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# **THE INFLUENCE OF SEX HORMONES ON NEUROMUSCULAR FUNCTION AND PREMENSTRUAL SYMPTOMS**

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The influence of sex hormones on neuromuscular function  
and premenstrual symptoms  
THESIS FOR DOCTORAL DEGREE (Ph.D.)

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*“ Ju mer man tänker desto mer inser man att det inte finns något enkelt svar”*  
*” The harder you think the more you realize there is no simple answer”*

*Nalle Puh*

## **ABSTRACT**

The menstrual cycle with fluctuation in female sex hormones has been suggested to influence neuromuscular function. However, variation in ligament laxity, soft tissue stiffness, and skeletal muscle strength across the menstrual cycle has led to conflicting results.

Furthermore, premenstrual symptoms (PMS) have been suggested to have a negative impact on neuromuscular performance and contribute to the risk of musculoskeletal injury in physically active women. On the other hand, the use of oral contraceptives (OCs) might protect from injury. The mechanisms of the influence of sex hormones on neuromuscular function and PMS are not fully understood.

The overall aim of this thesis was to explore the effect of endogenous sex hormones and OCs on muscle strength, postural control, and PMS in healthy women with moderate physical activity. A further aim was to explore the expression of sex hormone receptors in skeletal muscle during three well-defined phases of the menstrual cycle.

Postural control was measured during the active and hormone-free phase of OC treatment in physically active women with or without PMS as evaluated by prospective symptom rating. In the same cohort of women, muscle strength and hop performance were measured during one OC cycle and one normal menstrual cycle at three specific phases, using a cross-over design. Furthermore, changes in PMS in the same women starting to use or discontinuing the use of OCs were evaluated. In another cohort of healthy women, muscle biopsies were collected from the musculus vastus lateralis in the follicular phase, the ovulatory phase, and the luteal phase of the menstrual cycle for determination of mRNA and protein levels of sex steroid hormone receptors.

Women with PMS displayed a significant change in postural control (greater displacement area) during the active OC phase compared to the withdrawal phase of OC treatment, whereas women without such symptoms showed no variation in postural control during OC treatment. Muscle strength and hop performance did not vary during the different phases of the normal menstrual cycle, or during OC treatment. In women with PMS, OC treatment decreased ratings of premenstrual somatic symptoms, but not of negative mood symptoms. Gene and protein levels of estrogen receptor alpha and the progesterone receptor varied significantly during the three hormonally confirmed phases of the normal menstrual cycle.

The results of this thesis indicate that PMS influences postural control and OC treatment decreases PMS of somatic type. Furthermore, muscle strength and hop performance are not influenced by endogenous and exogenous sex hormones. The variation in expression of sex hormone receptors in skeletal muscle may have an impact on the effects of muscular training and sports injuries in women.

*Key words: postural control, balance, muscle strength, estrogen, progesterone, hormone receptor, oral contraceptives.*

## SAMMANFATTNING

Kvinnor har en ökad skaderisk för muskuloskeletala skador inom idrott i jämförelse med män. Menstruationscykeln med variation av könshormoner har föreslagits kunna påverka neuromuskulär funktion. Dock har studier visat varierande resultat när laxitet i ligament, muskulär stelhet och muskelstyrka har relaterats till olika faser i menstruationscykeln. Vidare förefaller förekomst av premenstruella symtom (PMS) kunna bidra till högre skadefrekvens genom påverkan på balans och neuromuskulär funktion. Däremot kan p-piller möjligen minska risken för muskuloskeletala skador hos aktiva kvinnor. Mekanismerna för hur könshormoner påverkar neuromuskulär funktion och PMS är inte helt kartlagda.

Syftet med föreliggande avhandling var att undersöka effekten av kroppsegna könshormoner, p-piller samt PMS på balans, muskelstyrka och hoppfunktion. Ett annat syfte var att studera uttrycket av hormonreceptorer i muskelvävnad vid tre väldefinierade faser under menstruationscykeln.

Balans mättes i enbensstående på en kraftplatta under den aktiva samt den hormonfria fasen vid p-pillerbehandling hos fysiskt aktiva kvinnor med och utan PMS. Detta utfördes med prospektiv daglig självskattning av symtom. I samma studiekohort mättes muskelstyrka och hoppfunktion under en p-pillercykel samt vid tre specifika faser i en menstruationscykel med crossover design. Dessutom undersöktes förändringar av PMS hos kvinnor som började använda eller slutade använda p-piller. I en annan studiekohort av friska kvinnor togs muskelbiopsier från lårmuskulaturen i follikelfas, ovulationsfas samt lutealfas under menstruationscykeln för att analysera mRNA- och proteinnivåer av könshormonreceptorer.

Kvinnor med PMS uppvisade signifikanta förändringar i balans under den aktiva p-pillerfasen i jämförelse med uppehållsfasen. Dessa förändringar sågs inte hos kvinnor utan PMS under p-pillermedicinering. För muskelstyrka och hoppfunktion påvisades ingen variation under olika faser i menstruationscykeln eller under p-pillermedicinering. För kvinnor med PMS, visade sig p-piller signifikant kunna minska fysiska symtom, såsom bröstspänning och svullnad, men inte negativa psykologiska symtom, såsom irritabilitet och nedstämdhet. Genuttryck och proteinnivåer av östrogen receptor alpha och progesteron receptorn visade sig variera signifikant under tre hormonellt verifierade faser av menstruationscykeln.

Resultatet av denna avhandling visar att PMS påverkar balans och att p-pillermedicinering kan ge minskade fysiska symtom. Dessutom visades att muskelstyrka och hoppfunktion inte påverkas av vare sig av kroppsegna hormoner eller syntetiska hormoner i p-piller. Hypotetiskt kan variationen i uttrycket av könshormonreceptorer i skelettmuskulatur påverka muskulär träning och risken för idrottsskador hos kvinnor under olika faser av menstruationscykeln.

## LIST OF SCIENTIFIC PAPERS

- I. **Ekenros L**, Hirschberg AL, Bäckström T, Fridén C  
Postural control in women with premenstrual symptoms during oral contraceptive treatment. *Acta Obstetrica et Gynecologica Scandinavica* 2011; 90: 97-102
- II. **Ekenros L**, Hirschberg AL, Heijne A, Fridén C  
Oral contraceptives do not affect muscle strength and hop performance in active women. *Clinical Journal of Sports Medicine* 2013; 23: 202-207
- III. **Ekenros L**, Hirschberg AL, Bäckström T, Fridén C  
Premenstrual symptoms in women using and not using oral contraceptives.  
In manuscript
- IV. **Ekenros L\***, Papoutsi Z\*, Fridén C, Dahlman Wright K, Hirschberg AL  
Expression of sex steroid hormone receptors in human skeletal muscle during the menstrual cycle. *Acta Physiologica* 2017; 219: 486-493

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## LIST OF ABBREVIATIONS

ACL	Anterior Cruciate Ligament
ANOVA	Analyses of Variance
AR	Androgen Receptor
CI	Confidence Interval
CNS	Central Nervous System
CoP	Center of Pressure
CoG	Center of Gravity
ER	Estrogen Receptor
E2	Estradiol
FP	Follicular Phase
Free T	Free Testosterone
FSH	Follicular Stimulating Hormone
GABA	Gamma( $\gamma$ )-Amino Butyric Acid
GnRH	Gonadotrophin Releasing Hormone
GRF	Ground Reaction Force
ICC	Intraclass Correlation Coefficient
ICD	International Classification of Diseases
IGF-I	Insulin-like growth factor-I
LH	Luteinising Hormone
LP	Luteal Phase
OC	Oral Contraceptive
OP	Ovulatory Phase
PR	Progesterone Receptor
P-4	Progesterone
PMDD	Premenstrual Dysphoric Disorder
PMS	Premenstrual Symptoms
RBA	Relative binding affinity
SHBG	Sex Hormone-Binding Globulin
SSRIs	Selective Serotonin Reuptake Inhibitors
T	Testosterone

## LIST OF DEFINITIONS

In the present thesis, the following abbreviations were used to define a certain feature.

CD scale	Assessment tool used for rating of premenstrual symptoms
Cyclic	A cyclic increase in premenstrual symptoms during the luteal phase compared to the follicular phase of the menstrual cycle
Cycle	The entire menstrual cycle from menses to menses, in both a normal menstrual cycle or during oral contraceptive cycle
Endogenous sex hormones	The natural sex hormones in females (estradiol, progesterone, testosterone).
Exogenous sex hormones	The synthetic hormones in oral contraceptives
Muscle torque	A force that produce a rotation or torsion
Non-OC phase	The withdrawal phase of the oral contraceptive cycle
Non OC-Starters	The women who were enrolled having a normal menstrual cycle (not using oral contraceptives)
Non-OC user	A woman having a normal menstrual cycle
OC user	A woman during oral contraceptive use
OC phase	The active hormone phase of the oral contraceptive cycle
OC-Starters	The women who were enrolled and were taking oral contraceptives.
Sex hormones	Umbrella term used in this thesis to describe the endogenous sex steroid hormones (estradiol, progesterone, testosterone) and the synthetic hormones in oral contraceptives (ethinyl estradiol and progestagens)

# 1 INTRODUCTION

The benefits of regular physical activity for somatic and mental health are remarkable for humans of all ages. The number of women taking part in sports at all levels is steadily increasing; for example, there are races devoted to women only, such as “Tjejmilen,” “Tjejvasan,” and “Tjejvättern.” Also in team sports, there is a growing number of female participants. Disquietingly, women seem to be more vulnerable to sustaining traumatic injuries during jumping and cutting sports (Arendt et al., 1999; Boden et al., 2000; Huston et al., 2000 *review*; Myklebust et al., 1997; Åman et al., 2016) and they also have a higher rate of overuse injuries compared to their male counterparts (Brukner & Bennel, 1997; Ross et al., 2015; Schroeder et al., 2015). Besides, it has been shown that these injuries occur earlier in life in females (Myklebust et al., 1998; Schroeder et al., 2015).

There are obvious anatomical and physiological differences between the mature female and male that explain the mean difference in physical performance between the sexes (Drinkwater, 2008). However, the most basic feature in women is the menstrual cycle with the fluctuation in sex hormones throughout the month. The sex hormones involved in the menstrual cycle are estradiol (E2), progesterone (P-4), testosterone (T), follicle-stimulating hormone (FSH), and lutenizing hormone (LH). Some of these hormones have been suggested to play a role in the etiology of musculoskeletal injuries in women. Additionally, the presence of premenstrual symptoms (PMS), such as breast tenderness, depressed mood, and irritability, has also been suggested to be a risk factor for injuries in women (Möller-Nilsen & Hammar, 1989). PMS of variable severity are common in fertile-aged women. Despite the well-known symptoms, the etiology of PMS has still not been fully elucidated. PMS seems to be caused by mechanisms in the central nervous system (CNS) and has been shown to alter postural control in women (Fridén et al., 2003b; 2005).

As a physiotherapist, working at a clinic specialized in sports medicine, I meet females with traumatic and overuse injuries. They describe how debilitating the injury is and how it interferes with their work and social and physical activities. Besides, it is also known that there might be increased risks of developing osteoarthritis after having a serious traumatic injury: for example, rupture of the anterior cruciate ligament (ACL) in the knee. To gain a better understanding of the mechanisms of musculoskeletal injuries in women, it is important to find out more about the hormonal effects, both endogenous and exogenous, as in the case of oral contraceptives (OCs), and the eventual effects of PMS on physical performance.

This thesis is focused on studies aimed at evaluating postural control, muscle strength, and hop performance in relation to OC use and PMS in physically active women. In addition, we characterized the expression of sex steroid hormone receptors during the menstrual cycle. The main concepts of this thesis are illustrated in Fig. 1.

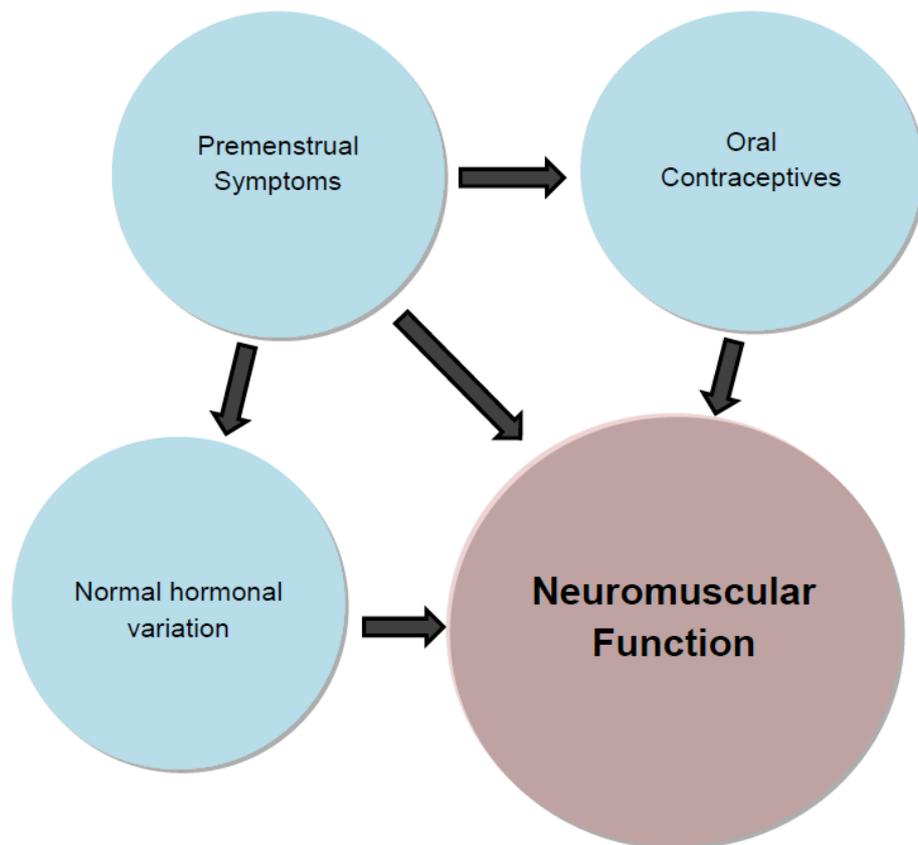


Fig. 1. The main concepts of the present thesis.



## 2 BACKGROUND

### 2.1 THE SKELETAL MUSCLE

#### 2.1.1 Skeletal muscle physiology

The skeletal muscle system is one of the largest systems in the body. Skeletal muscles act on the skeleton via voluntary control through the CNS (Watras, 2008). There are several functions of the skeletal muscles: maintenance of posture, locomotion, and respiration. The basic structure of the skeletal muscle is shown in Fig. 2. The skeletal muscle comprises numerous elongated cells called muscle fibers, which are grouped together into fascicles that, together, form the muscle. Connective tissue surrounds each muscle fiber (endomysium), each fascicle (perimysium), and the entire muscle (epimysium). At the end of the muscle, the connective tissue layers emanate together to form a tendon that attaches the muscle to the skeleton (Watras, 2008).

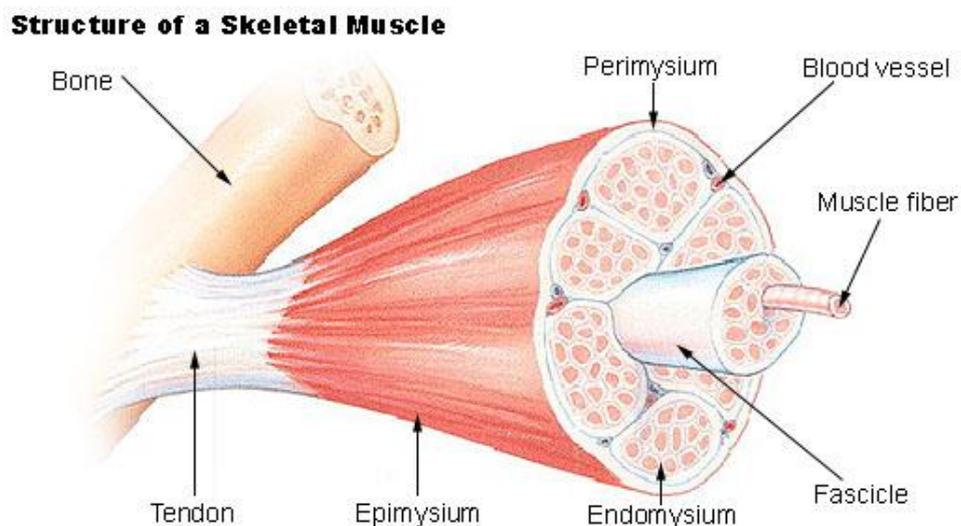


Fig. 2. Structure of the skeletal muscle.

Adapted from [https://upload.wikimedia.org/wikipedia/commons/e/ef/Skeletal\\_muscle\\_diagram.jpg](https://upload.wikimedia.org/wikipedia/commons/e/ef/Skeletal_muscle_diagram.jpg)

Muscle fibers are supplied by  $\alpha$ - and  $\gamma$ -motor neurons which, together with muscle fibers, form the motor unit. The size of the motor unit depends on how precise a control of the muscle is required. For example, in the finger muscles, a single  $\alpha$ -motor neuron supplies only a few muscle fibers, compared to the muscle in the thigh, where a single  $\alpha$ -motor neuron can

supply numerous muscle fibers. The motor unit is the functional contractile unit since all the muscle fibers within the motor unit contract synchronously when the motor nerve fires (Rubinson & Lang, 2008). Contracting muscles require large quantities of energy. Depending on the duration and intensity of the work, as well as the availability of oxygen to meet the demands of the working muscle, there are three integrated systems of metabolism in the muscle fibre: aerobic metabolism (e.g., during endurance exercises), anaerobic metabolism (e.g., during high power output), and alactacid breakdown of adenosine triphosphate (ATP) (Gastin, 2001 *review*). It should be mentioned that these systems interact in the muscle in various proportions (Gastin, 2001 *review*).

The muscular system comprises over 40% of the body's mass in males and about 23% of the body's mass in females (Cureton, 1998). This explains the mean difference in muscle strength between the sexes. The difference in muscle mass and strength is greater in the upper body, compared to the lower limbs (Hewett, 2000 *review*).

## **2.2 POSTURAL CONTROL**

### **2.2.1 Postural control – definition**

Postural control is used synonymously with postural balance and is defined as the ability to achieve a state of equilibrium by controlling the body's center of mass (CoM) in relation to the body's base of support (BoS) in standing, as well as during complex motor tasks (Horak & Macpherson, 1996; Lephart & Fu, 2000). The functional goal of postural control is to maintain postural equilibrium and postural orientation (Horak, 2006). Postural control is complex and is influenced by several bodily systems, such as the sensory, neuromuscular and cognitive systems within the CNS and the peripheral nervous system (PNS). It includes systems involved in higher-level planning (frontal cortex and motor cortex in the forebrain), coordination (in the brainstem and spinal networks coordinating muscle response synergies), and generation of forces (in the motor neurons and the muscle) which produce effective movements to control the body's posture in space (Shumway-Cook & Woollacott, 2012).

