual cycle. These results were later confirmed by Janse de Jonge *et al* [42].

The reason for the contradictory results from the above studies could be the different ways of menstrual cycle phase determination. In the earlier studies [18, 76, 84] the menstrual cycle phase was determined by counting days of the menstrual cycle and not by hormonal analyses. Since there are inconclusive data of muscle strength during the menstrual cycle further studies are needed.

## Knee joint kinesthesia

Kinesthesia, or sense of joint movement, is associated with sensations of joint movement either from internal forces (active) or external forces (passive), and is a submodality of proprioception [51]. Proprioception is related to the senses of position and movement of limbs. These senses are mediated through neural input from peripheral mechanoreceptors in the skin, muscles, tendons, ligaments and joint capsules [51]. Impaired proprioception in the knee joint has been considered as one of the risk factors for knee injuries [29]. To our knowledge, knee joint kinesthesia has not previously been studied during the menstrual cycle.

## Neuromuscular coordination

Neuromuscular coordination is defined as the ability to move different parts of the body smoothly and at the same time and involves both the nervous and muscular systems. Posthuma *et al* [77] found that the neuromuscular system is affected by the hormonal influence during the menstrual cycle. Women with PMS had a decreased fine motor function during the luteal phase compared to the other phases and compared to women without PMS during the same phase. However, using a finger-tap test Epting *et al* [26] were not able to detect a significant difference in fine motor function between the menstruation phase and midluteal phase. Neuromuscular coordination in the lower extremities has, to our knowledge, not previously been studied during the different phases of the menstrual cycle.



## Postural control

The ability to maintain the position of the center of body mass within the stability limits is termed postural stability or balance [87]. Postural control is defined as the ability to maintain control over posture [51] and is a complex function involving the somatosensory, vestibular and visual systems as well as muscle activity [38, 108]. From these systems the CNS receives information regarding body position and balance, which contributes to the maintenance of postural control. To assess postural control in stance, center of pressure (COP) displacements can be registered by stabilometry with different kinds of force platforms. Evaluation of postural control has several implications in sports medicine and has been used to identify subjects with increased risk for sport injuries and to evaluate rehabilitation programs [82]. However, it is still controversial if a larger displacement of the center of pressure is related to an increased risk of sport injuries.

Recent studies have indicated that sex steroids might have an effect on postural control. In postmenopausal women, the risk of a skeletal injury has been related to low levels of estrogen [33, 68]. This may partly be explained by impaired postural control which leads to a greater risk for fall-induced fractures [68], since long-term estrogen supplementation has been demonstrated to enhance balance in postmenopausal women [33, 68]. Whether improved postural control is due to a central nervous effect of estrogen, improved muscle strength, altered soft tissues, or a general improvement in the quality of life is not known. However, there are also studies that have not been able to demonstrate positive effects of estrogen supplementation on postural control [24, 52, 85].

The influence of hormonal variations during the menstrual cycle on postural control is less investigated. Darlington *et al* [17] detected a greater postural sway in the luteal phase and the early follicular phase of the menstrual cycle. Since impaired postural control have been suggested as a part of the explanation for sports related injuries, it is important to further study the relation between postural control and hormonal variations during the menstrual cycle.

# AIMS

The present study was designed to explore neuromuscular performance and postural control across three well defined and hormonally confirmed phases of the menstrual cycle: the early follicular phase (low levels of estradiol and progesterone), the ovulatory phase (high levels of estradiol) and the mid luteal phase (high levels of progesterone and estradiol) in moderately active women.

The specific aims were:

- To evaluate muscle strength and muscle endurance during the three phases of the menstrual cycle (paper I).
- To evaluate knee joint kinesthesia and neuromuscular coordination during the three phases of the menstrual cycle (papers II, III).
- To evaluate postural control during the three phases of the menstrual cycle (papers II, IV).
- To relate the results of neuromuscular performance and postural control to hormone levels (papers I, II, III, IV) and premenstrual symptoms (papers I, II, III, IV).

# MATERIALS AND METHODS

## Subjects

Thirteen female subjects participated in studies I and II and another 25 subjects in studies III and IV. Subjects were recruited among students at the Karolinska Institutet (Stockholm, Sweden). All subjects were healthy, non-smokers with a moderate activity level of up to two (I, II) and four (III, IV) sessions per week. No medication was allowed and oral contraceptives or other hormones had not been taken for at least three months before entering the studies. All participants gave their written informed consent prior to entering the study. The studies were all approved by the Ethical committee at the Karolinska Institutet, Stockholm, Sweden (dnr 00-102 and 01-311).

## Study design

After the subjects had performed one (I, II) or three (III, IV) familiarization trials at different occasions, the subjects were tested in two (I, II) or three (III, IV) consecutive menstrual cycles respectively and at three test sessions during each cycle (I, II, III, IV). The first test session was in the follicular phase (named eFP in paper I and II, MP in paper III and FP in paper IV), i.e. cycle day 3-5 of the menstrual cycle. The second test session was in the ovulatory phase (OP), which was detected by identification of the LH-surge in urine. The test protocol for the OP was performed within 24-48 hours after detection of the LH-surge. The third test session was in the luteal phase (named mLp in paper I and II, pMP in paper III and LP in paper IV), which was defined as 7 days after ovulation and with a simultaneous increase in serum progesterone. To minimize the problem of diurnal variation, the tests were performed at the same time of the day each test session for each subject. In the summary of this thesis the follicular phase is abbreviated as FP, the ovulatory phase as OP and the luteal phase as LP.

## Hormonal assays

For hormone determinations, blood samples were collected in the morning (7.30-10.00 AM) after 15 minutes of bed rest and preceding the physical test session in the FP, OP and LP in the three consecutive menstrual cycles. After centrifugation, serum was separated and stored at  $-20^{\circ}\text{C}$  until analyzed. Follicular stimulating hormone (FSH), luteinising hormone (LH), estradiol (E2), progesterone (P-4) and sex hormone-binding globulin (SHBG) were analyzed in serum with chemiluminescent enzyme immunometric assays (IMMULITE Automated Analyser, DPC).

Serum levels of testosterone (T) (III, IV) were determined by radioimmunoassay using a commercial kit obtained from Diagnostic Products Corp (Coat-account<sup>®</sup> Testosterone, Los Angeles, CA). Apparent concentrations of free T were calculated from values of total testosterone, SHBG and fixed albumin concentration of 40 g/L by successive approximation using computer program based on an equation derived from the law of mass action (III, IV) [99]. Detection limits and within and between assay coefficients of variation were for FSH 0.1 U/L, 5.4 % and 8.1 %; for LH 0.7 U/L, 4.8 % and 9.4 %; for E2 55 pmol/L, 9.3 % and 10.6 %; for P-4 0.6 nmol/L, 8.2 % and 9.2 %, for SHBG 0.2 nmol/L, 6.5 % and 8.7 %, and for T 0.1 nmol/L, 6 % and 10 %, respectively. Ovulation was determined by Ovustix<sup>®</sup> (Clearplan, Unipath Limited, Bedford, England), detecting the LH-surge in urine.

## Cyclicity Diagnosis

The women performed a prospective rating of PMS symptoms every evening during the test period (I, II, III, IV). A cyclicity diagnosis of PMS was made using a previously validated rating scale called Cyclicity Diagnoser (CD) [97]. The CD scale consists of four negative mood parameters (depression, fatigue, irritability, tension), three positive mood parameters (cheerfulness, friendliness, energy), and four somatic symptoms (headache, swelling, breast tenderness and menstrual bleeding). In addition, the CD scale contains a score for measuring every-day social function and work performance. The CD scale is a Likert scale ranging from 0-8, with 0 as complete absence of a particular symptom, and 8 as the maximal severity of the symptom.

In study I and II, subjects were considered to have PMS if they had a significant increase in at least three negative symptoms during nine premenstrual days compared to nine mid-follicular days according to the criteria described by Hammarbäck *et al* [34]. In studies III and IV, only one symptom was used for diagnosis of PMS according to The International Statistical Classification of Diseases and Related Health Problems, tenth revision (ICD-10). In all studies the diagnostic procedures were chosen according to the DSM-IV criterion [2] with prospective ratings of PMS in at least two menstrual cycles. All patients displayed at least one week of sparse symptomatology (scores less than 2) in the follicular phase.

## Muscle strength measurements

### Handgrip strength (I)

Handgrip strength of the dominant hand was measured with a handgrip strength dynamometer (Jamar<sup>®</sup>, Sammons Preston, Bolingbrook, IL, USA) previously tested for reliability (inter and intra-test reliability  $r=0.98$  and  $r=0.94-0.98$ , respectively) [74]. The strength was measured with the arm extended hanging along the body side. The best result (in kg) of three trials was recorded.

## One-leg hop test (I)

The one-leg hop test, with high test-retest reliability (Intraclass correlation coefficient, (ICC) value,  $r=0.96$ ) [1], was performed by jumping and landing on the right leg. The best jump (in cm) of three trials was recorded.

## Isokinetic muscle torque and endurance (I)

Measurements of maximal muscle torque (Nm) were performed with a standard isokinetic device (Biodex®, Corp, Shirley, NY, USA), after a 10-min warm-up period on a cycle ergometer. The device is previously tested for reliability and validity with acceptable results [23]. The subjects sat with the back supported at a hip angle of  $85^\circ$  and the trunk and hips were stabilized with straps. The lever was attached just above the ankle and the axis of rotation was aligned with the center of femoral condyles estimated by palpation. The knee extensors of the right leg were measured at  $120^\circ/\text{sec}$ , in a range of motion (ROM) of  $90\text{-}10^\circ$  knee flexion. Five consecutive concentric contractions were performed and the peak torque of the best contraction was recorded. After a short rest period of three minutes the subjects performed 50 concentric contractions of the knee extensors, at the same angular velocity and ROM, in order to estimate muscle endurance. The mean value from the last five contractions was compared to the first five ones and expressed in percent.

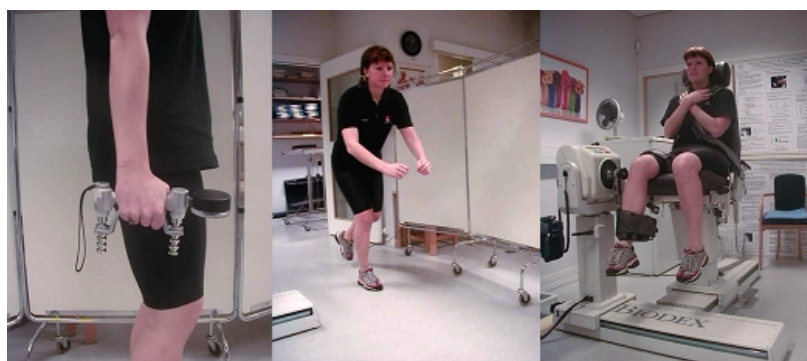


Fig 2. Measurements of handgrip strength, one-leg hop test and isokinetic muscle torque.

## Knee joint kinesthesia

Knee joint kinesthesia was measured (II, III) with a custom made device developed at the University of Vermont (Burlington, Vermont, USA). The device and software is described in greater detail by Beynnon *et al* [8], along with measures of its reliability and precision. Tests of knee joint kinesthesia were performed with the subjects sitting in a chair with both feet placed in air cuffs. These cuffs were attached to strings connected to an electric motor producing flexion or extension of the knees at an angular velocity of  $0.1 \text{ deg}/\text{sec}$ . Knee joint angles were registered by four electrolytic rotation sensors placed on the thigh and calf of each leg. Prior to the test, the subjects sat with the knee joints in approximately  $20 \text{ deg}$  of knee flexion as determined with a manual goniometer.

The blindfolded subjects pushed a button to indicate when they were able to detect a movement of their knee joints, and they were also asked if the direction of movement was extension or flexion. Prior to testing, the subjects were informed that the start of the movement could be delayed up to 30 sec. One test session included 12 movements, three in flexion and three in extension of the right leg and left leg, respectively. The order of knee joint movements (flex/ext and right/left, respectively) were randomised by the custom made software of the device. The difference in degrees of the knee joint angle from the start until the subject detected the movement was recorded. The mean values for the right and left leg, respectively, were used for analysis.

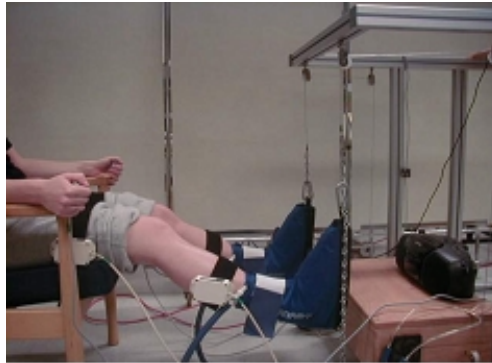


Fig 3. Measurements of knee joint kinesthesia (University of Vermont, Burlington, Vermont, USA).

## Neuromuscular coordination

### Square hop test (III)

Square hop test is a functional multi-directional test of the lower extremity [116]. This test has been evaluated for inter-and intra-test reliability with acceptable results ( $r=0.94$  and  $0.74$ , respectively) [116]. The subjects performed one trial during 30 seconds on each leg, clockwise in and out of a  $30 \times 35$  cm square marked with tape on the floor (Fig. 4.). The number of successful jumps inside the square without touching the tape, as judged by the test leader was recorded. The mean number of successful right and left leg jumps was used for analysis.