#### **2.2.1.1 Neuromuscular control**

Neuromuscular control is the dynamic input that assists in the maintenance of equilibrium via afferent information and efferent motor response (Riemann & Lephart, 2002). The

control of balance involves continuous feedback and feed-forward in processing visual, vestibular, and somatosensory inputs and executing neuromuscular actions (Shumway-Cook & Woollacott, 2012). The somatosensory system consists of specifically designed peripheral receptors that exist in the muscles (the muscle spindle), tendons (Golgi tendon organ) and the joints, and in the skin. These receptors detect changes in the specific structure, such as stretch and pressure and send afferent information about changes in body position to higher centers within the CNS (Riemann & Lephart, 2002; Shumway-Cook & Woollacott, 2012). This information is processed in the CNS and efferent information is sent out via  $\alpha$ -motor neurons to the muscles in action (Fig. 3). The feed-forward control is an anticipatory strategy used to prevent disturbance of the CoM with small, unconscious body movement before and during voluntary movements (Shumway-Cook & Woollacott, 2012).

### Postural Equilibrium & Postural Orientation

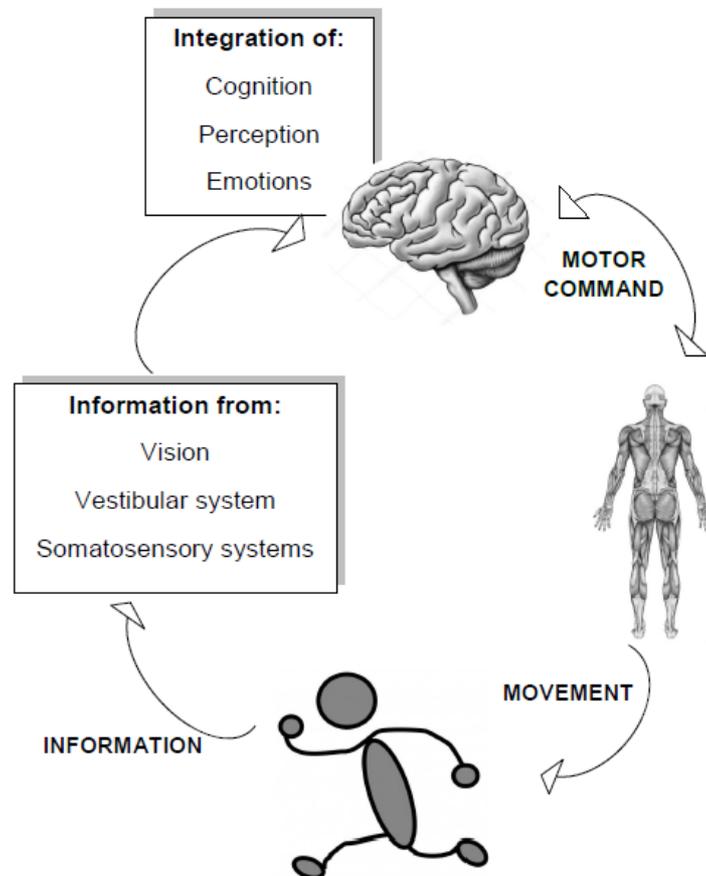


Fig. 3. Illustration of the systems involved in neuromuscular control.

### **2.2.2 Postural control – evaluation**

Stabilometry or kinetic analyses are techniques used to evaluate the forces that contribute to movement or stability. It can be assessed by using a force platform that measures ground reaction forces (GRFs), which are the forces exerted by the ground on the body in contact with it. The resultant force of all the GRFs emanates to the point of attack and this is the center of pressure (CoP) (Winter, 1995;1998). Quiet stance is characterized by small amounts of spontaneous postural sway that contribute to postural stability in this situation (Shumway-Cook & Woollacott, 2012; Winter, 1998). The size and shape of the BoS can be varied, e.g., due to two-legged stance, one-legged stance, and tandem stance in different settings.

Dynamic postural control and neuromuscular movement patterns can be evaluated by kinematic analyses. Kinematics measures motion independent of the causative forces by tracking body segments during movement tasks (Winter, 1995) There are different techniques in the laboratory setting to measure the body's movement in space: for example, by a three-dimensional optoelectronic system (Wikström et al., 2004; Winter, 1995).

In clinical settings, several validated measurement tools and test batteries have been developed to evaluate balance and postural control in specific patient groups with altered postural control (e.g., neurological diseases) (Horak et al., 2009) and for sports medicine purposes (Wikström et al., 2006). The assessment of postural control has several implications for identifying subjects with an elevated risk of musculoskeletal injuries and evaluating the effects of rehabilitation programs (Rogind et al., 2003).

### **2.2.3 Neuromuscular aspects of musculoskeletal injuries in females**

Females have a three- to six-fold higher incidence of sustaining an ACL injury in sports than males. Evidence in the literature suggest poor neuromuscular control in the biomechanics of the lower limb, and in particular the knee joint during the execution of potential hazardous sporting movements, is a primary contributor to the female ACL injury mechanism (Hewett, 2000 *review*; Hewett et al., 2005; McLean et al., 2004, Renström et al., 2008 *review*).

Differences between females and males in neuromuscular recruitment patterns and forces of landing have been proposed to explain the discrepancy. Females have been suggested to be dominant in their quadriceps muscle with response to an anterior tibial translation and extension of the knee, during landing from a jump (Hewett et al. 2006; Renström et al., 2008

*review*). The knee extensor muscles are antagonists to the ACL and increase strain on the ACL in knee flexion angles below 45°. In contrast, males are more hamstrings dominant and, by landing in a knee flexion pattern, the recruitment of hamstring muscle contributes to stabilization of the knee as a dynamic agonist to the ACL (Hewett, 2000 *review*; Renström et al., 2008 *review*). Further, females have shown differences in hip muscle activity with a greater femoral internal rotation and hip adduction that place the knee in a valgus position (Zeller et al., 2003).

Disturbance in postural control and neuromuscular function seems to be a cornerstone for understanding the elevated risk of musculoskeletal injuries in women. In the present thesis, postural control was evaluated in quiet standing in relation to hormonal variation during the use of OC, as well as the influence of PMS (Paper I).

### 2.3 THE MENSTRUAL CYCLE

An average menstrual cycle lasts for 28 days in regularly menstruating women. The menstrual cycle involves the interaction of several endocrine glands and a responsive uterus which are controlled by a feedback system in the hypothalamus–pituitary–gonadal axis (Fig. 4). The most important hormones that control the menstrual cycle are gonadotropin-releasing hormone (GnRH), produced in the hypothalamus, FSH and LH released from the anterior pituitary, and E2 and P-4 from the ovaries (Beshay & Carr, 2013; Drinkwater, 2008;).

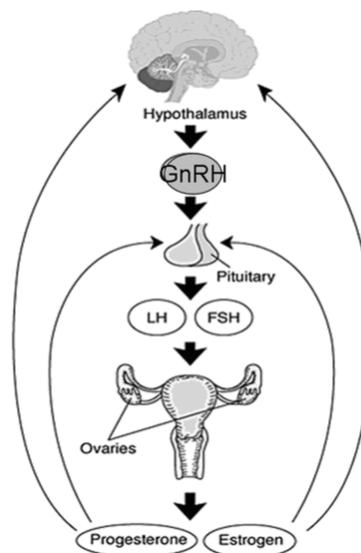


Fig. 4. The hypothalamus–pituitary–gonadal axis feed-back system for production of sex hormones. Adapted with the kind permission of CCC Rights Link® (Popat, Prodanov, Calis & Nelson, 2008).

The menstrual cycle can be divided into three hormonally different phases: the follicular phase, the ovulatory phase, and the luteal phase (Fig. 5). The follicular phase starts with the first day of menstrual bleeding (cycle day 1). During the follicular phase, FSH from the pituitary stimulates the maturation of antral follicles in the ovaries. After about one week, a selection of a predominant follicle occurs. When this follicle grows, the level of E2 steadily increases to reach the highest levels just before ovulation. At the peak of the follicular growth, ovulation takes place, with a rapid increase of LH (the LH surge) from the pituitary. LH is released as a result of a positive feedback action from high circulation levels of E2 on the anterior pituitary. An oocyte is released about 12 h after the LH surge and, with this action, the levels of E2 decline for a few days. The secretion of androgens such as androstendione and T from the ovary is stimulated by LH and the androgen level peaks simultaneously with the LH surge. The action of the androgens at this point is to enhance atresia of non-dominant follicles and to stimulate libido (Beshay & Carr, 2013; Fritz & Speroff, 2011). During the luteal phase, the ruptured follicle is formed into the corpus luteum, which secretes both E2 and P-4. One week after ovulation, during the mid-luteal phase, the levels of P-4 peak together with a secondary rise of the E2 levels. In the case of non-fertilization, the corpus luteum will gradually degenerate and the production of sex hormones will decline. The withdrawal of E2 and P-4 causes a degradation of the endometrium and next menstruation starts (Drinkwater, 2008).

Although the median duration of a menstrual cycle is 28 days, the normal range can vary between 25–30 days (Beshay & Carr, 2013). The luteal phase is about 14 days in most regularly menstruating women, but the follicular phase can vary more in length. The biological variety in cycle length between individuals and the fact that spontaneous anovulatory cycles are common causes methodological problems in menstrual cycle-related studies (Metcalf et al., 1980). In these kinds of studies, it is therefore necessary to determine different cycle phases with hormone analyses or a gynaecological evaluation (Sarwar et al., 1996). Since several factors such as diurnal variation, stress, and physical activity might affect the hormone levels, a standardized protocol for blood sampling is a prerequisite (Constantini et al., 2005; Vescovi, 2011).

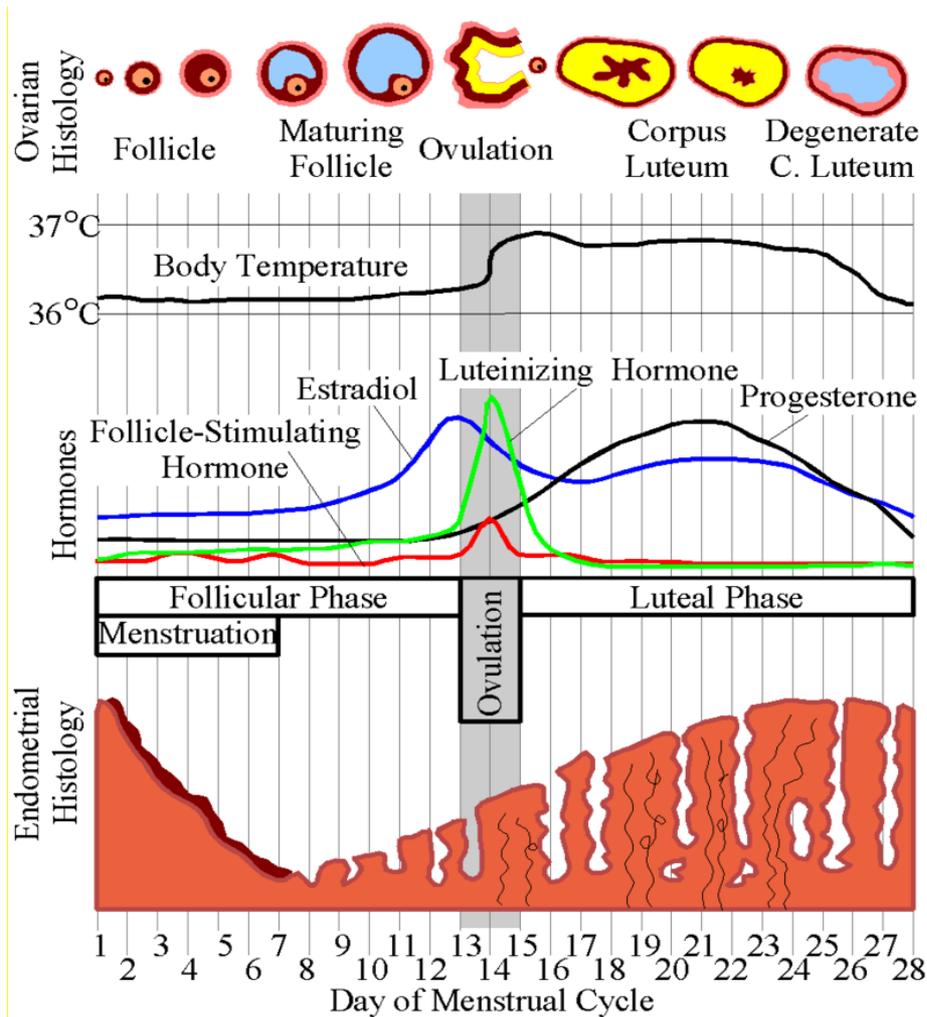


Fig. 5. A normal menstrual cycle lasts in most regularly menstruating women for 28 days and can be divided into the follicular phase, the ovulation phase, and the luteal phase.

The menstrual cycle: <http://commons.wikimedia.org/wiki/File:MenstrualCycle.png> by Chris73 (Licens Creative Commons BY NC SA)

## 2.4 SEX HORMONES

The sex hormones belong to a group of structurally related hormones, steroid hormones that are synthesized from cholesterol. Steroid hormones are lipophilic and can readily cross both the cell membrane and the nucleus membrane (Lackie, 2015). The biological effects of sex steroids can be displayed through genomic mechanisms by ligand binding to intracellular nuclear receptors or, more rapidly, by a non-genomic mechanism through membrane-bound receptors (Fig. 6) (McEwen, 2012 review; Sakamoto et al., 2012). After the ligand binding, the regulation of gene expression and production of proteins is initiated (McEwen, 2002).

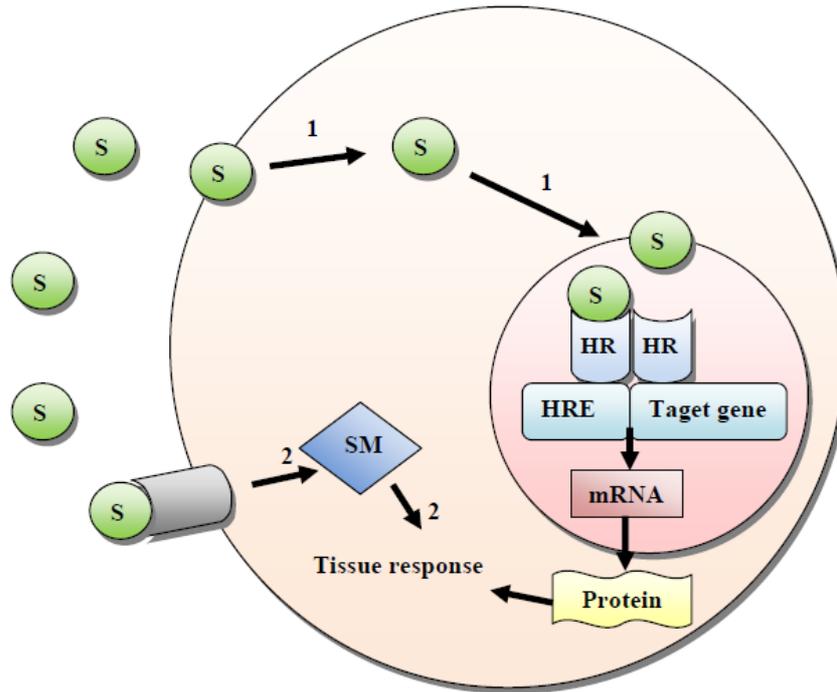


Fig. 6. Simplified illustration of the mechanism of signalling pathways for steroids: *Pathway 1*, genomic binding (classical, transcriptional), the steroid (S) binds to an intranuclear receptor the receptor dimerize and binds to the genome and activates the hormone responsive element (HRE), leading to transcription of messenger ribonucleic acid (mRNA) and protein synthesis. *Pathway 2*, non-genomic binding, the steroid (S) binds to a membrane-bound receptor for rapid signalling via flux of ions through the membrane or second-messenger (SM) signalling usually changing the membrane potential making it easier or more difficult for action potential generation.

### 2.4.1 Estrogen

Three types of estrogens are present in the human body: the most dominant and potent estrogen is E<sub>2</sub>, which is produced by the granulosa cells in the ovary. Additionally, E<sub>2</sub> can be produced by aromatization from androgens in other tissues like brain, fat, and in the liver (Brodie & Inkster, 1993; Miller, 1991) (Fig. 7). The other less potent estrogens, estrone (E<sub>1</sub>) and estriol (E<sub>3</sub>) are produced from E<sub>2</sub> by conversion in peripheral tissues. Most of the estrogens are bound to carrier proteins and are transported to the target tissues. Albumin carries 60% of the estrogens and 38% is carried by sex hormone-binding globulin (SHBG). The remaining 2% is free in the blood circulation and this fraction can enter the cells in the human body and bind to its specific receptor. There are two known nuclear estrogen receptors (ERs) in the human body, ER $\alpha$  and ER $\beta$ . The functions of estrogens are varied with actions on the reproductive system, the nervous system (Joels, 1997; McEwen, 2012 *review*), the

skeletal system (Fritz & Spearoff, 2011) the immune system (Carlsten et al., 1989), and the muscular system (Wiik et al., 2003; 2005).

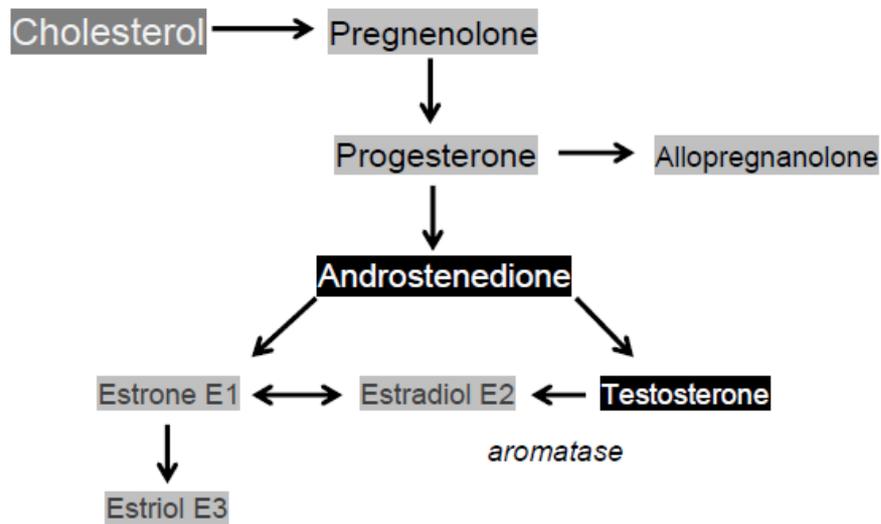


Fig. 7. Illustration of steroid hormone synthesis.