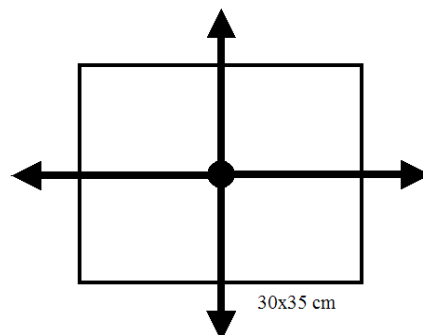


Fig 4. The Square hop test.

# Postural control

## Measurements on a Statometer

Postural control in study II was measured with an ankle disc placed on a Statometer developed at the Karolinska Institutet [48] (Fig 5). The Statometer is a circular platform supported by three cantilevers equally spaced around its periphery. Strain gauges are attached to each cantilever to give a continuous sampling of the load at a frequency of 10 Hz. The signals were amplified, A/D converted and analysed by a personal computer (Toshiba T3200). The ankle disc was placed on the Statometer supported by four bars. The test started with the ankle disc stabilized by the bars. After approximately 5 seconds the bars were suddenly and simultaneously removed, without warning, making the ankle disc unstable for the following 25 seconds. The subjects stood on one leg with their arms hanging along the body side. The exact position of the foot was marked on the ankle disc and the same foot placement was used for all three tests. The mean value of the three tests of the dominant leg, expressed in arbitrary units (AU), was recorded as the result of total postural displacement. The reliability of this method has earlier been investigated (ICC,  $r= 0.84-0.87$ ) [Lindgren & Leanderson, 1999, unpublished data].

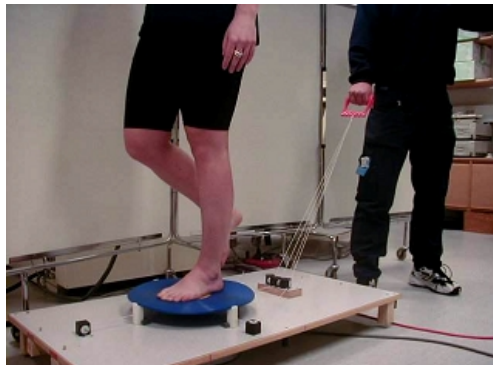


Fig 5. Measurements of postural control on a Statometer developed at the Karolinska Institutet

## Measurements on a force plate

For measurements of postural control in study IV, an AMTI<sup>®</sup> force platform (model LG6-4-2000, Advanced Mechanical Technology, Inc., Watertown, MA, USA) was used. Subjects stood barefoot in a comfortable two-legged stance with eyes open (EO) and closed (EC) for 2 minutes respectively, in one-legged stance EO for 1 minute, and finally in one-legged stance EC for 30 seconds (Fig 6). The subjects stood quietly and remained as still as possible with the arms hanging at the sides of the body. For the EO trials, the subjects focused on a target placed at eye level and located approximately 2 m in front of them. For EC trials, the subjects were instructed to focus on the same target before closing their eyes. To ensure accurate and repeated foot placement, the middle of the force plate was marked. The subjects were instructed to place their feet in the same position, with the mark placed between the center of both feet (two-legged stance) or at the center of the support foot (one-legged stance). The foot position for the two-legged stance was standing with the feet tight together. During the one-legged stance, the subjects were instructed to correct their position as soon as possible if disturbed. The non-supported limb was flexed to 90 degrees at the knee. If arms were used for balancing, subjects were asked to correct arm position by placing them at their side. The trials were separated with a 1-minute break with the subject resting in a chair. All experiments were performed in a quiet room and in the same order each test session.

Ground reaction force (GRF) data was sampled at 50 Hz, A/D converted at 12-bit resolution (Axiom<sup>®</sup>, Axiomtek Deutschland GMBH, Langenfeld, Germany) and stored on a dedicated signal analysis computer for later analysis. The force plate measured the three force components,  $F_x$ ,  $F_y$  and  $F_z$ , and the three moment components,  $M_x$ ,  $M_y$  and  $M_z$  ( $x$ ,  $y$ , and  $z$  are the medial-lateral, anterior-posterior, and vertical directions, respectively). The Center of Pressure (COP) position was calculated as follows:

$$COP_x = [(M_y + (Z_{off} * F_x))/F_z]*(-1) \quad (1)$$

$$COP_y = [(M_x - (Z_{off} * F_y))/F_z], \quad (2)$$

where  $Z_{off}$  = the vertical offset from the top plate to the origin of the force platform (a negative #).

The COP datum is given as a location (two dimensional) on the surface of the force plate. These two coordinates are identified in relation to the orientation of the subject: anterior-posterior (a-p) direction and medial-lateral (m-l) direction.



## Data analysis of force plate measurements

For detailed characterizations of COP displacements total displacement ( $dCOP$ ) in the a-p and m-l directions and the area encompassed by the COP displacement trace were studied. These were computed off-line using custom software written in MATLAB<sup>®</sup> (The MathWorks, Inc. Natick, MA, USA). The area (the plot of the COP displacement in the a-p direction vs. the COP displacement in the m-l direction) was computed using the ellipse area method and the principal axes of the ellipse were determined by the principal-component analysis (PCA) [70]. In this method, a subtotal of 85.35% of samples lie within the perimeter of an ellipse (as defined by the Rayleigh probability distribution [72]).



Fig 6. Measurements of postural control on an AMTI<sup>®</sup> force platform (model LG6-4-2000, Advanced Mechanical Technology, Inc., Watertown, MA, USA)

## Dropouts

The dropout rate of subjects was high in all studies in this thesis. In study I and II, two subjects out of 18 dropped out due to personal reasons before the study was finished, another subject was excluded due to a cold the last test occasion and two subjects missed to detect the LH-surge in the first menstrual cycle. In study III and IV four subjects out of 32 dropped out after the first test session due to various personal reasons. An additional three subjects were excluded since they failed to detect their ovulation with the Ovustix<sup>®</sup> in all three cycles. Twenty-five women completed at least one hormonally verified menstrual cycle and were included in study III and IV.

## Statistics

All data are presented using descriptive statistics, mean, standard deviation (SD) and number of observations. Data for muscle strength, neuromuscular coordination, knee joint kinesthesia and postural control were analysed with analysis of variance (ANOVA), repeated measurements design. Fisher LSD Post Hoc test was used for multiple comparisons (III) and Tukey HSD Post Hoc test was used to assess differences between groups (cyclic, non-cyclic) and phases (IV). All tests were two-sided and the level of significance was set at 0.05. The Pearson coefficient of correlation was used for analysis of correlations between variables. A non-parametric method (one-tailed Mann-Whitney U-test) was used to evaluate each symptom of the PMS-diagnosis.

In study I two consecutive menstrual cycles were analysed and only subjects with complete measurements in both menstrual cycles were analysed (n=10). In study II only the second menstrual cycle was included in the results (n=13). This was due to a learning effect in the postural control measurements. The learning effect was probably limited in study III and IV since the subjects performed three sessions of familiarization trials on three different days before entering the study. Since the dropout rate in study III and IV was high and several anovulatory cycles were identified, only one menstrual cycle for each subject was included in the results. For subjects with two or three accepted menstrual cycles the hormonally most representative cycle was included in study III. The selection of the menstrual cycle was performed before analysing data of the physical tests. In study IV, the cycle with the largest cyclic symptom changes was selected for individuals with PMS. The reason for this was to compare the groups with the largest cyclic symptom changes with the group without PMS. The menstrual cycle was chosen before analysing the data of the physical tests.

# RESULTS

## Hormones

Mean values of serum FSH, LH, E2 and P-4 in the FP, in the OP and in the LP, confirmed that the tests were performed in the specific phases of the menstrual cycle in all papers (I, II, III, IV). E2 levels were low in the FP and increased as expected in the OP and LP. P-4 levels were increased in the LP compared to the other phases. In paper I, LH was increased in the ovulation phase of the two consecutive cycles in all subjects but the increase was less than in the first cycle. In paper III and IV the LH-surge was clear in all included cycles. Only cycles with detected ovulation were included in the results. In study III/IV, four (6%) of all the 68 menstrual cycles investigated were anovulatory and therefore excluded in further analyses. The mean (SD) of serum P-4 in the LP in the anovulatory cycles was 6.3 (4) nmol/L.

## Cyclicity

When all physical tests had been performed, the prospectively collected CD scores were used as a grouping variable. In study I/II, eight out of thirteen subjects (62%) had at least three symptoms of PMS, while three (23%) had one and two (15%) had none of the symptoms. Most of the PMS symptoms were physical such as breast tenderness, feeling of swelling and fatigue. Out of the 64 ovulatory cycles in study III/IV, the CD scores of 20 (31%) cycles showed no cyclical changes in PMS symptoms and 44 (69%) cycles showed a follicular to luteal phase change in one or more symptoms. The classification of PMS differed in study I/II and III/IV. However, in both subject populations the pattern of symptoms was substantially different between the PMS group and non-PMS group. There were no significant differences in hormone levels between the PMS group and the non-PMS group (II, IV). In paper IV, there was a tendency ( $p=0.06$ ) towards higher T in the LP in the PMS group compared to the non-PMS group. However, no difference was seen in levels of free T.

## Muscle strength measurements

No significant variation was found with respect to handgrip strength, isokinetic muscle torque of the knee extensors (peak torque), muscle endurance (fatigue) or one-leg hop test between the phases in two consecutive menstrual cycles (Fig 7). There were no correlations between muscle strength tests and levels of LH, FSH, E2 and P-4 and there was no difference between the PMS and non-PMS group (data not shown).

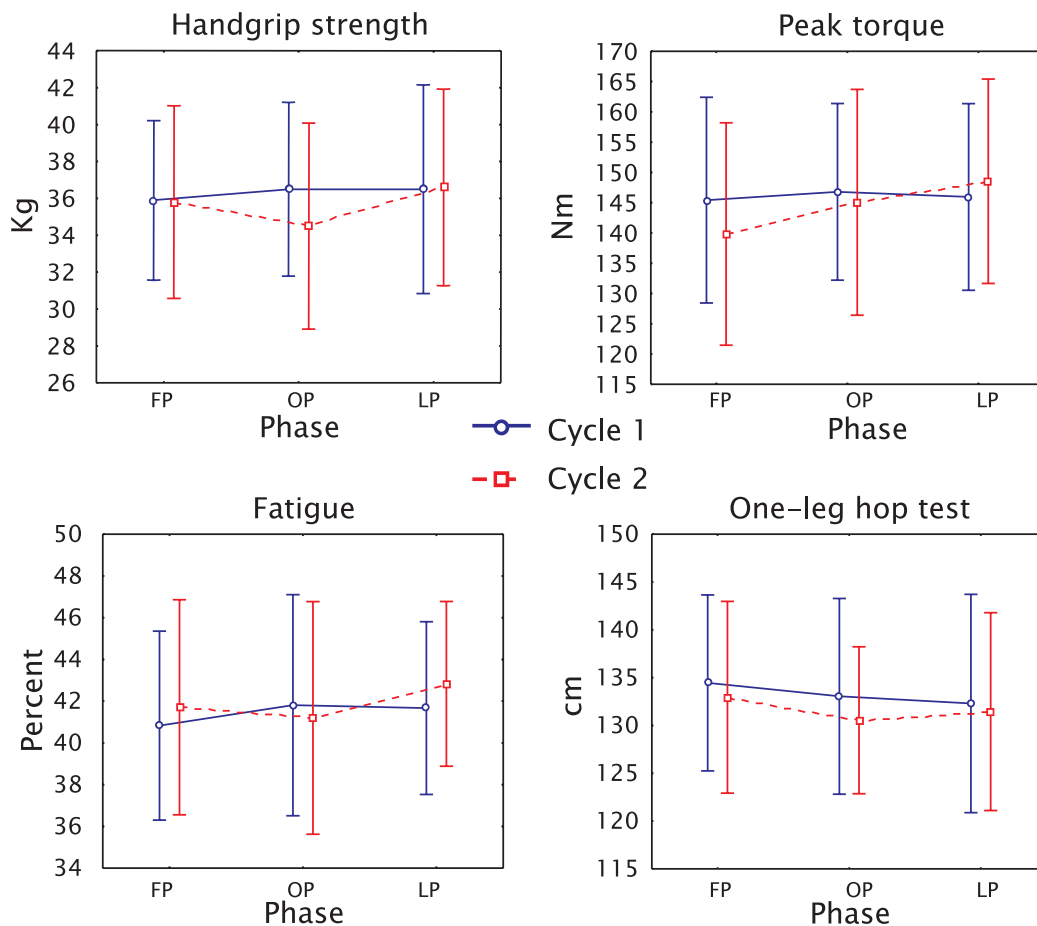


Fig 7. Mean values and CI for handgrip strength, isokinetic muscle torque, muscle endurance (fatigue) and one-leg hop test in the follicular phase (FP), ovulatory phase (OP) and luteal phase (LP). No significant difference was detected between cycles or phases.

## Knee joint kinesthesia

In paper III there was a significantly higher threshold to detect a movement in the LP compared to the FP and OP ( $p < 0.05$ ). The detection threshold in degrees (SD) was 1.0 (0.5), 1.0 (0.5) and 1.2 (0.7) in the FP, OP and LP, respectively. There was no significant difference between the PMS group and the non-PMS group (Fig 8).

However, the PMS group had the highest threshold to detect a movement in the LP. In study II there was no significant difference in knee joint kinesthesia between phases. However, subjects with PMS showed an overall greater threshold to detect a passive movement in the knee joint compared to subjects without PMS. No correlation was seen between knee joint kinesthesia and hormone levels in the respective phase.