#### 2.4.1.1 Estrogen effects on skeletal muscles

Receptors for estrogens, both the ER $\alpha$  and the ER $\beta$ , have been demonstrated in human skeletal muscle cells (Lemoine et al., 2003; Wiik et al., 2003; 2009). Furthermore, receptors for estrogens have been detected in human ACL (Lie et al., 1997; Liu et al., 1996; Yu et al., 1999). Wiik et al., (2005) have shown that ERs' in the skeletal muscle are up regulated in response to endurance training in men. The effects of estrogen on human skeletal muscle function have not, however, been fully elucidated. Animal studies support the view that estrogen has anabolic properties, including contractile function, as well as playing an important role in stimulating muscle repair and regenerative processes with an enhanced activation of silent satellite cells (Enns & Tiidus, 2010 *review*; Skelton et al., 1999). In humans, studies on the acute effects of high levels of estrogen on muscle strength during the menstrual cycle have shown contradictory results. Phillips et al., (1996) and Sarwar et al., (1996) have demonstrated an increased maximal voluntary isometric muscle force during ovulation (cycle days 12–18) in healthy women, when the levels of E2 are at the highest. Other authors have not been able to show any differences in muscle strength during the menstrual cycle in healthy fertile-aged women (Fridén et al., 2003; Gür, 1997; Gür et al.,

1999; Janse de Jonge et al., 2001; Lebrun et al. 1995; Montgomery & Shultz, 2010). In two recent studies on postmenopausal women, estrogen replacement therapies demonstrated positive effects on reduction of the age-related decline in muscle strength (Dieli - Conwright et al., 2009; Ronkainen et al., 2009). However, other studies have not been able to show any effects of estrogen supplement on muscle strength in menopausal women (Enns & Tiidus, 2010 *review*).

Estrogen has also been suggested to affect muscular stiffness and ligament laxity in normally menstruating women. Eiling et al., (2007) found a significantly lower active stiffness in the muscle of the lower extremities during ovulation (high E2), compared to the early follicular phase (low E2). Similar, a negative relationship between estrogen and muscle stiffness was shown by Bell et al., (2012). Hansen et al., (2013) did not, however, find differences in soft tissue stiffness in relation to menstrual cycle phases in female athletes. Some authors have demonstrated an increase in ligament laxity in relation to specific phases of the menstrual cycle when estrogen levels are high (Deie et al., 2002; Heitz et al., 1999; Shultz et al., 2004). These findings and conclusions have been refuted by other reports (Arnold et al., 2002; Bennon et al., 2002; Karageanes et al., 2000). Beynnon et al., (2005) concludes the ligament laxity to be greater in women compared to men, however, the menstrual cycle with fluctuation in E2 and P-4 was reported not to affect the ligament laxity.

Periodized high intense resistance training in women during the follicular phase (totally high levels of E2) has been shown to increase leg muscle strength in untrained to moderately trained women (Reis et al., 1995; Sung et al., 2014). These results were more recently confirmed by Wikström-Frisén et al., (2015). They showed improved performance in squats, countermovement jumps and peak torque for hamstring muscles after periodized resistance training with more frequent training during the follicular phase. Furthermore, an increase in lean body mass was also observed (Wikström-Frisén et al., 2015). However, in the above study, both women with a regular normal menstrual cycle and women on different types of oral contraceptives were mixed.

#### **2.4.2 Progesterone**

Progesterone, secreted from the corpus luteum, is mainly bound to albumin in the circulation and only a few percent is free and could easily enter the cells and bind to its specific receptor (Fritz & Speroff, 2011). There are two main isoforms of the classical nuclear receptors for progesterone, the progesterone receptor A (PR-A) and the progesterone receptor B (PR-B)

(Conneely & Lydon, 2000). Progesterone is a key hormone for conception and pregnancy maintenance (Fritz & Speroff, 2011).

#### *2.4.2.1 Progesterone effects on skeletal muscles*

Very little is known about the physiological effects of progesterone on human skeletal muscle. Copas et al., (2001) have demonstrated PR in the skeletal muscle of the pelvic floor in postmenopausal women. In a recent review by Oosthuysen & Bosch, (2010), circulating P-4 was suggested to correlate with an increased protein catabolism due to a greater oxidation of amino acids in the luteal phase.

### **2.4.3 Androgens**

The term androgen is used to describe all male sex hormones. Testosterone and dihydrotestosterone are both synthesized from androstenedione (Fig. 7). Testosterone is considered to act as an anabolic hormone by increasing protein synthesis and decreasing protein degradation (Crewther et al., 2011 *review*). There is one known nuclear receptor for androgens in central and peripheral tissue, the androgen receptor (AR).

#### *2.4.3.1 Androgen effects on skeletal muscles*

Increased protein metabolism, muscle growth, and improvement in physical performance with the influence of exogenous testosterone in combination with training are well documented in men (Crewther et al., 2011 *review*; Hartgens & Kuipers, 2004 *review*).

Receptors for androgens have previously been found in human male skeletal muscle. Kadi et al., (2000) found that the distribution of AR per fiber cross-section differs between muscles types. A higher distribution was demonstrated in the upper body (back and chest), compared to in the lower limbs, in male subjects (Kadi et al., 2000). The proportion of AR per fiber cross-section area was significantly higher in power lifters than in untrained controls, but only in the upper body. For the group of power lifters using exogenous androgen steroids, this up-regulation in AR was significantly elevated over those not using drugs, and still only in muscles of the upper body (Kadi et al., 2000). In the case of females, AR has previously only been demonstrated in skeletal muscles of the pelvic floor of postmenopausal women. The muscular function of the AR was, however, not investigated

in this study (Copas et al., 2001). Less is known about the occurrence and effects of androgens on muscle performance in fertile-aged women. However, female athletes with polycystic ovary syndrome (PCOS) were shown to have higher maximal oxygen uptake ( $VO_2\max$ ) and the highest performance values compared to non-PCOS athletes (Constantini, 1995; Rickenlund et al., 2003).

The demonstration of hormone receptors in human muscle tissue is quite new. There are few studies on hormone receptors in female muscle tissue, but no available research has evaluated the variation of sex hormone receptors during the menstrual cycle in fertile-aged women. In the present thesis, the aim of Paper IV was to explore the representation of hormone receptors in skeletal muscles during three specific phases of the menstrual cycle.

#### **2.4.4 Neurosteroids**

The sex hormones, E2, P-4, T, and their potent metabolites, are synthesized and released in the central and peripheral nervous system and can therefore be classified as neurosteroids (Compagnone & Mellon, 2000; McEwen, 2012 *review*). These sex steroid hormones may act directly by binding to their specific receptors, but can also exhibit modulatory effects on the synaptic transmission involved in emotional and cognitive control: for example, the noradrenergic, the dopaminergic, the serotonergic and the  $\gamma$ -aminobutyric acid (GABA)-ergic systems (Toffoletto et al., 2014 *review*). Allopregnanolone, a metabolite of progesterone, increases during the luteal phase and is considered to be of importance for PMS by influencing the GABA and the serotonin system (Sundström, 1999).

## **2.5 PREMENSTRUAL SYMPTOMS**

### **2.5.1 Prevalence and diagnostics**

More than 75% of women of fertile age experience one or more symptoms of PMS. The symptoms are characterized by negative mood and/or somatic disturbance during the luteal phase (Angst et al., 2001). The severity of the symptoms gradually increases during the late luteal phase and usually peaks about two days before the menses and subsides a few days after the onset of menses (Bäckström et al., 1983; Pearlstein et al., 2005). The symptoms are mild in most women and cause no impairment in daily activity and could be considered physiological rather than pathological. However, about 20% of fertile-aged women have

premenstrual complaints of clinical relevance (Yonkers et al., 2008 *review*) and about 3–8% have symptoms that are severe and associated with substantial distress and functional impairment that markedly affect the activity of daily living and require medical management (Sveindottir & Bäckström, 2000). The most frequently reported somatic symptoms are breast tenderness and bloating and the most common mood symptoms are irritability, depressed mood, and fatigue.

The former disagreement about terminology and diagnostic criteria led to a consensus report on the severe form of PMS. The proposed criterion was adopted by the American Psychiatric Association's Diagnostic and Statistical Manual V (DSM-5). The disorder was entitled premenstrual dysphoric disorder (PMDD) (APA, 2013). The diagnosis of PMDD (3–8 % of fertile-aged women) requires at least five luteal phase disorders, whereof at least one must be a negative mood symptom (e.g., irritability, depressed mood, fatigue, tension). The timing of the symptoms must be recorded by daily prospective ratings during at least two cycles and the symptoms should be severe enough to cause functional impairment. Additionally, the symptoms should not be an exacerbation of another psychiatric disorder. To differentiate PMS/PMDD from other psychological issues, the symptom-free interval before ovulation is crucial in the diagnostics of PMS (Bloch et al., 1997; Hammarbäck et al., 1989). PMS is not a laboratory-directed diagnose, since levels of hormones are often equal in women with and without PMS/PMDD (Rubinow & Schmidt, 1995).

### **2.5.2 Etiology and pathophysiology**

It is still a challenge to understand the etiology and pathophysiology of PMS, but advances have been made in recent years. Since mood and behavioural symptoms are key features of PMS, the underlying mechanism should involve the brain and neurotransmitters within the brain (Yonkers et al., 2008 *review*). Theories suggest symptoms to be triggered by the postovulatory peak in progesterone with the metabolites of progesterone acting in the brain (Schmidt et al., 1998; Nevatte et al., 2013; Ismaeli et al., 2016). Since the symptoms disappear during anovulatory cycles, the formation of corpus luteum is necessary for the development of PMS (Hammarbäck et al., 1988; 1991). The increased levels of progesterone metabolites acting on the inhibitory transmitter systems in the brain seem to be important for the development of PMS in women (Bäckström et al., 2011). Since there are no differences in serum levels of sex hormones in women with and without PMS (Rubinow & Schmidt, 1995), there are indications that some women are more sensitive to the normal fluctuation of sex

hormones and especially to the neuroactive metabolites allopregnanolone (3 $\alpha$ -OH-5 $\alpha$ -pregnan-20-one) and pregnanolone (3 $\alpha$ -OH-5 $\beta$ -pregnan-20-one) (Rapkin et al., 1997). Gamma-butyric acid and its A-receptor (GABA-A) constitute the most important inhibitory system in the brain and the site of action for benzodiazepines, barbiturates, and ethanol, as well as for endogenous neuroactive steroids (Sundström et al., 1999). Allopregnanolone and pregnanolone exert a rapid, non-genomic inhibitory effect on excitability neurons that directly modulate the activity of the GABA-A receptor (Biggo et al., 2001). Normally, positive modulation of the GABA-A receptor gives rise to anxiolytic and calming effects. However, in some individuals, particularly women with severe PMS, the effects of the modulation are the opposite and cause irritability and anxiety. This reaction is called a paradoxical reaction where low doses of allopregnanolone cause a negative mood and high doses a calm mood (Bäckström et al., 2014 *review*).

Estrogen metabolites are also neurotransmitters with an action in the nervous system that regulates and controls, e.g., fine motor function, coordination, pain, mood, cognitive function, and neuroprotection (McEwen, 2012 *review*).

It is unclear whether somatic symptoms of PMS are caused by changes in hormone-responsive peripheral tissue or if they might be a result of reduced tolerance of physical discomfort while in a dysphoric mood state. Studies on premenstrual-related fluid retention, breast enlargement, and weight gain have failed to show such a relation (Yonkers et al. 2008 *review*). It should be mentioned that menstrual-related headache and dysmenorrhoea are not somatic premenstrual symptoms, but separate conditions (Yonkers et al., 2008 *review*).

### **2.5.3 Therapeutic management of PMS**

#### **2.5.3.1 *Medical treatment with selective serotonin re-uptake inhibitors, GnRH analogues, and OCs***

The most common medical treatments for PMS and PMDD are aimed at influencing the neurotransmitters in the brain, such as the selective serotonin re-uptake inhibitors (SSRIs) (Nevatte et al., 2013). In a meta-analysis, Dimmock et al., (2000) considered SSRIs to be effective first-line therapy for severe PMS. SSRIs affect the serotonergic transmitter system without affecting ovarian steroid production. Ovulation persists as normal with this treatment. Frequently reported side effects of SSRI treatment are decreased libido, insomnia, fatigue, and nausea (Dimmock et al., 2000).

Since PMS does not occur in anovulatory cycles, treatments that suppress ovulation are particularly interesting (Wyatt et al., 2004 *review*). GnRH analogues downregulate the GnRH receptors and, by this action, inhibit ovulation. In a meta-analysis of seven randomized controlled trials, GnRH has shown high efficacy in the treatment of PMS/PMDD (Wyatt et al., 2004 *review*). There is, however, a risk of a low estrogenic state due to the inhibited ovulation. Add-back therapy of E2 and progesterone has been shown not to reduce the treatment effects of GnRH (Segebladh et al., 2009).

Oral contraceptives (see part 2.6 later in this thesis) have been shown to improve somatic symptoms (Bäckström et al., 1992). Concerning mood disorders, there are still contradictory results and the effect seems to depend on the type of progestagen and the treatment regimen, where continued use or shortening the withdrawal phase has been shown to improve mood symptoms, at least in women with mild symptoms (Nevatte et al., 2013). Mood disorders are, however, the major reason for discontinuation of OC treatment (Larsson et al., 1997). A novel OC containing the progestagen, drospirenone, which is derived from spironolactone instead of testosterone, has been demonstrated in studies to have properties that influence fluid balance (Kurshan et al., 2006; Pearlstein et al., 2005; Yonkers et al., 2005). In a Cochrane report, drospirenone has been considered to be treatment for PMS and PMDD, with improvement in both somatic and mood-related disorders (Lopez et al., 2012).

### *2.5.3.2 Non-medical management with physical exercise*

Few studies have evaluated the effect of physical exercise on cyclical symptoms and PMS. Among the studies found in the literature, all have demonstrated improvement in negative cyclical symptoms as a result of aerobic exercise (Aganoff & Boyle, 1994; Prior et al., 1997; Steege & Blumenthal, 1993; Stoddard et al., 2007). Unfortunately, these studies did not verify cycle phase by hormone analysis, but relied on self-reports or unreliable measures by counting days. Furthermore, they did not use prospective daily ratings of PMS. Instead, a retrospective assessment was used. Aganoff & Boyle, (1994) used a cross-sectional study design. In addition, it should be mentioned that none of these studies were aimed at treating women with a medical diagnosis of PMS/PMDD. In the most recent study by Stoddard et al. (2007), a significant improvement was found in cyclical symptoms using a prospective study design comprising 14 sedentary women performing a 24-week exercise training program. Some analysis of hormones was performed; however, the precise procedure was not defined. The study group was small and had no control group.

#### **2.5.4 The relation between PMS, postural control, and musculoskeletal injuries**

Möller-Nielsen & Hammar, (1989) have shown women with PMS to be at greater risk for soccer-related musculoskeletal injuries. In general, for both men and women, depressed mood has been suggested to be a reliable predictor to utilize in sports injuries (Galambos et al., 2005).

Fridén et al., (2003; 2005) have shown alterations in postural control during different hormonally verified phases of the menstrual cycle. Healthy women with PMS showed an altered postural control during the luteal phase. This difference was not seen in women without PMS (Fridén et al., 2003a; 2005). Abt et al., (2007); Ericksen & Gribble, (2012); Hariell et al., (2010), and Hertel et al., (2006) found no differences in postural control during different phases of the menstrual cycle. However, no ratings and analyses for PMS were done in any of these studies. Posthuma et al., (1987) demonstrated worse coordination during the luteal phase in females with PMS than in females without such symptoms. A correlation between low mood, anxiety, and postural control has been reported earlier (Wada et al., 2001; Bolmond et al., 2002). Additionally, Kitaoka et al., (2004) have studied the effects on mood state on anticipatory postural adjustment and shown a significant correlation between low mood and reaction time. The proposed mechanism has been suggested to be mood-related effects on central interactions of visual, vestibular, and somatosensory inputs in the maintenance of postural control (Wada et al., 2001; Bolmond et al., 2002). Fridén et al., (2003a; 2005) suggested that progesterone metabolites interact with the GABA-ergic system in women with PMS, causing a disturbance in postural control.

The presence of PMS seems, as a result of transmission action within the central and peripheral nervous system, to be involved in disturbance of postural control and balance. It is unclear to what extent OC use relieves PMS and if it affects postural control in healthy women. This is studied in Paper I in this thesis.

## **2.6 ORAL CONTRACEPTIVES**

Oral contraceptives are the most common reversible contraceptive method in European countries, with a user rate of 30–45% in fertile-aged women (Skouby, 2010). Higher frequencies are reported at the lower ages (Lindh et al., 2000; Skouby, 2010). In female

athletes, OC use is as common as in the general population (Hagmar et al., 2009). Besides contraception, OCs are commonly used as treatment of dysmenorrhea, bleeding disorders, endometriosis, and polycystic ovary syndrome (PCOS) (Fortney et al. 1986), Another purpose of OC treatment is time shifting of the menstrual cycle (Constantin et al., 2005).

Combined OCs contain both an estrogen component (in most cases synthetic ethinyl estradiol) and a synthetic progesterone (progestagen) component which, in most OCs, are structurally related to testosterone (Stanczyk, 2003 *review*) or spironolactone for the progestagen drospirenone (Lopez et al., 2012). The first OCs were introduced in the 1960s and contained 150 µg ethinyl estradiol. The dosage has today been markedly decreased to 20–35 µg. The different progesterone derivatives have unique biological properties and profiles with different metabolic effects depending on their relative binding affinity (RBA) (van Rooijen et al., 2002; Stanczyk, 2003 *review*). Progestagens with a high RBA to PR requires a relatively low dose to inhibit ovulation and progestagens with a high RBA to AR are known to produce undesirable side effects such as acne and hirsutism (Carr, 1998). However, combined OCs usually have anti-androgenic effects with suppression of free testosterone, which reduce acne and hirsutism (Stanczyk, 2003 *review*).

The contraceptive effect is accomplished by suppression of the hypothalamus-pituitary system, induced by ethinyl estradiol and progestagen in combination. This will prevent the surge of GnRH, LH, and FSH, which inhibits the secretion of endogenous female sex hormones (E2 and P-4), thus preventing ovulation and subsequent pregnancy (Mishell et al., 1977). In monophasic OCs, the dosage of ethinyl estradiol and the progestagen component is constant throughout the active treatment period, usually for 21 days, followed by seven days of withdrawal (menstrual bleeding) (Fig. 8) (Rapkin et al., 2006). The levels of ethinyl estradiol peaks about 1 h after ingestion, falls rapidly for the following six h and then slowly declines. Ethinyl estradiol is detectable for up to two days after discontinuation, and some progestagens for up to five days (Rechichi et al., 2009 *review*). The endogenous hormones that control the menstrual cycle are not completely suppressed by OCs; the levels of E2 are similar to the levels during the early follicular phase in a normal menstrual cycle and, during the withdrawal phase, the E2 levels rise (Rapkin et al., 2006; Rechichi et al., 2006). To achieve a more complete suppression of endogenous hormones, the withdrawn phase can be shortened or excluded (Rapkin et al., 2006).