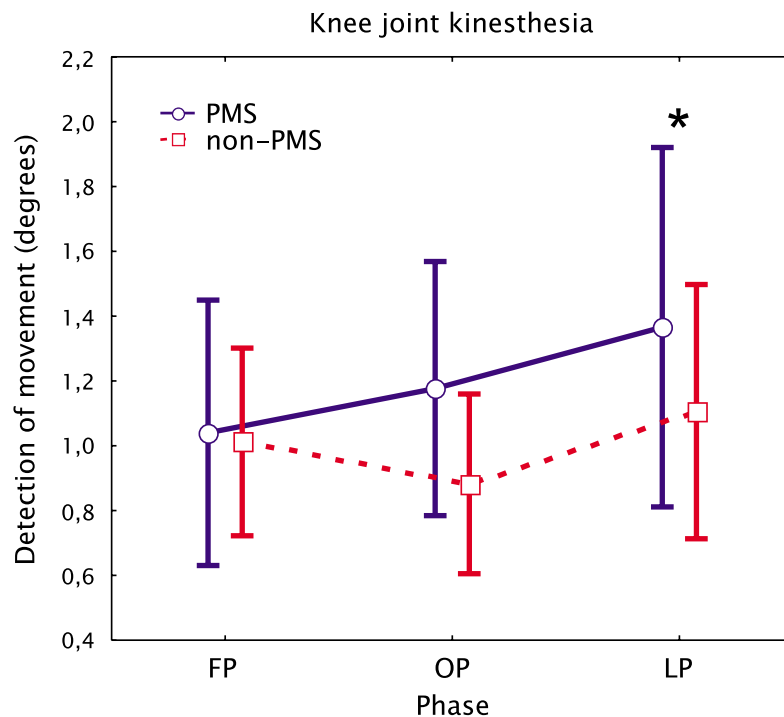


Fig 8. Knee joint kinesthesia in the follicular phase (FP), ovulatory phase (OP) and luteal phase (LP) when subjects were grouped in a PMS group and a non-PMS group. There was a significantly higher threshold to detect a movement in the LP compared to the other phases but no significant difference between the PMS and non-PMS group. \* Represents difference from FP and OP, ( $*p < 0.05$ ).

# Neuromuscular coordination

## Square hop test

The subjects performed significantly greater number of jumps in the OP compared to the FP and the LP ( $p < 0.001$ ). There was no significant correlation between number of jumps and hormone levels in the respective phase. However, the levels of total and free testosterone levels were increased in the OP as was the performance of square hop test (Fig 9).

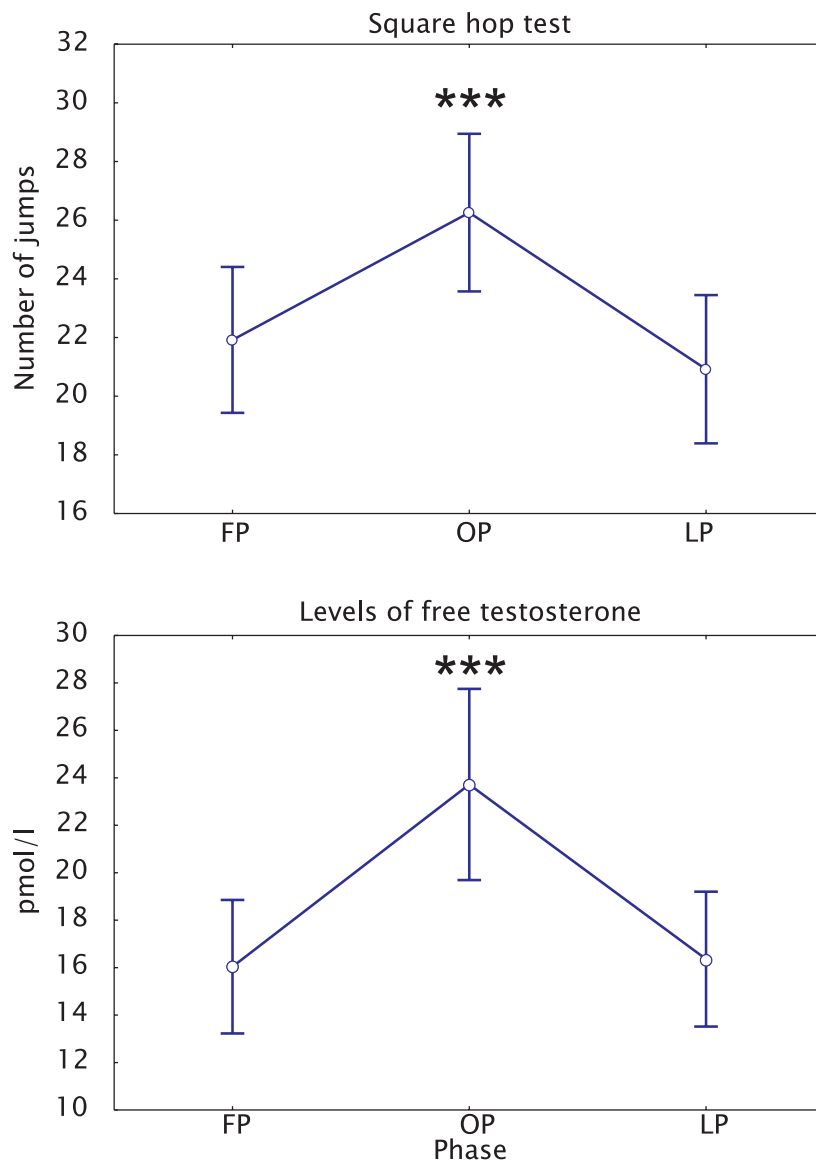


Fig 9. Square hop test (number of jumps) and levels of free testosterone (pmol/l) in the follicular phase (FP), the ovulatory phase (OP) and the luteal phase (LP). The performance of square hop test and levels of free testosterone were significantly increased in the OP. \*\*\* Represents difference from FP and LP, (\*\*\*) $p < 0.001$ .

There was no significant difference in square hop test between the PMS group and the non-PMS group. However, the values were lower for the PMS group than for the non-PMS group (Fig 10).

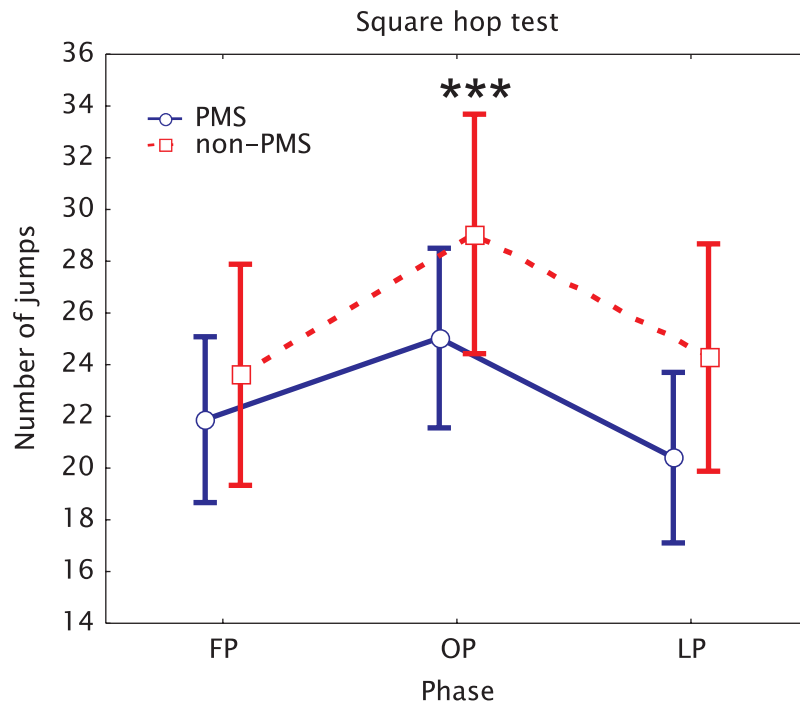


Fig 10. Mean number of jumps in the follicular phase (FP), ovulatory phase (OP) and the luteal phase (LP) when subjects were grouped in a PMS group and a non-PMS group. Neuromuscular coordination was significantly enhanced in the OP. There was no significant difference between the groups. \*\*\* Represents difference from FP and LP, (\*\*\*) $p < 0.001$ .

## Postural control

### Measurements on a Statometer (II)

Subjects with PMS (n=7) had a significantly greater postural displacement compared to subjects without PMS (n=5), ( $p=0.002$ ). Postural displacement (arbitrary units) among women with PMS during the FP, OP and LP was 64.1, 58.4 and 69.8, respectively, and 53.5, 46.7 and 47.0, respectively, for the non-PMS group. Among subjects with PMS, there was a tendency towards greater postural sway in the LP ( $p=0.06$ ). However, among women without PMS, no difference in postural displacement could be detected between phases.

## Measurements on a force plate (IV)

When the subjects were divided into the groups of PMS (n=16) and non-PMS women (n=9), an ANOVA for repeated measures detected a significant interaction between phase and group for both COP a-p direction ( $p<0.05$ ) and displacement area ( $p<0.05$ ), but no difference was seen in COP m-l direction. Post-hoc analysis revealed that in the PMS group, the total COP displacement in the a-p direction was significantly larger in the LP compared to the FP ( $p<0.05$ ) and there was a tendency towards a larger displacement in the LP compared to the OP ( $p<0.08$ ). The displacement area in the PMS group was significantly larger ( $p<0.05$ ) in the LP compared to the FP and OP. In the non-PMS group, no significant differences were found between the phases in either COP a-p direction or displacement area. All one-legged stance EC measurements were excluded since very few subjects were able to maintain their balance for 30 sec in this position. The difficulties of the one-legged stance EC were evenly distributed between the PMS group and the non-PMS group.

The criteria for PMS was different in study I/II and III/IV. Figure 11 shows the results of the displacement area when subjects in paper IV were divided into three groups: one group with three or more symptoms of PMS (n=8), one group with one to three symptoms (n=8) and one group with no symptoms at all (n=9). There was no significant difference between the three groups. However, the group with most symptoms showed the largest displacement area in the LP.

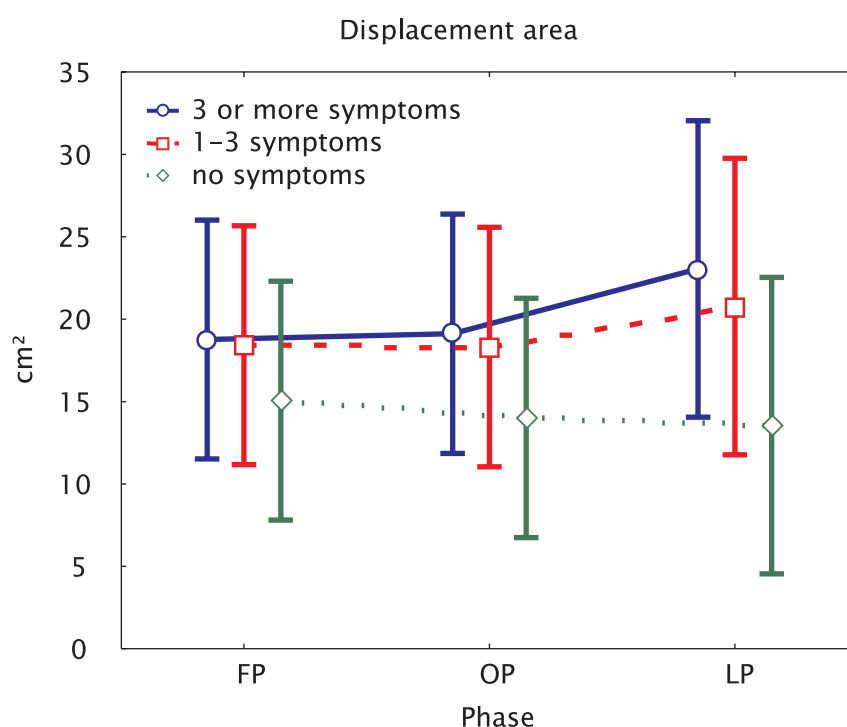


Fig 11. Results of displacement area (CI) in the follicular phase (FP), ovulatory phase (OP) and the luteal phase (LP) when subjects in paper IV were divided into three groups: one group with three or more PMS symptoms (n=8), one group with one to three PMS symptoms (n=8) and one group with no PMS symptoms at all (n=9).

There were no significant differences between groups. When comparing subjects without symptoms with subjects having one or more symptoms, the PMS group displayed altered postural control in the LP.



There were no significant differences in the two-legged stance, either with EO or EC, between the different phases of the menstrual cycle and there were no differences between the PMS and the non-PMS group in these variables.

In order to investigate the possibility of a learning effect in the testing of postural control in paper IV, subjects having performed all the tests during three complete menstrual cycles were grouped (n=18). No significant differences were found in postural control measures between the three consecutive menstrual cycles or for each phase within the menstrual cycle under any test condition.

No significant correlations between hormone levels and measurements of postural control were found in the respective phase.

## DISCUSSION

This thesis demonstrates a variation of knee joint kinesthesia, neuromuscular coordination and postural control during the menstrual cycle, whereas no difference in muscle strength and endurance was found. The menstrual phase variation of postural control was only shown in women with PMS.