OCs are usually well tolerated and, in a recent comprehensive study, as many as >90% of women on OC treatment reported high-level of satisfaction (Skouby, 2010). The most frequently reported side effect is depressed mood and it emerges from the progestagen

component in some OCs and affects some of the women. Still, for the modern OCs, the most serious adverse effect is venous thromboembolism, which occurs in rates of 9–10/10,000 OC-using women /year, comparable with a yearly incidence for non-OC users of 4–5/10,000 women (Reid et al., 2010). The risk of hypercoagulation is estrogen-dose-dependent (Reid et al., 2010).

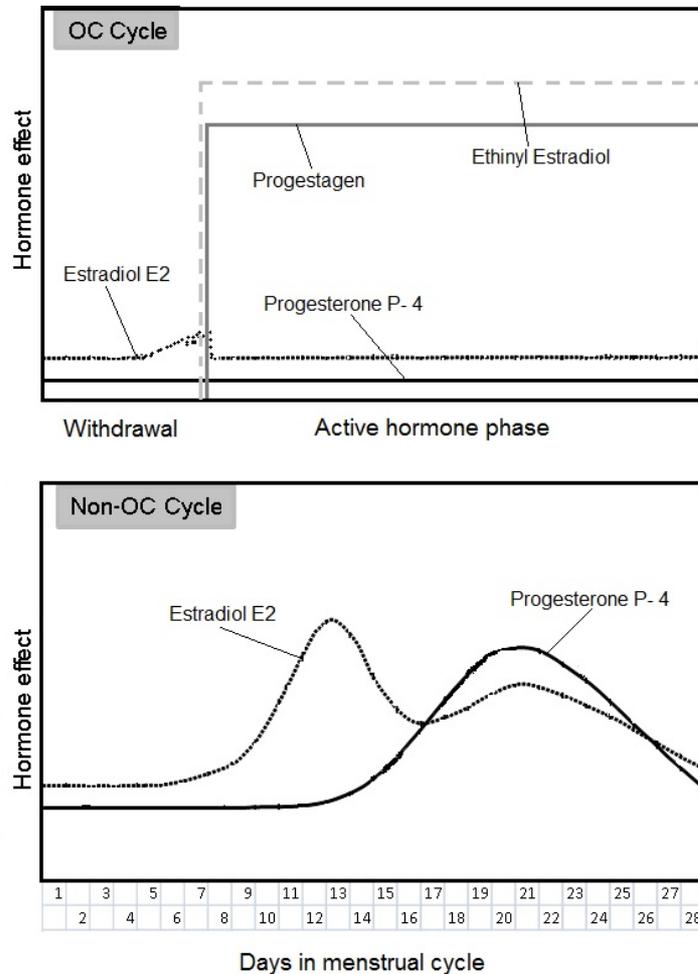


Fig. 8. Illustration displays the effects of oral contraceptives on the endogenous hormone production (top figure), in comparison to the regular menstruating cycle.

### 2.6.1 Oral contraceptives in relation to neuromuscular function and musculoskeletal injuries

The literature on OCs in relation to soft tissue function has shown contradictory results. OCs have been shown to lower the collagen fraction synthesis rate in the patellar tendon in young OC users, compared to non-OC users (Hansen et al., 2008). These results were explained by the OC-induced reduction of the bioavailability of insulin-like growth factor-I (IGF-I), which

has an important effect on tendon collagen synthesis (Hansen et al., 2008; Hansen et al., 2009). In a more recent study from the same research group, no impact of the use of OC and menstrual phase was found on patellar tendon morphology, biochemical composition, or biomechanical properties in female team handball athletes (Hansen et al., 2013). Bryant et al., (2008) observed strain reduction of the m. triceps surae aponeurosis during maximal isometric plantar flexion in OC users. Conversely, the same authors showed, in a more recent study, OC users to maintain dynamic control more constantly during different hopping tasks, compared to the non-OC users. The authors suggest that this explains the lower rate of lower limb musculoskeletal injuries in the OC users (Bryant et al., 2011). The use of OCs has been suggested to protect from ligament injuries in female athletes. Martineau et al., (2004) observed a significant decrease in anterior translation of the tibia (reduced laxity in the ACL) in female athletic OC users compared to non-OC users. Conversely, Pokoney et al., (2000) found no differences in ligament laxity between OC users and non-OC users. In none of these studies (Martineau et al., 2004; Pokoney et al., 2000) were sex hormones assessed. The methodology used was to count cycle days and OC use by self-report.

Data on anaerobic power, jump performance, and muscle strength during OC use are minimal and have shown various results. On evaluating anaerobic power, Redman and Weatherby, (2004) showed a greater performance in 10-second all-out rowing during the withdrawal phase compared with the active treatment phase with triphasic OCs. No mechanism was proposed. However, this claim was refuted in other studies (Bushman et al., 2006; Giacomoni et al., 2000; Rechichi & Dawson, 2009), who showed no differences in anaerobic performance based on cycling power, jumping power, and stair climbing. Rechichi and Dawson, (2009) evaluated reactive strength with a drop jump test and found altered performance during the late withdrawal phase, compared to the active treatment phase, suggesting that the rise in endogenous E2 may negatively impact neuromuscular timing (Rechichi & Dawson, 2009). To evaluate muscle strength, four studies, comparing active OC treatment phase with the withdrawal phase, have failed to demonstrate any significant differences in maximal force-generating capacity within a monophasic OC cycle (Elliot et al., 2005; Lebrun et al., 2003; Peters & Burrows, 2006; Sarwar et al., 1996).

#### *2.6.1.1 Training effect and injury rate with OC use*

Rickenlund et al., (2004) investigated physical performance before and after 10 months of OC treatment in endurance athletes and found no change in endurance capacity and strength,

except a small decline in performance by the beep test. In a double-blind study, Nichols et al., (2008) were not able to show any differences in muscle strength development during a 12-week training program for female athletes using or not using OCs. Reiger & Yingling, (2016) did not demonstrate any differences in jumping height and ground reaction forces when comparing OC users with regularly menstruating women after a three-week jump protocol with five training sessions/week.

Regarding musculoskeletal injuries and OC use, Möller-Nielsen & Hammar, (1998) have shown women on OC treatment to be less affected by traumatic sport injuries. Two epidemiological case-control studies based on two different ligament reconstruction registers have both demonstrated a lower likelihood of young females who use OCs to sustain an operatively treated ACL injury (Rahr-Wagner et al., 2014; Gray & Gugala, 2016).

The effect of exogenous hormones in OCs on neuromuscular performance in healthy fertile-aged women is not fully understood. In Paper II, the effect on muscle strength and hop performance of OC use and hormonal variation during the menstrual cycle was evaluated.

## **2.7 RATIONALE**

Females are reported to have a three- to six-fold higher incidence of sustaining a traumatic knee injury (Boden et al., 2000; Huston et al., 2000 *review*; Myklebust et al., 1997; Åman et al., 2016). Several intrinsic and extrinsic factors have been proposed to explain the mechanisms behind the increased injury rate. Hormonal factors have been studied to some extent by, e.g., observing differences in ligament laxity, neuromuscular performance, and muscle strength during different phases of the menstrual cycle and with OC use.

The aim of his thesis with an experimental design was to evaluate the effects of sex hormone (both endogenous and exogenous) on muscle strength, postural control, and PMS in healthy fertile-aged women. In doing so, the results of the papers included might, to some extent, help to gain an understanding of the mechanisms of differences in neuromuscular performance in women.

### **3 OVERALL AIM**

The overall aim of this thesis was to evaluate the influence of sex hormones and OC treatment on skeletal muscle function and premenstrual symptoms.

#### **3.1 SPECIFIC AIMS**

The specific aims were:

- To evaluate the effect of OC treatment on postural control and the influence of PMS in physically active women (Paper I).
  
- To evaluate and compare muscle strength in the upper and lower limb, as well as hop performance in active women during OC use and non-OC use in the same woman (Paper II).
  
- To evaluate changes in premenstrual symptoms in women starting to use and discontinuing the use of OCs (Paper III).
  
- To investigate mRNA and protein levels of sex steroid hormone receptors in skeletal muscle in three different phases of the menstrual cycle (Paper IV).



## **4 METHODS**

### **4.1 STUDY DESIGN**

In Papers I and IV, a prospective design was used and, in Papers II and III, a cross-over design was used.

### **4.2 RECRUITMENT**

In this thesis, two different study cohorts of women were recruited. In Papers I–III, the participants were recruited among students at Karolinska Institutet and at the Swedish School of Sport and Health Sciences. In Paper IV, the subjects were recruited among students at Karolinska Institutet.

### **4.3 ETHICAL APPROVAL**

The women who participated in the work on this thesis gave their written informed consent prior to entering the study. The papers in this thesis were approved by the Stockholm Regional Ethical Review Board (Dnr 2001-311 and 2006/198-31/1). These studies were conducted according to the Helsinki Declaration (ethical principles for medical research involving human subjects).

### **4.4 INCLUSION AND EXCLUSION CRITERIA**

The inclusion criteria were: healthy women aged 20–35, non-smokers, without any regular medication or hormonal treatment. In Papers I–III, they were supposed to have a normal menstrual cycle or to use low-dose OCs and participate in moderate physical activity consisting of two–four sessions/week. In Paper IV, they were supposed to have a normal menstrual cycle and be sedentary or participate in light recreational physical activity.

The exclusion criteria were: chronic diseases, a past or present neurological disorder, a recent musculoskeletal injury.

## **4.5 SAMPLE SIZE ESTIMATIONS**

In Papers I and II, the sample size calculation was based on previous studies with a clinically relevant difference in maximal muscle strength of 20% using a comparable methodology (Fridén et al., 2003; 2005). For a power of 80% with a 2-sided alpha level of 5%, 30 participants were required, 15 in each group (OC Starters and non-OC Starters). Paper III was based on the same study sample as used in Papers I and II.

In Paper IV, the sample size was based on an earlier study on ER receptor expression (Wiik et al., 2003). To demonstrate a hypothesized variation between menstrual cycle phases, with a power of 80% and a 2-sided alpha level of 5%, 20 subjects were required.

## **4.6 PARTICIPANTS**

### **4.6.1 Papers I–III**

In Papers I–III, a total of 30 women received written and oral information about the study and filled out a health declaration form. Based on the inclusion criteria, 24 women were enrolled (three women did not match the inclusion criteria and another three women regretted participation after receiving information) and gave their written informed consent prior to entering the study. These 24 subjects were divided into two different groups depending on OC or non-OC use when entering the study. The term “OC Starters” referred to the subjects on OC treatment when enrolled ( $n = 12$ ) and “Non-OC Starters” to the subjects who were menstruating normally from the start ( $n = 12$ ). Fig. 9 shows a flowchart of the participants in three different studies, Papers I–III.

In Papers I–III, women with both normal menstrual cycles and women on OCs were included. Out of the 24 subjects enrolled in the study, the OC Starters ( $n = 12$ ) had been using low-dose monophasic OCs containing different progestagen components for at least three months, whereas the non-OC Starters ( $n = 12$ ) were regularly menstruating. After the assessment in the first cycle (OC or menstrual cycle), the subjects switched to OC vs. non-OC in a cross-over design (Fig. 8). The OC Starters discontinued their OC use and after a wash-out period of one to three months the OC Starters then continued the study protocol with assessments during a normal menstrual cycle (non-OC cycle). The non-OC Starters, were instead prescribed low-dose monophasic OCs and were assessed at the start of the use of the second pill chart (OC cycle).

All women were using low-dose monophasic OCs of a similar type (Table 1). The regimen for the OC treatment was 21 days of active hormone treatment followed by seven days of withdrawal (no active hormone). Screening for contraindications of OC treatment was based on anamnestic data of risk for thromboembolism and blood pressure measurement prior to prescribing OCs.

Table 1. The different types of monophasic OCs used in the present thesis (n = 19).

Estrogen	Progestagen	n
Ethinyl estradiol 30 µg/day	Levonorgestrel 0.15 mg/day	10
Ethinyl estradiol 35 µg/day	Norgestimat 0.25 mg/day	3
Ethinyl estradiol 30 µg/day	Drospirenone 3 mg/day	2
Ethinyl estradiol 20 µg/day	Desogestrel 0.15 mg/day	2
Ethinyl estradiol 35 µg/day	Noretisterone 0.5 mg/day	1
Ethinyl estradiol 35 µg/day	Lynestrenol 0.75 mg/day	1

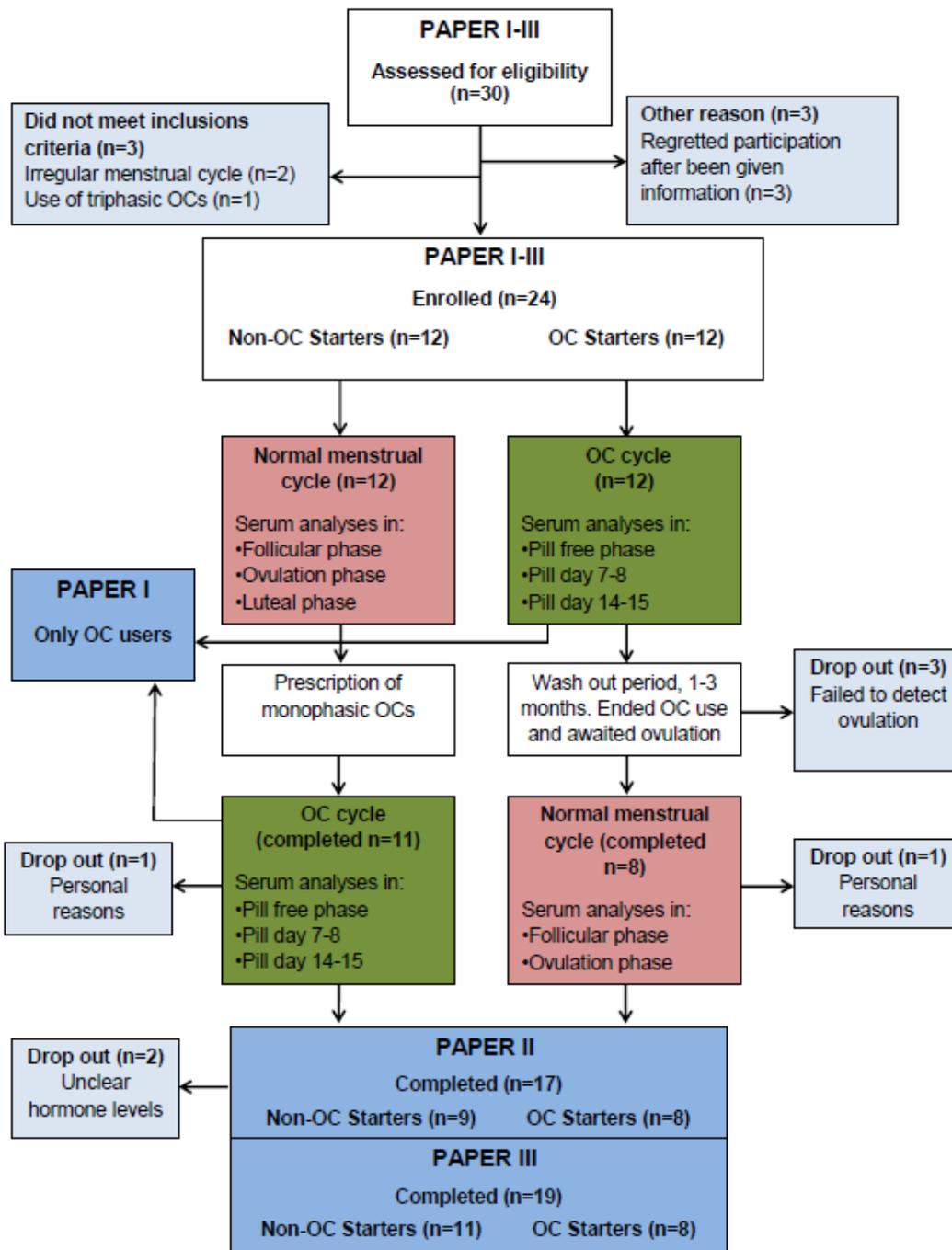


Fig. 9. Flowchart for Papers I-III.

#### 4.6.2 Paper IV

In Paper IV, all women enrolled (n = 36) had normal menstrual cycles, received written and oral information about the study, and filled out a health declaration form. Based upon the inclusions criteria, 30 women were enrolled in the study. For data collection, the women were divided according to three different schedules during a period of three menstrual cycles (Fig. 10). The characteristics of the enrolled participants of the two different cohorts (n=24 and n=30) are presented in Table 2.

Table 2. Characteristics of the participating women of cohort I for Paper I-III (n = 24), divided into regard to entering the study with OCs or without OCs and cohort II for Paper IV (n = 30).

	<b>Cohort 1</b>		<b>Cohort 2</b>
	<b>OC Starters (n = 12)</b>	<b>Non-OC Starters (n = 12)</b>	<b>(n = 30)</b>
Age (yrs)	24.6 (2.5)	27.0 (4.8)	29.0 (1.4)
Height (cm)	164.5 (6.3)	166.8 (5.2)	167.5 (3.5)
Weight (kg)	61.9 (9.4)	61.8 (6.5)	61.8 (1.8)
BMI (kg/m <sup>2</sup> )	23.7 (6.9)	23.1 (1.1)	21.9 (1.6)
Physical activity level (session/ week)	3.1 (1.1)	2.1 (1.1)	

Values in mean (SD)

Abbreviation: BMI (Body mass index)

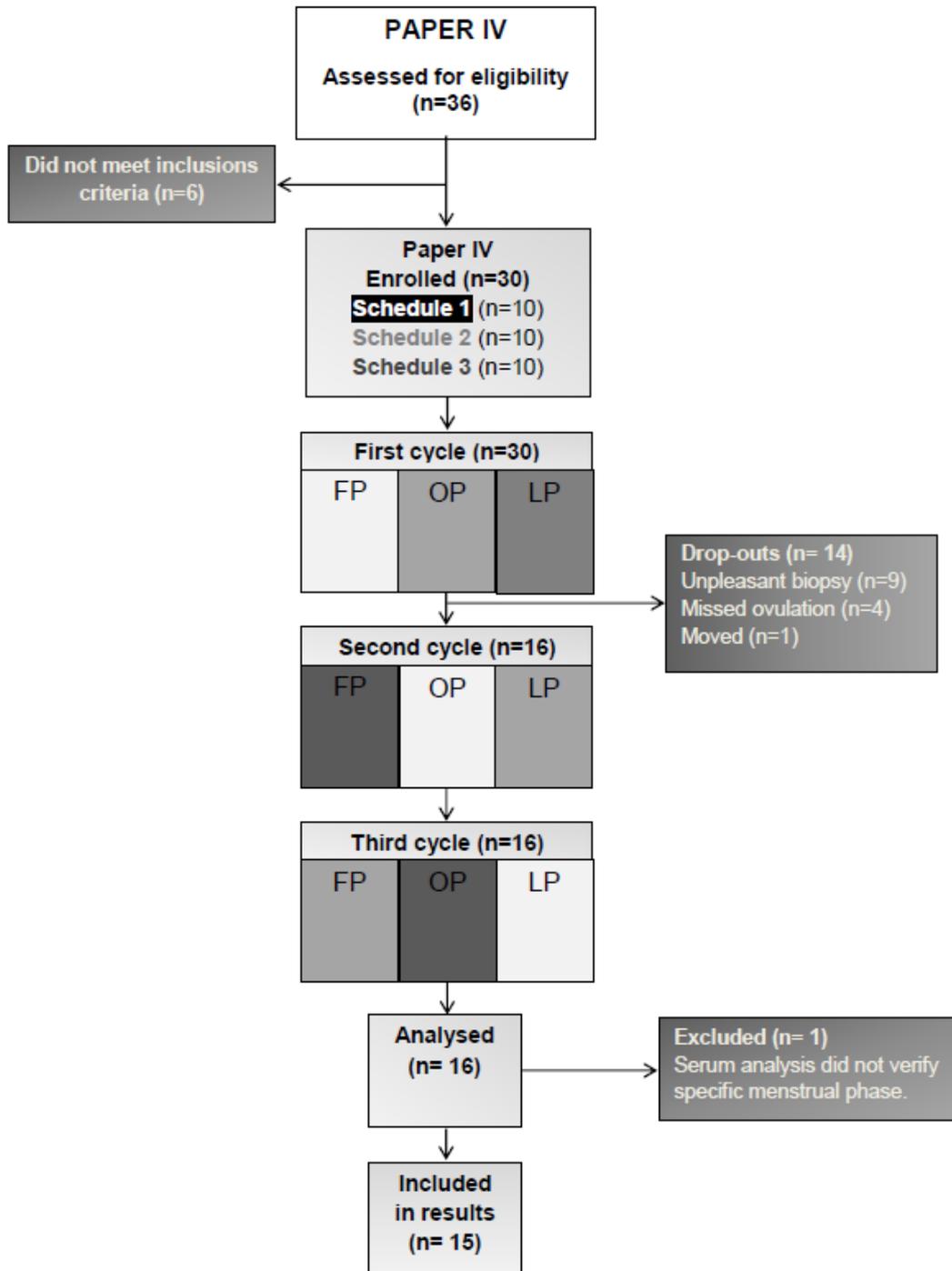


Fig. 10. Study protocol and sample size for Paper IV. The subjects were divided according to three different schedules, for each schedule (n = 10), one muscle biopsy specimen was collected in one specific phase per cycle.