### Muscle strength

The influence of the female sex steroids on muscle strength and muscle endurance has been discussed. It has been suggested that estrogen has an anabolic effect on the muscle by increasing glucose uptake and glycogen storage [12]. Circulating estradiol also influences exercise-induced GH secretion, which might be of importance for anabolic adaptations to exercise such as muscle hypertrophy and bone mineralization [39]. In contrast, progesterone impairs contraction-mediated glucose uptake and therefore might have catabolic effects on muscle metabolism [12].

However, in agreement with the most recent and hormonally controlled studies [31, 32, 42, 50], we were not able to detect any differences in muscle torque of the knee flexors and extensors during the menstrual cycle (I). The reason for the conflicting results from earlier studies showing phase differences [18, 75, 76, 84] might be the different ways of determining the menstrual cycle phases. In those studies, the phases were determined by counting the days of the menstrual cycle and no hormone analyses were performed. From our results, we conclude that muscle strength and endurance are not influenced by the hormonal variation during the menstrual cycle.

In spite of our results it cannot be excluded that the hormonal fluctuations during the menstrual cycle might be used for optimizing training programs for female athletes. In a study by Reis *et al* [79], the frequency of strength training was periodized in the menstrual cycle. The authors showed that an increased number of strength training sessions during the follicular phase compared to the luteal phase were beneficial for development of muscle strength. However, their study was performed on well-trained athletes and not moderately trained subjects like in the present study (I).

## Knee joint kinesthesia and neuromuscular coordination

Knee joint kinesthesia was impaired in the luteal phase compared to the follicular and ovulatory phases (III). Although the mean difference was small (0.2 degrees or 2 sec), 0.2 degrees represents approximately a 20 percent lowering of the detection ability and that might be of clinical importance. In this study, there was no significant difference between women with PMS compared to women without these symptoms. However, the PMS women had higher values for detection of movements in the luteal phase than non-PMS women. In study II, subjects with PMS had an overall greater threshold for detection of passive movements but no differences in knee joint kinesthesia could be detected between the three phases of the menstrual cycle. It may be explained by the small number of subjects in the study.

Neuromuscular coordination during the menstrual cycle was significantly enhanced during the ovulation phase compared to the other phases (III). However, there was no significant difference between the PMS group and the non-PMS group. Neuromuscular coordination was measured with square hop test, which is a functional test that requires repeated changes of directions [116]. Östenberg *et al* [116] showed that the square hop test did not correlate to isokinetic muscle torque and suggested that the square hop test also involves other skills than muscle strength such as endurance, neuromuscular coordination and postural control. Multi-directional, cutting movements are characteristic for many team sports like soccer and handball and in these sports, non-contact knee ligament injuries are common in women [65, 66]. It seems possible that the results of performance with the square hop test may be of importance for the risk of injury in these sports. It seems possible that performance with the square hop test may be related to the risk of injury in these sports.

Significant variations of knee joint kinesthesia and neuromuscular coordination during the menstrual cycle have previously not been reported and the mechanisms behind these changes are not known. Kinesthesia and neuromuscular coordination are complex functions regulated by the nervous and muscular systems. It can only be speculated whether sex hormones affect these functions via peripheral or central mechanisms. Although the influence of PMS on knee joint kinesthesia may suggest central mechanisms, fluctuations of estradiol and progesterone during the menstrual cycle might also affect peripheral receptors on tendons and ligaments and thereby alter kinesthesia. Previous studies have suggested that the variation of estradiol and progesterone during the menstrual cycle influences neurological and motor functions [88, 89, 114]. Increased levels of progesterone metabolites during the luteal phase are known to affect various transmitter and hormone systems, for example in the cerebellum, resulting in effects on motor function [90].

However, no correlation between knee joint kinesthesia and progesterone or estradiol levels during the luteal phase could be detected. Instead there may be a difference in the CNS sensitivity to these hormones e.g. at the receptor level, which may affect neurological and motor functions [95]. Poor concentration is one of the symptoms of PMS [2]. It cannot be excluded that impaired concentration during the luteal phase may be of importance for the results of knee joint kinesthesia. The mechanisms for variations in the performance of square hop test during the menstrual cycle seem to be different since the performance was enhanced in the ovulatory phase. The simultaneous increase of testosterone in this phase may play a role, since increased testosterone levels in women have been associated with enhanced physical performance [81].

## Postural control

This thesis demonstrated an altered postural control in the luteal phase in women with PMS but there were no differences in women without PMS. The “term altered postural control” in this thesis signifies an increased displacement of COP. We chose to not use the term “impaired postural control”, since it is controversial whether an increased displacement implies impairment or not [69]. In some neurological disorders a small displacement might be pathological.

Postural control was evaluated with two different methods. In paper II, postural control was measured with an ankle disc supported by four bars placed on a Statometer [48]. This method measures the reaction to a sudden perturbation and may be more like a real injury situation compared to measurements of quiet stance on a solid surface. However, there are some limitations to this method (see Methodological aspects below), and therefore an AMTI<sup>®</sup> force platform, which is well evaluated, was used in the following study. The results obtained from the two methods were similar. In study II, there was a tendency towards a larger displacement in the luteal phase in the PMS group but the small number of subjects limited that study. Paper IV confirmed an altered postural control in the luteal phase of women having PMS.

To our knowledge, there are no previous reports on postural control during the menstrual cycle in women with PMS and only one previous study investigating postural control in premenopausal women. Darlington *et al* detected a greater displacement in the luteal phase and the early follicular phase of the menstrual cycle [17]. However, the influence of PMS was not investigated in that study.

The mechanisms of our findings are not elucidated. There were no differences in hormonal levels between women with PMS compared to women without PMS, which is in agreement with the findings of Bäckström *et al* [11]. Thus, differences in steroid hormone levels do not seem to explain the differences in postural control in the two groups. Instead there may be a difference in the CNS sensitivity to circulating sex steroids [95]. Progesterone increases during the luteal phase and is metabolised to allopregnanolone (3 $\alpha$ -OH-5 $\alpha$ -pregnan-20-one) and pregnanolone (3 $\alpha$ -OH-5 $\beta$ -pregnan-20-one) [19]. Both are neurosteroids and active agonists on the GABA-A receptor [57] with an action similar to benzodiazepins and ethanol. In animal studies, it is shown that some of the effects of alcohol are mediated via increased productions of allopregnanolone [47, 62, 63]. Long-term treatment with allopregnanolone has been reported to have anxiolytic effects in animals [105], while short-term treatment has been reported to induce anxiety [30]. Furthermore, allopregnanolone has been shown to inhibit learning and memory [43], and increase appetite in animal studies [15]. In humans, a high dose of pregnanolone is hypnotic and anesthetic [13, 95]. In parallel with inducing sedation, allopregnanolone has also been shown to induce disturbances in motor function [92, 103]. These effects are related to the CNS and cerebellum. Furthermore, anxiety and adverse changes in mood states have recently been reported to alter postural control [10, 113]. Adverse moods during the menstrual cycle develop shortly after ovulation, at the same time as the plasma progesterone and allopregnanolone concentrations start to increase [11, 102].