Abbreviations: (FP) follicular phase, (OP) ovulation phase, (LP) luteal phase.

## **4.7 PROCEDURS**

### **4.7.1 Procedure, Paper I**

Prior to the assessments, all subjects performed three familiarizations trials on separate occasions in order to get used to the equipment and the test procedure of physical performance, as well as to avoid learning effects. The subjects were tested for postural control during three different occasions on the pill chart in one OC cycle. The first test session was at days 3–5 in the withdrawal phase (non-OC phase), the second test session was during days 7–8 on the pill chart (OC phase) and the third test session was on days 14–15 on the pill chart (OC phase). The two latter occasions were then calculated together as one OC phase since the hormonal status is equal in the OC phase.

Based on the results of the PMS screening (see below), the subjects were divided into a PMS group and a non-PMS group.

### **4.7.2 Cyclicity diagnosis**

Prospective ratings of PMS were used in Paper II and Paper III during the OC cycle and non-OC cycles for both groups (OC Starters and Non-OC Starters). A previous validated cyclicity diagnostic tool, the Cyclicity Diagnoser (CD), was used (Sundström et al., 1999). The subjects were supposed to rate their symptoms every evening during the test period. The CD scale, which complies with the description in the World Health Organization International Classification of Diseases (ICD-10) and the American Collage of Obstetrics and Gynecology (ACOG) (WHO, 1996; ACOG, 2000) criteria for PMS and with the DSM IV (APA, 1994) criteria for PMDD, consists of four negative mood parameters (depression, fatigue, irritability, and tension), two positive mood parameters (friendliness and energy) and three somatic symptoms (swelling/bloating, breast tenderness and menstrual bleeding). In addition, the CD scale consists of scores for measuring various effects on daily life using seven different parameters: appetite, activity of daily life, general impairment of daily life, sleeping disorders, temprament, difficulty in concentrating, and feeling out of control (Fig. 11).

The CD scale is a nonparametric Likert scale with a range of 0–8, with 0 indicating the absolute absence of a particular symptom and 8 the maximal severity of the symptom. A significant increase in at least one negative mood symptom and/or somatic symptom during the cycle during the premenstrual days, days 24–28 and days 1–2, compared with cycle days 4–10, indicates PMS (referens).

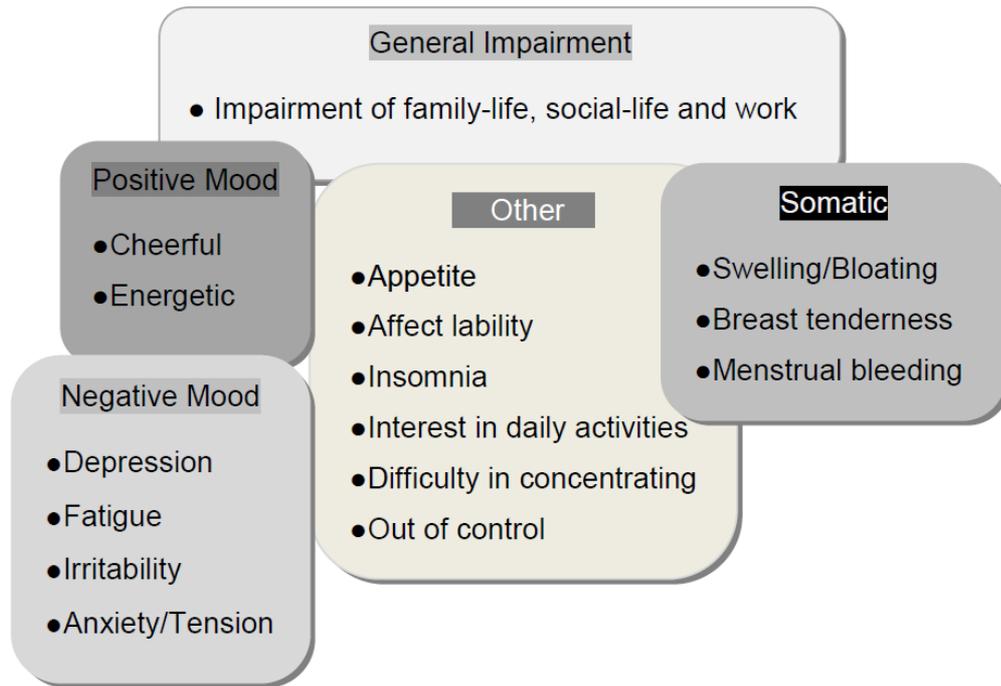


Fig. 11. The different modules of PMS described in the CD scale, used in this thesis.

#### 4.7.3 Procedure, Paper II

After the subjects had performed three familiarizations trials on separate occasions, they were tested in two hormonally different cycles: a normal menstrual cycle (non-OC cycle) and an OC cycle.

For the non-OC cycle, the first test session was in the follicular phase (FP) (cycle days 3–5). The second test session in the non-OC cycle was in the ovulatory phase (OP), which was detected by an LH surge in urine. After detection of an LH surge, the subject was assessed within 24–48 h. The third test session was in the luteal phase, which was defined as seven days after ovulation with a simultaneous increase in serum progesterone.

For the OC cycle, the first test session was on days 3–5 in the withdrawal phase (non-OC phase). The second test session was during days 7–8 on the pill chart (OC phase) and the third test session was on days 14–15 on the pill chart (OC phase).

In order to minimize diurnal variation, the assessments were performed at the same time of day at each session.

#### 4.7.4 Procedure, Paper III

The results in Paper III were based on the CD scales, together with serum analyses as described in Paper II.

#### 4.7.5 Procedure, Paper IV

Regularly menstruating women were included in Paper IV. The protocol for blood sampling and collection of muscle biopsy specimens at three different phases during three consecutive menstrual cycles is illustrated in Fig. 12. The first assessment was in the follicular phase (FP) (cycle days 3–5). The second assessment was in the ovulatory phase (OP), which was detected by an LH surge in the urine. The third assessment was in the luteal phase (LP), which was defined as occurring seven days after ovulation with a simultaneous increase in serum progesterone.

	Follicular phase	Ovulatory phase	Luteal phase
<b>Schedule 1</b>			
Menstrual cycle 1	Muscle biopsy 1		
Menstrual cycle 2		Muscle biopsy 2	
Menstrual cycle 3			Muscle biopsy 3
<b>Schedule 2</b>			
Menstrual cycle 1		Muscle biopsy 1	
Menstrual cycle 2			Muscle biopsy 2
Menstrual cycle 3	Muscle biopsy 3		
<b>Schedule 3</b>			
Menstrual cycle 1			Muscle biopsy 1
Menstrual cycle 2	Muscle biopsy 2		
Menstrual cycle 3		Muscle biopsy 3	

Fig. 12. The protocol for blood sampling and muscle biopsy specimen collection followed three different schedules with one assay in different phases of each menstrual cycle.

## 4.8 DROP OUTS

In Paper I, seven participants dropped out: four due to missing values on the force platform; two did not show totally clear hormone levels, and one for personal reasons (Fig. 9).

In Paper II, seven participants dropped out: three due to failure to detect ovulation during the non-OC cycle, two did not show totally clear hormone levels, and two for personal reasons (Fig. 9).

In Paper III, five participants dropped out: three due to failure to detect ovulation during the non-OC cycle, two for personal reasons (Fig. 9).

In Paper IV, 14 participants dropped out: four due to failure to detect ovulation, nine had an unpleasant biopsy and one for personal reasons (Fig. 10).

## **4.9 DATA COLLECTION**

### **4.9.1 Blood sampling and analyses**

In all Papers of the present thesis blood samples for determination of hormone levels were collected in the morning after 15 min of rest before every test occasion. After centrifugation, serum was separated and stored at -20°C until analyzed. E2, P-4, and SHBG were analyzed in serum using chemiluminescent enzyme immune-metric assays (IMMULITE Automated Analyser, DPC). Serum levels of T 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 T, fixed albumin concentration of 40g/L by successive approximation using computer program based on equation derived from the law of mass action (Södergård et al., 1982). Detection limits and coefficients of variation were standardized for each hormone. For a more detailed description of the serum analyses, see the Method section in each of the respective Paper I-IV.

### **4.9.2 Measurement of postural control**

Measurements on an AMTI<sup>®</sup> force platform were used to evaluate postural control in a quiet stance, expressed as displacement of CoP. Subjects were asked to stand barefoot in a comfortable one-legged stance on a marker at the center of the platform. Postural control was measured for one minute. The subjects stood quietly with the arms relaxed and with the non-supported leg flexed to 90° at the knee, with their eyes focused on a target placed at eye level approximately 2 m in front of them (Fig. 13). If the arms were used for balancing, subjects

were asked to correct the position as soon as possible. Three trials, one for each leg, were performed, with a one-minute break with the subject resting in a chair. All experiments were performed in a quiet room and in the same order at each test session.



Fig.13. Measurements of postural control on an AMTI<sup>®</sup> force platform (model LG6-4-2000, Advanced Mechanical Technology, Inc., Watertown, MA, USA).

Ground reaction force data were sampled at 50 Hz and the AMTI MSA-6 amplifier system was used together with a PC and an analogue data actuation system. The force platform measured three force components,  $F_x$ ,  $F_y$  and  $F_z$ , and three moment components,  $M_x$ ,  $M_y$ , and  $M_z$  ( $x$ ,  $y$ , and  $z$  are the medio-lateral ( $x$ ), anterior-posterior ( $y$ ), and the vertical ( $z$ ) directions) (Fig. 14).

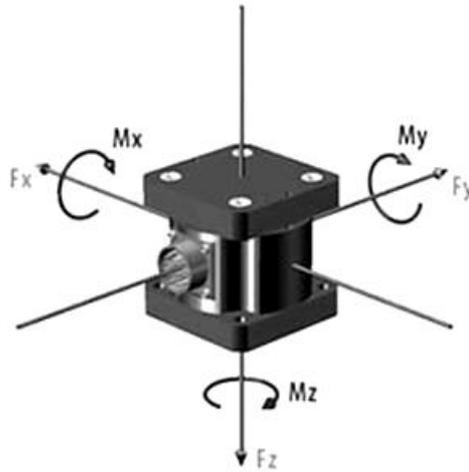


Fig. 14. Illustrates the force components ( $F_x$ ,  $F_y$ , and  $F_z$ ), together with the moment components ( $M_x$ ,  $M_y$ , and  $M_z$ ), measured using the force platform.

The CoP position was calculated as follows:

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

$$\text{CoPy} = [(M_x - (Z_{\text{off}} * F_y)) / F_z]$$

Where  $Z_{\text{off}}$  = 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 medio-lateral (m-l) direction.

#### 4.9.2.1 Data analysis of measurements on force platform

To study in detail the CoP displacements, total displacement (dCoP) in the a-p and m-l directions and the area encompassed by the CoP displacement trace were analyzed. These characteristics were computed off-line using the software of MATLAB<sup>®</sup> (The Math Works, Inc. Natick, MA, USA). The area was computed using the ellipse area method and the principal axes of the ellipse were determined by the principal-component analysis (PCA) (Oliveira et al., 1996). Subtotals of 85.35% of samples lie within the perimeter of an ellipse when using this method (Papoulis, 1984).

### 4.9.3 Measurements of muscle strength and hop performance

#### 4.9.3.1 Isokinetic muscle torque

A standardized isokinetic device (Biodex<sup>®</sup>, Corp, Shirley, NY, USA) was used for measurements of maximal muscle torque (Nm) in the leg extensor muscles. This device has been tested previously for mechanical reliability with good results and, concerning the validity of isometric torque and position measurements, the Biodex<sup>®</sup> device was considered acceptable for both clinical and research purposes at velocities below 300°/seconds (Drouin et al., 2004).

The subjects sat upright with the back supported, at a hip angle of 85°. 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 the femoral condyles (estimation by palpation). Fig. 15 illustrates the test position. The knee extensor muscles of the right leg were measured at 120°/seconds, in a range of motion (RoM) of 90°–10° of knee flexion. Five consecutive concentric contractions were performed and the peak torque of the best contraction was recorded.



Fig. 15. Measurement of maximal isokinetic muscle strength of the leg extensor muscles using a Biodex device (Biodex<sup>®</sup> Corp, Shirley, NY, USA).

*Picture adapted with the kind permission from the manufacture at biodex.com.*

#### 4.9.3.2 Handgrip strength

The dominant hand was tested for handgrip strength with a handgrip strength dynamometer (Jamar<sup>®</sup>, Sammons Preston, Bolingbrook, IL, USA). This device has been tested previously for reliability (inter- and intra-test reliability,  $r = 0.98$  and  $r = 0.94-0.98$ , respectively) (Peolsson et al., 2001). The subjects were tested for handgrip strength with the arms hanging along the side of the body. The best result (in kg) of three trials was recorded.

#### 4.9.3.3 One-leg hop test

The one-leg hop test was used to measure explosive muscular power. This test was originally described by Tegner et al., (1986) and a modified version allowing the use of arms to accelerating the jump was described later (Zetterström et al., 1992). The one-leg hop test has shown great test-retest reliability: ICC,  $r = 0.96$  (Ageberg et al., 1998). The test is performed by jumping off and landing on the dominate leg (right leg in all cases in this thesis) with maintained control of posture and orientation. The best jump out of at least three trials was recorded (until no increase in hop length was seen). Fig. 16 illustrates the performance.



Fig. 16. Illustration of the start and stop position of the one-leg hop test. The subject jumps off and lands on the same leg, with maintained control of posture and orientation.

#### **4.9.4 Muscle biopsy collection and analyses**

The muscle biopsy specimen was collected from the m. vastus lateralis of the quadriceps muscle, in rest and using a percutaneous needle biopsy technique. The collection of biopsy specimens was done in three consecutive menstrual cycles and followed a structured schedule (Fig. 12) where one biopsy specimen was collected each month during three different phases of the menstrual cycle in each subject. After the collection, all samples were immediately cooled in liquid nitrogen and frozen in isopentane and stored at  $-80^{\circ}\text{C}$  until analyzed.

In order to extract the RNA from the muscle biopsy specimen, a purification procedure was applied using a combination of Trizol reagent and RNeasy Clean Up Kit (Qiagen, Hilden, Germany). Specific management is described in the published paper (Ekenros et al., 2016). Total RNA was purified using the RNeasy Clean Up Kit (Qiagen) according to the manufacturer's instructions. One hundred nanograms of total RNA from each sample were then reverse transcribed into cDNA using TaqMan Reverse Transcription Reagents (Applied Biosystems, Stockholm, Sweden) with random hexamer primers. Real-time (RT) PCR assays were conducted using the Applied Biosystems 7500 fast RT-PCR system with SYBR green master mix RT-PCR reagent (Applied Biosystems). All RT-PCRs were performed in duplicate. Acidic ribosomal phosphoprotein PO (36B4) was used as an internal control gene (Akamine et al., 2007).

An enzyme-linked immunosorbent assay (ELISA) was used to determine protein levels of hormone receptors. The tissue homogenization procedure was carried out. Approximately 200 mg of muscle tissue were homogenized with 1 mL of ice-cold lysis buffer (see Ekenros et al., 2016 for more details). Tissue extracts were prepared by centrifugation at 10,000 g for 10 min at  $4^{\circ}\text{C}$ . The protein concentration of the tissue extract was calculated using the Bradford assay method (Bio-Rad Hercules, CA, USA). 20  $\mu\text{g}$  protein was used for the determination of ER $\alpha$  protein levels, while 30  $\mu\text{g}$  protein was used for the determination of PR and AR protein levels. In all cases, NR Sandwich ELISA by Active Motif was used according to the manufacturer's instructions.

#### **4.10 DATA ANALYSIS**

Statistical tests and analysis and assembling of descriptive data were performed using the STATISTICA software (Statsoft, version 12, Tulsa, OK, USA) and the Statistical Package for

the Social Sciences (SPSS) (SPSS Inc, version 17, Chicago,IL, USA). Table 3 lists the descriptive statistics and statistical analyses in the present thesis.

In Papers II and III an idealization of cycle length was done in order to make comparable cycle lengths when analyzing the CD scales. The menstrual cycle length was therefore idealized to 28 days in all regularly menstruating women. The most critical days to be measured were cycle days 3–5 (early follicular phase), days 12–24 h after the LH surge (ovulation phase) and 7 days after ovulation (mid-luteal phase). Preovulatory days were cut out in women with menstrual cycles longer than 28 days. In cases where the menstrual cycle was shorter than 28 days, the cycle was extended with preovulatory days.

Table 3. The statistical methods used in the respective Papers of this thesis.

	Paper I	Paper II	Paper III	Paper IV
<b>Descriptive statistics</b>				
Mean	●	●	●	●
Standard deviation	●	●	●	●
Median	●	●	●	
Standard error of mean	●	●		
IQR 25th - 75th percentile	●	●		
Min-Max	●	●	●	●
<b>Statistical methods</b>				
Mann Whitney U-test	●		●	
Wilcoxon Rank Sum Test			●	
Pearsons Corr. Coefficient				●
Spearman Rank Corr.				●
Repeated ANOVA	●	●	●	
Mixed models ANOVA				●
Fisher post Hoc	●	●	●	

Abbreviations: (IQR) Interquartile range, (Min) Minimum, (Max) Maximum, (Corr.) Correlation, (ANOVA) Analyze of varians

#### *4.10.1.1 Paper I*

The Mann-Whitney U-test was used to analyze each symptom on the CD scale and to identify women with cyclicity (Hammarbäck et al., 1989). Postural control data were analyzed using repeated measures analysis of variance (ANOVA). The Fisher post hoc test for multiple comparisons was used to assess differences between groups and phases.

#### *4.10.1.2 Paper II*

Analyses of muscle strength between groups and between phases within groups were performed using ANOVA.

#### *4.10.1.3 Paper III*

The Mann-Whitney U-test was used to analyze each symptom on the CD scale and to identify women with cyclicity (Hammarbäck et al., 1989). The Chi-square test was used to detect changes in the number of women with cyclicity between cycles. The Wilcoxon rank sum test was used to analyze the difference between the menstrual and premenstrual phases. Group data were further analyzed by ANOVA, comparing the seven days in the menstrual phase with the seven days in the premenstrual phase during the non- OC cycle vs. the OC cycle. The Fisher post hoc test for multiple comparisons was used to assess differences between the non-OC cycle and the OC cycle.

#### *4.10.1.4 Paper IV*

A mixed model was used with phase as a within-group factor and three levels (FP, OP, and LP). Correlations were performed using the Pearson correlation coefficient as well as the Spearman rank correlation. All tests were two-sided and the level of significance was set at 0.05.

## 5 RESULTS AND DISCUSSION

The main results are presented in this section; please see each publication and manuscript for detailed results.

### 5.1 OVERALL FINDINGS

The overall purpose of this thesis was to explore postural control and muscle function in relation to sex hormones and PMS. The thesis comprises four papers on the basis of which the main findings will be reviewed and discussed.

The main findings were:

- Postural control was altered in women with PMS on OC treatment compared to women on OC treatment without such symptoms.
- Treatment with OCs did not have an acute effect on muscle strength and hop performance in physically active women.
- Treatment with OCs reduced somatic PMS but had no effect on mood symptoms in this group of healthy regularly exercising women.
- Variations in mRNA and protein levels of ER $\alpha$  and PR were detected in skeletal muscle during the follicular, ovulatory, and luteal phase, respectively. However, no variation in AR was detected across the menstrual cycle.

### 5.2 HORMONE DETERMINATION

During the normal menstrual cycle (the non-OC cycle), the hormone levels in serum confirmed the different phases of the menstrual cycle (FP, OP, and LP). Serum analyses of endogenous hormone levels during the OC cycle were low, as expected, which confirmed the intake of OCs. The exogenous hormones in OCs are not measured in serum analysis. The hormone levels are displayed in Table 4.

Table 4. Mean (SD) of hormone levels during three phases in the normal menstrual cycle (non-OC cycle) and during the OC cycle (n = 19).

	Normal menstrual cycle (non-OC cycle)			OC cycle		
	FP	OP	LP	Withdrawal	Pill day 7-8	Pill day 14-15
FSH(mlU/ml)	6.5 (1.9)	7.4 (3.6)	3.6 (2.4)	5.9 (3.0)	4.5 (3.6)	2.3 (1.9)
LH(mlU/ml)	4.7 (1.4)	10.9 (7.0)	3.7 (1.9)	3.7 (1.8)	5.0 (3.4)	2.2 (2.8)
E2(pmol/l)	114 (30.5)	268 (171)	403 (220)	100 (43.7)	109 (23.7)	289 (274)
P-4(nmol/l)	1.7 (1.4)	8.3 (7.1)	36.5 (19.4)	1.2 (0.3)	1.2 (0.3)	1.4 (0.4)
SHBG(nmol/l)	55.0 (18.0)	53.7 (16.0)	57.2 (16.0)	110 (62.8)	107 (63.4)	117 (69)

Abbreviations: FP (follicular phase), OP (ovulation phase), LP (luteal phase), FSH (follicle stimulating hormone), LH (luteinizing hormone), E2 (estradiol), P-4 (progesterone), SHBG (sex hormone-binding globulin)

### 5.3 POSTURAL CONTROL

In Paper I, an altered postural control during the OC phase in women with PMS (n = 6) was demonstrated. However, there was no difference in postural control in women without such symptoms (n = 9). Results from the CD scale showed that six women had a significant increase in somatic and negative mood symptoms during the OC phase, compared to the non-OC phase (PMS group), whereas nine women did not show any cyclical changes between the phases (non-PMS group). Negative mood symptoms dominated over somatic symptoms in the PMS group (Fig. 17).

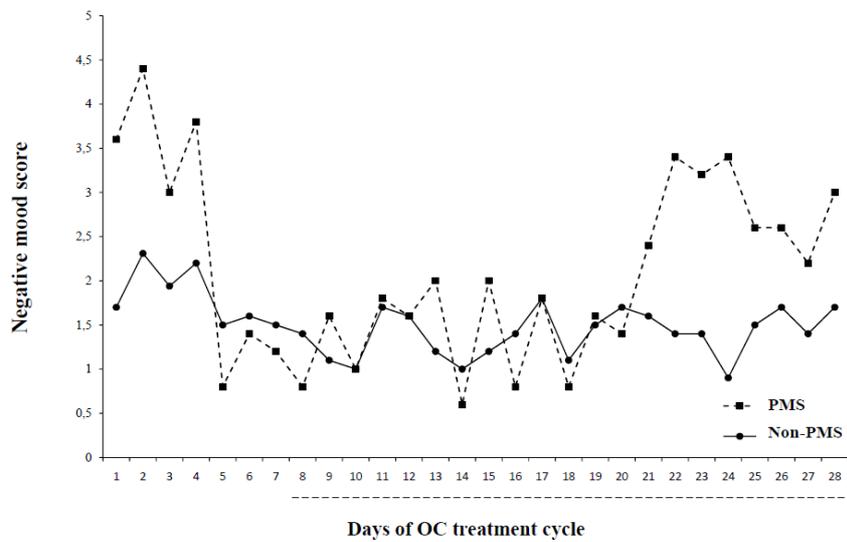


Fig. 17. Mean negative mood scores during a treatment cycle in the PMS group (n = 6) and the non-PMS group (n = 9) respectively.

Measurements of postural control on the force platform demonstrated a significant interaction ( $p < 0.05$ ) between phase and group. In the PMS group, the displacement area ( $\text{cm}^2$ ) was significantly greater in the OC phase, compared to the non-OC phase (Fig. 18). In the non-PMS group, no significant difference between phases was observed. A significantly greater displacement area was shown in the PMS group, compared to the non-PMS group, in the OC phase ( $p < 0.05$ ). No difference between groups was found in the non-OC phase (Fig. 16).

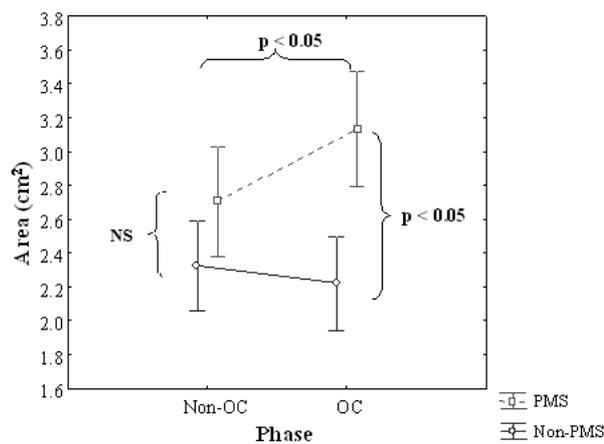


Fig. 18. Mean (SEM) displacement area during the non-OC phase and the OC phase of an OC cycle in the PMS group (n = 6) and the non-PMS group (n = 9), respectively.

### 5.3.1 Postural control in relation to sex hormones and PMS

Fridén et al., (2003a; 2005) studied regularly menstruating women during three different phases of the menstrual cycle and demonstrated that women with PMS had an altered postural control during the luteal phase, compared to the follicular phase and ovulatory phases. This difference in postural control was only seen in women with PMS. Darlington et al. (2001) detected a greater displacement in the luteal phase and in the early follicular phase. In this study the influence of PMS was not evaluated. Later, studies on postural control during different phases of the menstrual cycle have not been able to show any differences in postural control (Abt et al., 2007; Hertel et al., 2006; Harriell et al., 2010; Ericksen & Gribble, 2012). However, ratings of PMS were not performed in those studies.

Variations in E2 and P-4 levels during the menstrual cycle are known to affect the CNS (McEwen 2002). The progesterone metabolite and neurotransmitter, allopregnanolone, acts as a positive modulator of the GABA-A receptor, similar to the action of benzodiazepines (Gee et al., 1987; Majewska et al., 1986). Benzodiazepines and allopregnanolone have been reported to have several negative effects, including drowsiness, concentration difficulties, motor incoordination, muscle weakness, and memory impairment (Holbrook et al., 2000; Longo & Johnson, 2000; Sundstrom et al., 1999). Söderpalm et al., (2004) demonstrated a mild sedative effect of allopregnanolone, with impairment of smooth eye pursuit movement and self-reported fatigue in both men and women. In an animal study, Tamarova et al., (2007) showed a reduction in postural adjustment when the inhibitory GABA-A receptor in the brain was modulated, suggesting a connection between GABA-A receptor activation and postural control. Besides, modulation of GABA-A has, in some individuals (those with severe PMS), been shown to cause low mood, irritability, and anxiety (Bäckström et al., 2014 *review*). Low mood and anxiety have been suggested earlier to correlate with altered postural control (Bolmont et al., 2002; Wada et al., 2001). In addition, mood states have been shown to alter the reaction time on anticipatory postural adjustment (Kitaoka et al., 2004).

In the present thesis, some of the women showed PMS despite OC use. Negative mood symptoms were shown to dominate over somatic symptoms in this group. This is in agreement with OC treatment studies on PMS, where somatic symptoms have been shown to be relieved and mood symptoms remained (Bäckström et al., 1992). In the present thesis, serum levels of endogenous E2, P-4, and T were low during OC treatment, as expected. The exogenous hormones, e.g., the progestagen component in OCs, may, in some women, trigger the negative mood symptom, as proposed for the natural P-4 and its metabolites (Bäckström et al., 2003).

### 5.3.1.1 *Measurements of postural control*

In Paper I, only women on OC treatment were included and divided into two groups: PMS and non-PMS. Postural control was evaluated in a one-legged stance on an AMTI<sup>®</sup> force platform. Measurements with objective stabilometry using force platforms are considered to be the golden standard with acceptable test-retest reliability (Ek Dahl et al., 1989). A significant increase in the displacement area was demonstrated in the women with PMS during the OC phase (active pills), but not during the non-OC phase (withdrawal phase) and no difference was detected between phases in the women without PMS. However, when measuring postural control in a sports medicine context it is important to understand its complexity. Research suggests an individual nonlinear relationship between dynamic stability and the degree of performance and pathology for both females and males. The individual strategy for managing a particular motor task can vary greatly and has been suggested to be a cornerstone for understanding adaptable physiological systems (van Emmerik & van Wegen, 2002 *review*; Lipsitz, 2002). For example, no relationship between the strategy of balance in quiet one-legged stance and the strategy used in more complex and dynamic tasks, such as the star excursion balance test (Wikström et al., 2006 *review*) or in a single-leg landing from jumping task (Sell, 2012), has been demonstrated in the literature. Furthermore, there is still limited evidence to support that altered balance in the one-legged stance correlates with an increased risk of musculoskeletal injuries in the lower extremity (Steffen et al., 2016; Söderman et al., 2002; Tropp et al., 1984). However, there seems to be some correlation since several studies have shown proprioceptive balance training to be an effective prevention strategy for specific injuries in specific sports (in both males and females) (Bahr et al., 1997; Wester et al., 1996; Wedderkopp et al., 1999). No validated measurement of static or dynamic balance was performed, however, in the above referred studies at baseline or following the intervention. This makes it unclear whether the intervention of balance training directly affected balancing ability.

### **5.3.1 Altered postural control in relation to musculoskeletal injuries in females**

To consider altered balance as a risk factor for musculoskeletal injuries seems to be controversial. The literature suggests that humans have wide individual spectra when it comes to different strategies to solve different balance tasks (Wikström et al., 2006 *review*). One could expect, however, that within the same individual and the same balance task, one could expect the same pattern for test and re-test. In the present thesis (Paper I), a difference in postural

control strategies was demonstrated in the same women, with a greater displacement area correlating with cyclical symptoms of negative mood. Studies on neuromuscular characteristics measured by means of kinematics and kinetics have failed to show any differences in jumping and landing patterns in women during different, serum-verified phases of the menstrual cycle (Chaudhari et al., 2007; Abt et al., 2007) or on different occasions during an OC cycle (Chaudhari et al., 2007). In a prospective study of 838 basketball and soccer players, Steffen et al., (2016) found, no significant difference in sway velocity, excursion, or dynamic balance between injured and uninjured female elite athletes.

Training programs aimed at enhancing neuromuscular function and proprioception to prevent musculoskeletal injuries have shown promising results in reducing ACL injuries among female athletes (Hewett et al., 2000 *review*; Mandelbaum et al., 2005; Myklebust et al., 2003).

## **5.4 MUSCLE STRENGTH AND SEX HORMONES**

The aim of Paper II was to compare muscle strength and hop performance in women on OC treatment (OC cycle) with the same women during the normal menstrual cycle (non-OC cycle). The outcome showed no overall differences between the OC cycle and the non-OC cycle. The results for isokinetic muscle strength in the knee extensors (peak torque) showed no difference between the respective cycles ( $p = 0.78$ ) (Fig. 19). Similar findings of no significant differences in the different cycles were noted for measurements of handgrip strength ( $p = 0.76$ ) and for the one-leg hop test ( $p = 0.78$ ) (Fig. 20).

There were no differences in any of the parameters of strength between the different phases in the OC cycle (active hormone phase vs. withdrawal) or within the phases in the non-OC cycle, apart from a significant increase in peak torque in the luteal phase, compared to the follicular phase.

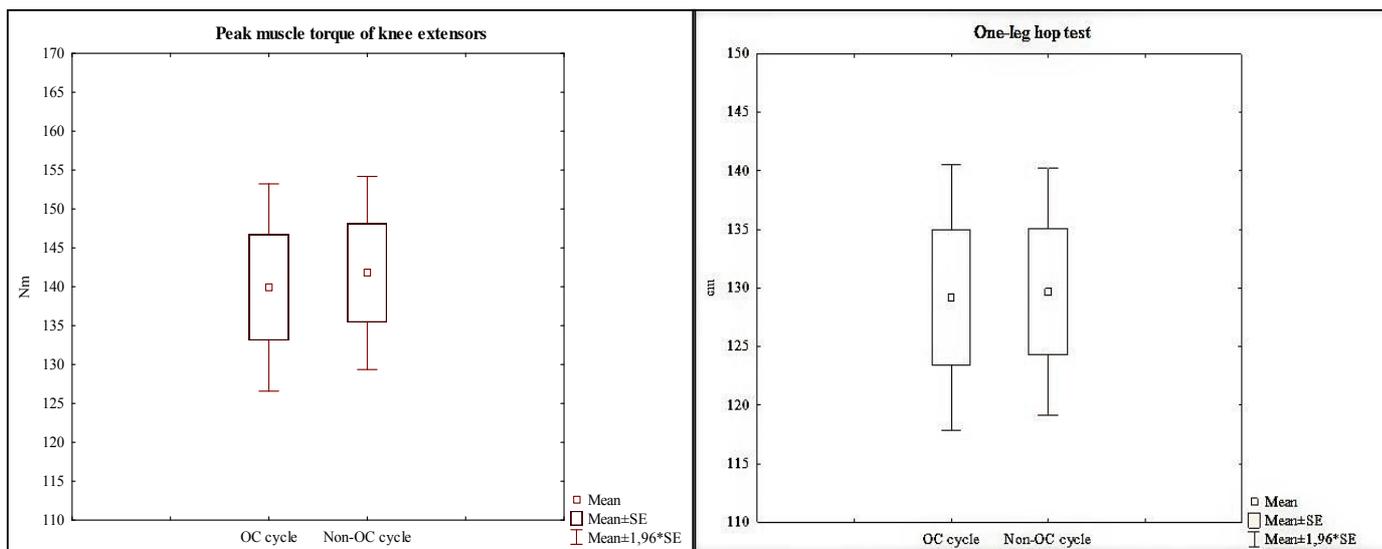


Fig. 19-20. Illustrates the result for isokinetic muscle strength (peak torque) in the knee extensors (Nm) and the performance of the one-leg hop test (centimeters). There were no significant differences in any of the evaluated parameters between the OC cycle and non-OC cycle.

The influence of female endogenous and exogenous sex steroids on neuromuscular function has been debated. Estrogen has been suggested to have anabolic effects on the skeletal muscle that may be explained by energy metabolism properties (Enns & Tiidus, 2010 *review*). Estrogen has been assumed to promote glycogen uptake and storage in the liver and skeletal muscles through increased lipid synthesis and enhanced lipolysis in the skeletal muscle (Braun & Horton, 2001). In the present thesis (Paper II), the endogenous, as well as the exogenous, sex hormones did not have any effect on skeletal muscle function with regard to strength and hop performance. These findings support previous studies on muscle strength in relation to OC use which suggest no difference in strength connected with OC treatment (Elliot et al., 2005; Lebrun et al., 2003; Ruzic et al., 2003). Elliot et al., (2005) studied 14 women on monophasic OC use and found no significant difference in maximal force production of the dorsal interosseous muscle. Lebrun et al., (2003) used the triphasic OC formulation in a study on 14 physically active women and were not able to show any significant difference in isokinetic muscle strength during OC treatment. However, Philips et al., (1996) showed that OC users had a significant increase in maximal force production in the adductor pollicis over a six-month training period, compared to non-OC users. Although the progestagen component in different OC formulations varies in its potency and androgenicity, OC use might be able to affect soft tissue and muscle strength.

Overall, it seems that the androgenic component is not large enough to influence muscle strength. The progestagen levonorgestrel, which belongs to the more androgenic type, was the most frequently used one (n=10) in the present thesis. Due to the small sample size, no stratification was performed on each OC formulation (Paper II). Since no conclusions regarding different OC formulations and muscle strength can be drawn, the choice of OC is still open to the particular female athlete.

In healthy, fertile-aged women, ingestion of OCs does not seem to have any effect on skeletal muscle strength. In animal studies, however, estrogen has been shown to possibly have an anabolic effect on skeletal muscle strength (Enns & Tiidus, 2010 *review*; Skelton et al., 1999). Additionally, in women with a low estrogenic state, as in postmenopausal women, replacement therapy of estrogens might have anabolic effects on muscle strength (Ronkainen et al., 2009; Dieli-Conwright et al., 2009).