A possible explanation for the altered postural control in the luteal phase of the PMS women may be an increased conversion rate of progesterone to neurosteroids affecting balance and motor function. We suggest that female sex hormones act in the CNS to influence postural control. Further studies are needed to clarify the precise mechanisms for menstrual cycle related variations in postural control.

## Clinical implications

A variation of knee joint kinesthesia, neuromuscular coordination and postural control during the menstrual cycle may be of importance for the increased risk of injuries among female athletes compared to male athletes [4, 9, 40, 65, 66]. Various factors have been suggested to be involved in the increased incidence of female ACL injuries, e.g. gender differences in muscle strength, muscular activation pattern, knee stiffness and jumping and landing characteristics [40, 109, 112]. This thesis (I) and several previous studies [31, 32, 42, 50] have shown that muscle strength does not vary across the menstrual cycle and thus, can not be related to the increased injury rate in women during specific menstrual phases. Gender differences in neuromuscular control [29] and the more “quadriceps dependent” knee in women [14, 41, 58] may be of importance for knee injuries in female athletes. Hewett [36] also suggested that females tend to be more “ligament-dominant” in their joint control strategy while male athletes seem to be more “muscle dominant”. Neuromuscular training, with the aim of stabilizing the knee joint, may decrease the injury rate in female athletes [36, 64]. Increased muscle strength and physical fitness may also prevent injuries. Jones *et al* [45] suggest that gender *per se* is not a risk factor for injury; it is more likely that the underlying risk factor is physical fitness. Even though muscle strength does not vary across the menstrual cycle, the hormonal fluctuations during the menstrual cycle might be used for optimizing training programs.

Möller Nielsen & Hammar and Myklebust *et al* [66, 67] have found an increased risk of injury in the luteal phase. Our results of impaired kinesthesia and altered postural control in the luteal phase, especially in women with PMS, may be related to this increased risk of injury. However, since the present thesis only included moderately active women, the findings cannot directly be applied to highly trained female athletes. Based on our results, the advice to physically active women is not to interrupt training and competing during the luteal phase. Instead it is recommended to make use of neuromuscular training programs in order to prevent ACL injuries [36, 64]. The knowledge from this thesis should also be considered in training and rehabilitation programs for female athletes. Further and extended research, including highly trained athletes, is needed to increase our knowledge about risk factors for injury in order to enhance the possibilities for prevention and to improve training advice to female athletes.

## Methodological aspects

The present thesis showed a high dropout rate. The reason for this was mainly due to difficulties for the subjects to detect ovulation with the Ovustix<sup>®</sup> even after directions from the test leader. In paper I, the subjects were tested a little too late in the ovulatory phase in the first menstrual cycle according to the rather small increase of serum LH. Several cycles were also anovulatory, which is known to be common in a test situation. The ideal situation would have been to analyse the blood samples immediately to insure that the subjects were tested in the right phase. Furthermore, the subjects were followed intensively for a relatively long period of time, two (I, II) and three (III, IV) menstrual cycles respectively, which most likely influenced the dropout rate.

The criteria for PMS differed between studies I/II and studies II/IV. One reason for the chosen criteria in studies I/II was the small material. If the subjects in these studies had been grouped according to ICD-10, only two subjects would have been in the non-PMS group. When the subjects in paper III/IV were divided into three groups according to the different criteria for PMS (no symptoms, one to three symptoms and three or more symptoms), the group with most symptoms showed the largest displacement area in the luteal phase. It may suggest an association to the degree of PMS symptoms although there were no significant differences between the groups.

Some limitations of the square hop test should also be considered. A possible learning effect is always a problem when performing physical tests. In the present study, this possibility was at least partially controlled by the subjects performing three sessions of familiarization on three different days before the study started. Another limitation of this test was the recording of number of jumps by the test leader. Still, the inter- and intra-test reliability for this method has been shown to be  $r=0.94$  and  $0.74$ , respectively [116]. The square hop test should be tested for validity, but since there is no apparent golden standard for measurement of neuromuscular coordination this is not easy to perform.

The measurement of postural control on the Statometer also has some limitations. The method includes a manual removal of the supporting bars that may be affected by the amount of force used to pull on the release handle. The ankle disc was also able to rotate when the disc became unstable. However, the method was previously tested for reliability with good results (ICC 0.84-0.87) [Lindgren & Leanderson, 1999, unpublished data].

In study IV, postural control was investigated with a well-proven AMTI<sup>®</sup> force platform standing on two legs as well as one leg with eyes open and closed [25]. However, no data are yet available to show which variable of the stabilometry test provides the best measure of postural control in one-legged stance [16]. The COP displacement in the a-p and m-l direction and the ellipse area method used in the present study (IV) are two commonly used variables. Center of pressure measures on an AMTI<sup>®</sup> force platform have previously been tested for reliability with acceptable variations [25]. Intra-subject variability has often been explained by a learning effect [100], but in the present study, the subjects performed three sessions of familiarization trials on three different days before the study started. This probably limited the effect of learning in the study and when analyzing data of the 18 subjects who completed all three consecutive menstrual cycles, no difference between cycles or between phases could be detected. The measurements of postural control on one leg with closed eyes had to be excluded since very few subjects were able to maintain their balance for 30 sec in that position. This is also reported by Ekdahl *et al* [25].

## Future prospects

In future studies the role of neurosteroids for balance and motor function will be addressed. Of particular interest is allopregnanolone, and we aim to study the association between serum levels of allopregnanolone and postural control. Furthermore, the effects of oral contraceptives (OC) on neuromuscular performance and postural control in women with PMS compared to women without PMS are of interest. This is an important issue since many women use OC. Furthermore, there is support that OC may protect from athletic injuries [67]. Still, the association between athletic injuries and the menstrual cycle is not fully elucidated. Further prospective studies in trained athletes are therefore warranted.



## GENERAL CONCLUSIONS

The major conclusions based on the results of the studies described in the papers (I-IV):

- Muscle strength and muscle endurance do not vary between different well-determined menstrual cycle phases in moderately trained young women (I).
- Knee joint kinesthesia is impaired in the mid luteal phase of the menstrual cycle (III) and women with premenstrual symptoms have an overall higher threshold to detect a passive motion in the knee joint compared to women without these symptoms (II). Neuromuscular coordination evaluated with square hop test is enhanced in the ovulatory phase of the menstrual cycle (III).
- Postural control is altered in the mid luteal phase in women with premenstrual symptoms but in women without these symptoms no significant difference could be detected (II, IV).
- No correlations between steroid hormone levels and the results of muscle strength, neuromuscular coordination, knee joint kinesthesia and postural control during the menstrual cycle could be detected.

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