Intensified strength training during the follicular phase (elevated levels of E2) has been suggested to have a positive effect on muscle strength in physically active women, compared to the same training protocol during the luteal phase (Reis et al., 1995; Sung et al., 2014; Wikström-Frisén et al., 2015). In a study by Wikström-Frisén et al., (2015,) no division into groups was done. Non-OC users and OC users (both monophasic and triphasic) were clustered and analyzed together. Mono- and triphasic OCs differs in the amount ethinyl estradiol and progestagen across the pill chart. Monophasic OCs have a constant hormone level while triphasic OCs vary in the hormone levels aimed a mimicking the natural menstrual cycle (Redman & Weatherby, 2004). Apart from the proposed estrogenic effect in the follicular phase, it can be speculated if cyclical symptoms in the luteal phase, which are experienced by more than 75% of fertile-aged women, would have an effect on the results. The mental states of, e.g., fatigue, lack of interest, and lack of concentration, might influence the motivation to train hard and, consequently, lead to a defaulted gain in muscle strength.

## **5.5 ORAL CONTRACEPTIVES AND PMS**

To the best of our knowledge, Paper III is the first prospective study of PMS in healthy, physically active women switching from not using OC to OC treatment or *vice versa*. The results of Paper III showed an overall (n = 19) significantly (p <0.05) higher rating of somatic symptoms during the premenstrual phase (seven days) in the normal menstrual cycle (non-OC), compared to the corresponding seven days in the OC cycle (Fig. 21). In contrast, the negative

mood scores did not differ between the two cycles (Fig. 22). Negative mood symptoms are, in general, less common regardless of OC treatment or non-OC treatment, which was also found in the present study in agreement with Sveinsdóttir and Bäckström, (2000). The progestagen component in OCs inhibits ovulation by a feed-back mechanism on the pituitary. The formation of the corpus luteum fails, which results in a reduced plasma concentration of neurosteroids in the brain (REF). Still, a subset of women on OCs experience negative mood symptoms, hypothetically due to a similar effect of the progestagens in the brain (REF).

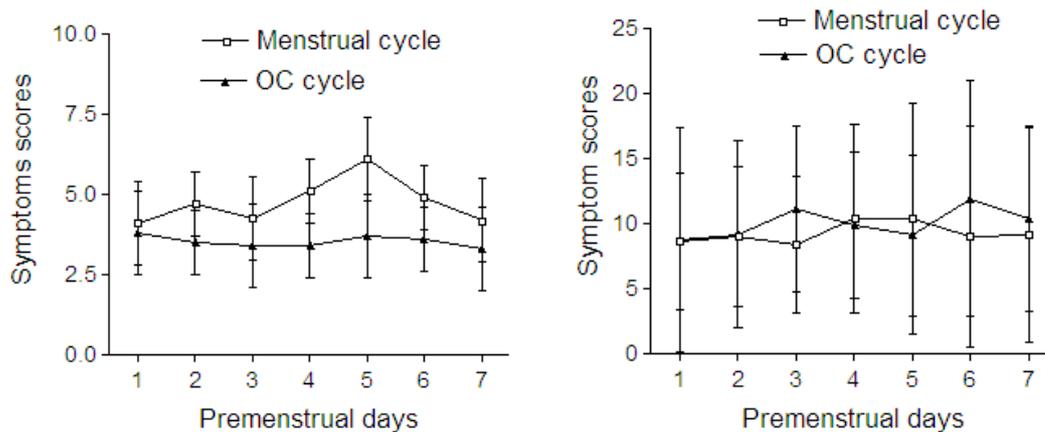


Fig. 21-22. Illustrates the symptom scoring for summarized somatic symptoms and for summarized negative mood symptoms in the entire study group (n = 19) during the normal menstrual cycle and the OC cycle, respectively.

The results of PMS in the Non-OC Starters (n = 11) and OC Starters (n = 8) showed that 8 out of 11 women among the Non-OC Starters had PMS of a somatic type ( $p < 0.05$ ) during the normal menstrual cycle. After having switched to OC treatment, only 4 out of 11 still showed PMS in symptoms of the somatic type ( $p = 0.09$ ). Fig. 23 illustrates the summarized somatic symptoms in one woman showing PMS during the normal menstrual cycle, but not during the following OC cycle. In contrast, the negative mood symptoms did not change between the normal menstrual cycle and the OC cycle.

Among the OC Starters, 2 out of 8 women showed PMS in symptoms of the somatic type during the OC cycle. After washout, there was no change in symptoms scores in the normal menstrual cycle. For the symptoms of negative mood, the proportion of women showing PMS

in the OC cycle was 2 out of 8. After washout, a proportion of 4 out of 8 women showed negative mood symptoms in the normal menstrual cycle (ns).

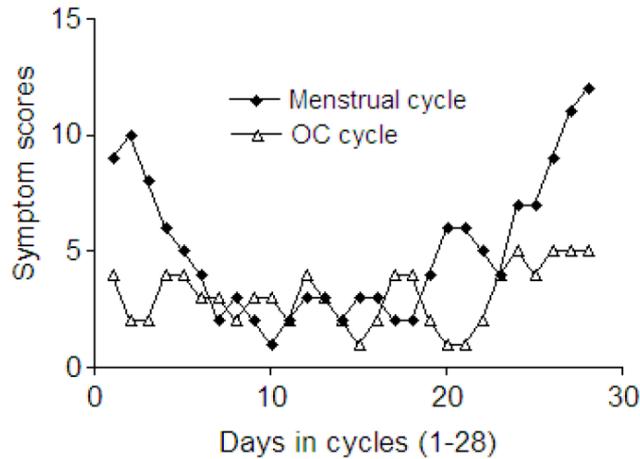


Fig. 23. Illustrates the summarized somatic symptoms scores for one woman among the Non-OC Starters. The premenstrual days were set to cycle days 24–28 and days 1–2 (total, 7 days).

Only a few randomized controlled trials have been published that evaluate the effect of OC on PMS (Graham & Sherwin, 1992; Bancroft & Rennie, 1993), and with conflicting results. The treatment effect of combined OCs on PMS and PMDD seems to be dependent on the type of progestagen, the dosage of ethinyl estradiol, and the treatment regimen. In the present study, a variety of OCs were used with different dosages of ethinyl estradiol (25–30 µg) and with different types of progestagen (levonorgestrel, norethisteron, norgestimat, drospirenone, and desogestrel combined with lynestrenol) and in a 21/7 regimen. A Cochrane review aimed at evaluating the relatively novel progestin, drospirenone, has shown a reduction in somatic PMS as well as in negative mood symptoms in both women with PMS and PMDD (Lopez et al., 2012). Compared to other progestagens, drospirenone has anti-androgenic and anti-aldosterone properties (Yonkers et al., 2005; Pearlstein et al., 2005). Furthermore, in a recent study, the progestagen norgestimate, combined with 35 µg ethinyl estradiol, has been shown to improve both somatic and mood symptoms in women with mild to severe PMS (Nyberg, 2013). In a review of continuous (no withdrawal) OC treatment with levonorgestrel and 20 µg ethinyl estradiol, a non-consistent treatment effect on the treatment of PMS and PMDD was demonstrated (Freeman et al., 2012). The regimen of 24 active-pill and 4 pill-free days has been shown to be more effective for reducing cycle-related symptoms, compared to the traditional

21/7 regimen, due to more complete suppression of ovarian activity and a lower release of endogenous FSH, E2, and P-4 (Yonkers et al., 2008).

In a sport-related perspective, the use of OC does not guarantee total relief of PMS. Women respond differently to different progestagens: some are still left with negative mood symptoms, even after some month or so of treatment, and some respond well, with a stabilized effect on both mood and somatic symptoms. The psychological negative mood symptoms may possibly affect the performance in sports and trainability. Some literature suggests a lower injury rate in females using OCs, compared to those who not use OCs (Gray & Gugala, 2016; Möller-Nielsen & Hammar, 1998; Rahr-Wagner et al., 2014).

#### *5.5.1.1 Effects of physical aerobic exercise on low mood*

Physical activity has the capacity to promote synaptic and functional plasticity in the brain and spinal cord (Vaynman & Gomez-Pinilla, 2005 *review*). Endurance exercise in particular has been shown to be effective for alleviating depressed mood (Vaynman & Gomez-Pinilla, 2005 *review*). Tozzi et al., (2016) showed in a prospective study that 16 weeks of aerobic exercise, twice a week, significantly reduced depressed mood and improved well-being in healthy men and women. According to a magnetic resonance imaging (MRI) evaluation, the improvements in mood were related to a strengthening of connectivity in the functional brain region that regulates mood. They also demonstrated a dose-dependent response (Tozzi et al., 2016). Literature on PMS and exercise (Aganoff & Boyle, 1994; Prior et al., 1997; Steege & Blumenthal, 1992; Stoddard et al., 2007) suggests an improvement of cyclical symptoms with regular aerobic exercises in sedentary females. However, the underlying mechanism of the improvement was not studied and it remains unclear whether exercise affects the PMS or whether it is the effect of well-being due to increased physical activity. Exercise therapies for the management of women diagnosed with PMS/PMDD have not yet been conducted in clinical trials.

## **5.6 HORMONE RECEPTORS IN SKELETAL MUSCLE**

In the present thesis (Paper IV), a significant variation in mRNA and protein levels of the steroid hormone receptors ER $\alpha$  and PR were detected in skeletal muscle during three confirmed phases of the menstrual cycle in 15 women. The mRNA levels of ER $\beta$  were undetectable in all

three phases and the mRNA and protein levels of AR were constant and showed no significant variation across the menstrual cycle. The relative mRNA and protein levels of steroid hormone receptors are displayed in Fig. 24-25.

There was a significant difference in mRNA levels of ER $\alpha$  between the three phases of the menstrual cycle (Fig. 24). The expression of ER $\alpha$  followed a reversed pattern as E2 levels in serum. The ER $\alpha$  was significantly higher in the follicular phase, compared to the ovulatory phase ( $p < 0.05$ ) and the luteal phase ( $p < 0.001$ ). Furthermore, ER $\alpha$  mRNA levels were significantly higher in the ovulatory phase compared to the luteal phase ( $p > 0.001$ ). The results for protein levels of ER $\alpha$  demonstrated a similar pattern as the mRNA levels across the menstrual cycle with the highest levels in the follicular phase (Fig. 25). The levels in the follicular phase were significantly higher than in the luteal phase ( $p < 0.01$ ).

A significant overall effect was detected on mRNA levels of PR between the phases ( $p < 0.05$ ) (Fig. 24). The PR mRNA levels were significantly lower in the luteal phase, compared to the ovulatory phase ( $p < 0.01$ ). There was no significant difference in mRNA levels of PR between the follicular and the ovulatory phase. The protein levels of PR displayed a significant overall effect across the menstrual cycle ( $p < 0.01$ ), although in a different pattern compared to the mRNA levels of PR (Fig. 21). The protein levels of PR were significantly higher in the luteal phase of the menstrual cycle, compared to the follicular phase ( $p < 0.01$ ) and the ovulatory phase ( $p < 0.01$ ) (Fig. 25).

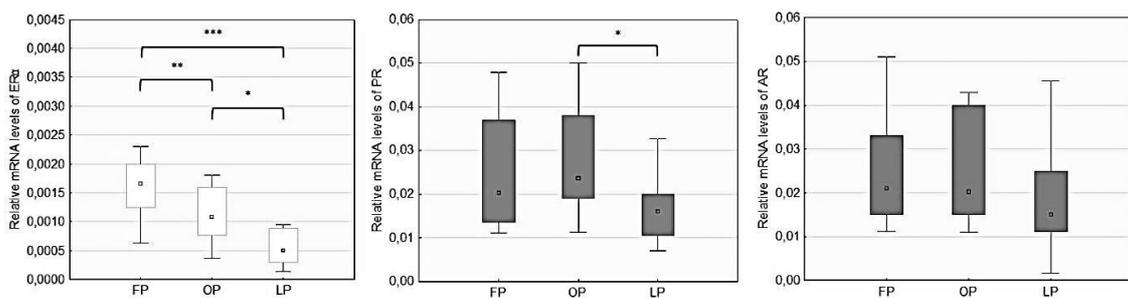


Fig. 24. Relative mRNA levels of ER $\alpha$ , PR, and AR in the follicular phase (FP, ovulatory phase (OP), and luteal phase (LP). The box plots show the median and 25–75%, and the bars show the non-outlier range. Levels of significance were set to: \*0.05, \*\*0.01, \*\*\*0.001.

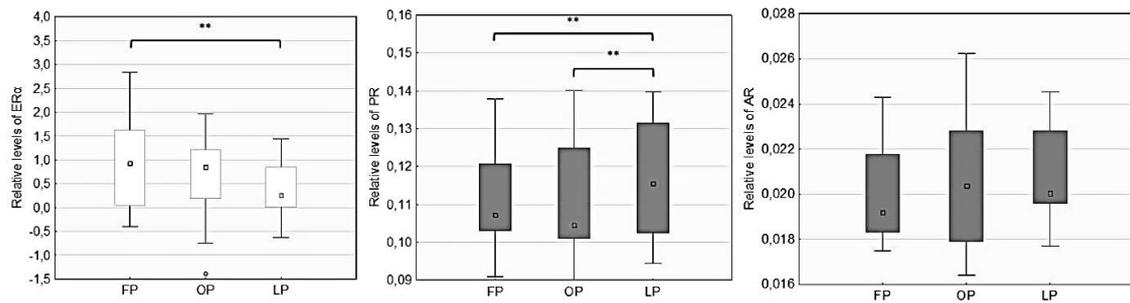


Fig. 25. Protein levels ER $\alpha$ , PR and AR in the FP, OP and LP. For ER $\alpha$  a recombinant protein was used to make a standard curve; for PR and AR, cell extract was used to make the standard curve. Measured levels of ER $\alpha$ , PR and AR are normalized to microgram of total protein. The box plots show the median and 25–75%, and the bars show the non-outlier range. Levels of significance were set to: \*0.05 and \*\*0.01.

Expression of mRNA of ER $\alpha$  (Lemonie et al., 2003) and expression of mRNA and protein levels of both ER $\alpha$  and ER $\beta$  (Wiik et al., 2003; 2009) have previously been demonstrated in human skeletal muscle tissue. The mRNA expression of ER $\alpha$  was shown to be 180-fold higher than the mRNA expression of ER $\beta$  (Wiik et al., 2003). The mRNA expression of ER $\beta$  was undetectable in the present thesis; thus an analysis of protein was not performed. In contrast, Wiik et al., (2003) demonstrated protein expression of ER $\beta$  in six human adults (three women and three men). The differences in results may be explained by the different methods used in the studies. In the studies by Wiik et al., (2003; 2009), an immunohistochemistry protocol was used to stain ER $\alpha$  and ER $\beta$  protein, while in the present thesis, an ELISA assay was used.

The present thesis is, to the best of our knowledge, the first one to explore ER $\alpha$  in skeletal muscle tissue during different phases of the menstrual cycle. The highest levels of mRNA and protein of ER $\alpha$  were demonstrated in the early follicular phase when the serum levels of E2 were at the lowest. Conversely, during the mid-luteal phase, the lowest levels of mRNA and protein of ER $\alpha$  were demonstrated when the serum levels of E2 were at their highest. Mertens et al., (2001) have studied variation in both ER $\alpha$  and ER $\beta$  in different cell types of the endometrium (epithelial, glandular, and stromal cells) and showed variation in the levels of the estrogen receptors across the menstrual cycle. The highest levels of ER $\alpha$  and ER $\beta$  in the endometrium were demonstrated during the early proliferative phase, which corresponds with the mid- follicular phase in the menstrual cycle. The endometrial cells were shown to gradually decline during the secretory (luteal) phase (Mertens et al., 2001). The physical functions of the

variation in estrogen receptors in human skeletal muscle are not known. In a cross-sectional study, Wiik et al., (2005) have demonstrated a greater mRNA expression in both ER $\alpha$  and ER $\beta$  in highly endurance-trained male athletes, compared to moderately active males. In some studies, estrogen has been suggested to contribute to better trainability of skeletal muscle strength (Reis et al., 1995; Sung et al., 2014).

Progesterone receptors have previously been demonstrated in the skeletal muscle of the pelvic floor in postmenopausal women (Copas et al., 2001). In Paper IV, mRNA and protein expression of PR were demonstrated in skeletal muscle tissue in the three different phases of the menstrual cycle. Progesterone has been suggested to have catabolic properties with regard to skeletal muscles (Oosthuysen & Bosch, 2010 *review*) and may contribute to reduced trainability in the luteal phase where serum levels of P-4 are at the highest (Reis et al., 1995; Sung et al., 2014).

The serum levels of T (both T and free-T) are generally low in the follicular and luteal phases, but a few days prior to the LH surge before ovulation, an approximately 30–45% increase has been demonstrated (Sinha-Hikim et al., 1998). This increase in serum T was also demonstrated in the present thesis (Paper IV). However, for the AR (at mRNA and protein levels), no significant change was recorded across the menstrual cycle (Paper IV). Testosterone is a well-known anabolic steroid, in the exogenous form in particular (Crewther et al., 2011 *review*). Endogenous testosterone in women is less well studied. No studies have yet been done dealing with the effect on skeletal muscle function of the peak in T during the normal menstrual cycle. Interestingly, adequate levels of T may compensate for the effect of fatigue in fast-twitch muscle fibers and then contribute to better neuromuscular efficiency (Bosco et al., 2000). The prevalence of hyperandrogenism and PCOS is common among female Olympic athletes with menstrual disturbances (Hagmar & Berglund, 2009). These athletes have been shown to have higher levels of T in the bloodstream with an enhanced effect on physical performance (Rickenlund et al., 2003).

## **5.7 MUSCULOSKELETAL INJURIES IN RELATION TO HORMONES AND PMS**

### **5.7.1 Musculoskeletal injuries in relation to normal hormonal variation and OCs**

Myklebust et al., (1998) reported a higher incidence of sports-related musculoskeletal injuries in female elite handball players during the late luteal phase and cycle days 1–2 of the menstrual cycle with an occurrence of 14 out of 17 injuries in the proposed phases. Similar findings were made by Slauterbeck et al., (2002), who reported the highest incidence during cycle days 1–2. Comparably, Möller-Nielsen & Hammar, (1989; 1991) showed, in a prospective study of 86 female soccer players, a higher injury rate in the luteal phase and at the start of menses in women with PMS. Some studies have, however, reported a potential for traumatic sports injuries to appear more frequently around the ovulatory phase (cycle days 10–14) (Arendt et al., 1999; 2002; Wojtys et al., 1998; 2002). Beynon et al., 2006 showed, in a cross-sectional (case control) study on recreational alpine skiers, a significantly higher injury rate in the preovulatory phase than in the postovulatory phase, based on serum analyses of progesterone collected a few hours post injury in combination with cycle length based on retrospective self-reports. In the study by Slauterbeck et al., (2002), the menstrual cycle phases were verified by analyzing levels of E2 and P-4 in saliva, in addition to which, self-reports in a later study by Wojtys et al., (2002) serum analyses were performed to verify the menstrual cycle phase.

Only a few prospective studies have divided the data on OC treatment. Möller-Nielsen & Hammar, (1989; 1991) showed that women using OCs to have less traumatic injuries compared to non-OC users. Ruedl et al., (2009) studied recreational female alpine skiers and found no differences in injury rates across the menstrual cycle and no difference between OC users and non-OC users. Similarly, Agel et al., (2006) showed no differences in injury rates (ACL injury and ankle sprain) among female athletes (basketball and soccer players) using and not using OCs. In two epidemiologic studies on ACL register data, women on OC treatment were less likely to have a surgical repair of the ACL compared to matched controls (Gray & Gugala, 2016; Rahr-Wagner et al., 2014).

Several studies related to the menstrual cycle in the context of sports medicine suffer from a methodological bias with unreliable verification of menstrual cycle phases. Counting days or relying on recall in self-reports to estimate cycle phase is feasible and quite usual in these kinds of menstrual cycle-related studies. However, these methods give no information about the actual levels of sex hormones and conclusions concerning a specific event (injury, muscle

strength, etc.) specifically related to a certain phase in the menstrual cycle will be doubtful (Vescovi, 2011). It might be inadequate to refer, e.g., to the ovulation phase when not having verified the LH surge and mid-luteal rise in P-4. De Jonge et al., (2012) reviewed 12 studies related to the menstrual cycle phases and exercise performance and found only five to accurately verify the menstrual cycle phases. It is well known that such individual factors as increased intensity of physical activity and physical and mental stress have been shown to highly influence the characteristics of the normal menstrual cycle (To & Wong, 2000; Russel, et al., 1984). In a study on 26 recreationally active women, Schaumberg et al. (2015) illustrated, by means of self-reported regular menstrual cycles, the need for adequate and frequent analyses of sex hormones during the different phases of the menstrual cycle. They found only 70% of their study sample to have an ovulatory menstrual cycle with a sufficient rise in progesterone during the mid-luteal phase. The women who did not show a mid-luteal rise in P-4 were considered to be anovulatory at that cycle (Schaumberg et al., 2012). Besides, in several menstrual cycle-related studies in the sports medicine context, OC users and non-OC users have been treated as one group. It might be misleading to compare studies discussing hormonal effects during OC ingestion based on endogenous hormone levels. It is well known that synthetic ethinyl estradiol has a higher ER affinity and is more potent than endogenous E2 in inducing estrogenic effects in women (Coelingh Bennink, 2004). Traditionally, the phases of the normal menstrual cycle (FP, OP, and LP) refer to hormonal variation. In an OC cycle, these specific phases do not exist. In monophasic OCs, there are only two different hormonal phases (active pill and withdrawal phase). Nevertheless, a serum analysis is needed to verify OC use. Serum measures the endogenous sex hormones during OC use, which are supposed to be depressed, and the levels of SHBG are expected to be elevated.

Another confounding factor when studying sex hormone levels in relation to tissue effects, is the possible lag between the change in serum hormone levels and the physiological impact on performance. For example, in a study by Shultz et al., (2004) using serum analyses on a daily basis, a four days delay was demonstrated between the rise in E2 and the effect on knee laxity.

## **5.8 METHODOLOGICAL CONSIDERATIONS**

### *5.8.1.1 Drop-outs*

In menstrual cycle-related studies aimed at exploring physical function, women are often followed for a long period of time, in this thesis, at least three months. For that reason, the sample size is often limited due to problems concerning compliance with the study protocol and treatment. The drop-out rate was high, mainly because women in the OC Starter group found it difficult to detect ovulation with the Ovustrix® after having ended the OC treatment. It is not unusual that it may take some time before the return of ovulatory cycles after stopping OC use. Nassarella et al., (2008) demonstrated a variation in menstrual cycle length of  $31 \pm 11$  days in women who had recently discontinued OC use. In Paper IV, several women had an unpleasant experience of the muscle biopsy collection and were unwilling to expose themselves to repeated biopsies.

### *5.8.1.2 OC prescription, PMS ratings and ethics*

Another aspect of an ethical nature is the use of low-dose monophasic OCs and the rating of PMS. The women who volunteered to participate in the present thesis study agreed to start OC treatment and were carefully examined for exclusion criteria for treatment before the prescribing of OCs. The cohort (Papers I–III) of healthy, physically active women showed, in general, mild PMS according to ICD-10 and none was diagnosed with PMS/PMDD. Unfortunately, in the OC treatment in Papers I–III, the progestagen component in the monophasic OCs was not equally distributed over the study group, a variety of six different progestagens being used. However, levonorgestrel, a second generation progestagen, was in the majority ( $n = 10$ ). Additionally, the duration of OC treatment varied widely, which might have influenced the outcomes.

### *5.8.1.3 Verification of cycle phase and OC treatment*

In the present thesis, the menstrual cycle phase and verification of OC use were determined by analyzing sex hormones in serum in the morning before breakfast. The blood sampling was performed at the same time as the collection of data on postural control (Paper I), muscle

strength, hop performance (Paper II), and muscle biopsy (Paper IV) was carried out. To confirm the different phases of the menstrual cycle, daily urinary testing, using Ovustix<sup>®</sup> (LH sticks), based on cycle length, was required; together with a serum analysis of hormones.

#### *5.8.1.4 ELISA*

In Paper IV, the expression of hormone receptors in skeletal muscle tissue was analyzed by the nonradioactive method, ELISA. The sandwich ELISA quantifies antigens (protein) between two layers of antibodies (i.e., capture and detection antibody). This method is considered to be highly specific (ELISA encyclopedia, 2017). Immunohistochemistry is another method used to study protein expression of hormone receptors and refers to the process of selectively imaging antigens by using the principle of antibodies binding specifically to antigens in the skeletal muscle tissue. In this method, the antibody-antigen interaction can be visualized and the specific site of the protein can be detected.

#### *5.8.1.5 Transmissibility*

The complex interplay between female sex hormones and their relationship to soft tissue-like ligament, tendons, skeletal muscles, and musculoskeletal injury remains unclear. As described in this thesis, several studies have demonstrated a relationship between the menstrual cycle and the OC cycle in relation to physical performance and the risk of sustaining a musculoskeletal injury. Most of these studies are, however, small and have important limitations. Since some of the studies rely on questionnaires for menstrual cycle days and premenstrual symptoms, the risk of a recall bias is notable. Our young, physically active or sedentary women in this thesis are probably representative of this population of women, but the sample size might be too small to be generalized to a larger group of women. Moreover, the findings might not be applicable to female athletes and younger teen-aged females.

## 6 CONCLUSION

- Postural control, measured according to the displacement area, was shown to be altered during the active hormone phase (OC phase) in healthy physically active women with PMS, compared to women without such symptoms. The displacement area was significantly greater in the OC phase than in the withdrawal phase in women with PMS. In women without PMS (non-PMS), no differences in postural control between these phases were detected.
- Skeletal muscle strength and hop performance did not vary between a normal menstrual cycle and an OC treatment cycle in the same women. Nor did it vary during the different phases of the normal menstrual cycle or during the active hormone phase and withdrawal phase in OC treatment, as measured in healthy physically active women.
- OC treatment decreased the ratings of PMS of the somatic type, but it did not affect negative mood symptoms in healthy women.
- Significant variations of mRNA and protein levels of ER $\alpha$  and PR were found in skeletal muscle tissue during three hormonally confirmed phases of the normal menstrual cycle. The levels of mRNA and protein AR were shown to be constant across the menstrual cycle.

## 7 CLINICAL IMPLICATIONS

- The effects of PMS, even mild PMS, influence balance and might even affect complex neuromuscular performance and should be accounted for in issues concerning sports injuries and the etiology of sports injuries in females.
- Neuromuscular training has been shown to prevent musculoskeletal injuries in female athletes. Hypothetically, it might be even more effective to train complex balance tasks in the luteal phase of the menstrual cycle.
- Since no conclusions regarding different OC formulations and muscle strength can be drawn, the choice of OC is still open to the individual female athlete.
- PMS of variable severity is common, also in healthy, regularly exercising groups of women and the ratings of symptoms can be decreased to some extent by the use of OCs.
- The variation in expression of sex hormone receptors in skeletal muscle may have an impact on the effects of muscular training and sports injuries in women.

## 8 FUTURE PROSPECTIVES

- There is a great need for continuing research in this area with studies on larger cohorts in carefully designed, controlled, and prospectively randomized trials.
- Studies on the effects of physical performance in relation to the menstrual cycle and OCs on well-defined subgroups of women, such as athletes, young women, and women with PMS.
- Studies on specified progestagen components and short-term vs. long-term use of OCs to evaluate eventual effects on skeletal muscle strength and neuromuscular performance.
- Clinical trials of individuals (subgroups based on exercise level, PMS, age) aimed at constructing tailored rehabilitation programs.
- Qualitative research-based studies on the individually perceived aspects of periodized training and time of injury.
- Treatment studies evaluating the effect on PMS of using different constructs of exercise therapy (e.g., endurance, strength and yoga) on well-defined groups of women diagnosed with PMS/PMDD.
- Further exploration of the physiological effects of the ER, PR, and AR in skeletal muscle tissues and the process of adaptation to physical activity.

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## 10 REFERENCES

- Abt, J.P., Sell, T.C., Laudner, K.G., McCrory, J.L., Loucks, T.L., Berga, S.L., & Lephart, S.M.** (2007). Neromuscular and biomechanical characteristics do not vary across the menstrual cycle. *Knee Surg Sports Traumatol Arthrosc*, 15, 901 - 907. doi: 10.1007/s00167-007-0302-3.
- Ashley, C.D., Kramer, M.L. & Bishop, P.** (2000), Estrogen and Substrate Metabolism, A Review of Contradictory Research. *Sports Med*, 29, 221 - 227 doi:10.2165/00007256-200029040-00001
- Aganoff, J.A., & Boyle, G.J.** (1994). Aerobic exercise, mood states and menstrual cycle symptoms. *Journal of Psychosomatic Research*, 38(3), 183 - 192. [http://dx.doi.org.proxy.kib.ki.se/10.1016/0022-3999\(94\)90114-7](http://dx.doi.org.proxy.kib.ki.se/10.1016/0022-3999(94)90114-7)
- Ageberg, E., Zetterström, R., & Moritz, U.** (1998). Stabiliometry and one-leg hop test have high test-retest reliability. *Scand J Med Sci Sports*, 8, 198 - 202. doi:10.1111/j.1600-0838.1998.tb00192.x
- Agel, J., Bershadsky, B., & Arendt, E.A.** (2006). Hormonal therapy: ACL and ankle injury. *Med Sci Sports Exerc*, 38, 7 - 12. Doi:10.1249/01.mss.0000194072.13021.78
- Akamine, R., Yamamoto, T., Watanabe, M., Yamazaki, N., Kataoka, M., Ishikawa, M., & Shinohara, Y.** (2007). Usefulness of the 5' region of the cDNA encoding acidic ribosomal phosphoprotein P0 conserved among rats, mice, and humans as a standard probe for gene expression analysis in different tissues and animal species. *J Biochem Biophys Methods*, 70, 481 – 486. <http://dx.doi.org.proxy.kib.ki.se/10.1016/j.jbbm.2006.11.008>
- American College of Obstetricians and Gynecologists.** Premenstrual syndrome, Washington , DC : National Guideline Clearinghouse, 2000.
- American Psychiatric Association.** (2013). *Diagnostic and Statistical Manual of Mental Disorders* (Fifth ed.). Arlington, VA: American Psychiatric Publishing. ISBN 978-0-89042-555-8.
- Angst, J., Sellaro, R., Merikangas, K.R., & Endicott, J.** (2001). The epidemiology of perimenstrual psychological symptoms. *Acta Psychiatr Scand*, 104,110 - 116. doi:10.1034/j.1600-0447.2001.00412.x
- Arendt, E.A., Bershadsky, B., & Agel, J.** (2002) Periodicity of noncontact anterior cruciate ligament injuries during the menstrual cycle. *J Gend Specif Med*, 5, 19 - 26. doi: not found.
- Arendt, E. A., Agel, J., & Dick, R.** (1999). Anterior cruciate ligament injury patterns among collegiate men and women. *J Athl Train*, 34(2), 86 - 92. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1322895/>

**Bahr, R., Lian, Ø. & Bahr, I. A.** (1997). A twofold reduction in the incidence of acute ankle sprains in volleyball after the introduction of an injury prevention program: a prospective cohort study. *Scand J Med Sci Sports*, 7, 172 - 177. doi:10.1111/j.1600-0838.1997.tb00135.x

**Bell, D.R., Blackburn, J.T., Norcorss, M.F., Ondrak, K.S., Hudson, J.D., Hackney, A.C., & Padua, D.A.** (2012). Estrogen and muscle stiffness have a negative relationship in females. *Knee Surg Sports Traumatol Arthrosc*, 20, 361 - 367. doi:10.1007/s00167-011-1577-y

**Beynnon, B.D., Bernstein, I.M., Belisle, A., Brattbakk, B., Devanny, P., Risinger, R., & Durant D.** (2002). The effect of estradiol and progesterone on knee and ankle joint laxity. *Am. J. Sports Med*, 33(9). doi: 10.1177/0363546505275149

**Beynnon, B.D., Johnson, R.J., Braun, S., Sargent, M., Bernstein, I.M., Skelly, J.M., & Vacek, P.M.** (2006). The relationship between menstrual cycle phase and anterior cruciate ligament injury. *Am. J. Sports Med*, 34, (5), 757-765. doi: 10.1177/0363546505282624

**Beshay, V., & Carr, B.** (2013). Chapter 2: The hypothalamic- pituitary- ovarian axis and control of the menstrual cycle. T. Falcone and W.W. Hurd (eds.), *Clinical Reproductive Medicine and Surgery: A Practical Guide*, doi: 10.1007/978-1-4614-6837-0\_2, © Springer Science+Business Media New York.

**Biggo, G., Follesa, P., Sanna, E., Purdy, R.H., & Concas, A.** (2001). GABA A-receptor plasticity during long-term exposure to and withdrawal from progesterone. *Int rev neurob*, 46, 207 - 241. [http://dx.doi.org.proxy.kib.ki.se/10.1016/S0074-7742\(01\)46064-8](http://dx.doi.org.proxy.kib.ki.se/10.1016/S0074-7742(01)46064-8)

**Boden, B.P., Dean, G.S., Feagin, J.A. Jr., & Garrett, W.E. Jr.** (2000). Mechanisms of anterior cruciate ligament injury, *Orthopedics*, 23, 573 - 578. <https://www.ncbi.nlm.nih.gov.proxy.kib.ki.se/pubmed/10875418?dopt=Abstract>

**Bolmont, B., Gangloff, P., Vouriot, A. & Perrin, P.P.** (2002). Mood states and anxiety influence abilities to maintain balance control in healthy human subjects. *Neurosci Lett*, 329, 96 – 100. doi: 10.1016/S0304-3940(02)00578-5

**Braun, B., & Horton, T.** (2001) *review*. Endocrine regulation of exercise substrate utilization in women compared to men. *Exerc. Sport Sci*, 29(4), 149 – 154. doi:10.2165/00007256-200636050-00003

**Brodie, A., & Inkster, S.** (1993). Aromatase in the testis. *J steroid Biochem Mol Biol*, 44, 549 - 555. doi: 10.1016/0960-0760(93)90258-X

**Brukner, P., & Bennell, K.** (1997). Stress fractures in female athletes diagnosis, management and rehabilitation. *Sports Med*, 24 (6), 419-429. doi:10.2165/00007256-199724060-00006

- Bryant, A.L., Clark, R.A., Bartold, S., Murphy, A., Bennell, K.L., Hohmann, E., & Crossley, K.M.** (2008). Effects of estrogen on the mechanical behavior of the human Achilles tendon in vivo. *J Appl Physiol*, 105, 1035–1043. doi: 10.1152/jappphysiol.01281.2007
- Bryant, A., Crossley, K., Bartold, S., Hohmann, E., & Clark, R.** (2011). Estrogen-induced effects on the neuro-mechanics of hopping in humans. *Eur J App Physiol*, 111, 245-252. doi:10.1007/s00421-010-1647-8
- Burrows, M., & Peters, C.** (2007) The influence of oral contraceptives on athletic performance in female athletes. *Sports Med*, 37(7), 557–574. doi: 10.2165/00007256-200737070-00001
- Bushman, B., Masterson, G., & Nelsen, J.** (2006) Anaerobic power performance and menstrual cycle: eumenorrhetic and oral contraceptive users. *J Sports Med Phys Fitness*, 46, 132 – 137.
- Bäckström, T., Sanders, D., Leask, R.M., Davidsson, D., Warner, P., & Bancroft, J.** (1983). Mood, sexuality, hormones and the menstrual cycle. II. Hormone levels and their relationship to premenstrual syndrome. *Psychosom Med*, 45, 503-507.
- Bäckström, T., Hansson-Malmström, Y., Lindhe, B.A., Cavalli-Björkman, B., & Nordenström, S.** (1992). Oral contraceptives in premenstrual syndrome: a randomized comparison of triphasic and monophasic preparations. *Contraception*, 46, 253-268. [https://doi.org/10.1016/0010-7824\(92\)90006-F](https://doi.org/10.1016/0010-7824(92)90006-F)
- Bäckström, T., Haage, D., Löfgren, M., Johansson, I.M., Strömberg, J., Nyberg, S., & Bengtsson, S.K.** (2011). Paradoxical effects of GABA-A modulators may explain sex steroid induced negative mood symptoms in some persons. *Neuroscience*, 15(191), 46-54.  
<http://dx.doi.org.proxy.kib.ki.se/10.1016/j.neuroscience.2011.03.061>
- Bäckström, T., Andreen, L., Birzniece, V., Björn, I., Johansson, I.M, Nordenstam-Haghjo, M., Zhu, D.** (2003). The role of hormones and hormonal treatment in premenstrual syndrome. *CNS Drugs*, 17, 325 - 342. doi 10.2165/00023210-200317050-00003
- Bäckström, T., Bixo, M., Johansson, M., Nyberg, S., Ossewaarde, L., Ragagnin, G., & van Wingen, G.** (2014) *review*. Allopregnanolone and mood disorders *Progress in Neurobiology* 113 (2014) 88–94  
DOI: 10.1016/j.pneurobio.2013.07.005
- Carlsten, H., Holmdahl, R., Tarkowski, A., & Nilsson, L.A.** (1989). Oestradiol- and testosterone-mediated effects on the immune system in normal and autoimmune mice are genetically linked and inherited as dominant traits. *Immunology*, 68, 209 – 214. doi: <https://www.ncbi.nlm.nih.gov.proxy.kib.ki.se/pubmed/2807378?dopt=Abstract>

**Caspersen, C.J., Powell, K.E., & Christenson, G.M.** (1985). Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public Health Rep*, 100, 126 - 131.  
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1424733/pdf/pubhealthrep00100-0016.pdf>

**Chaudhari, A.M.W., Lindenfeld, T.N., Andriacchi, T.P., Hewett, T.E., Riccobene, J., Myer, G.D., & Noyes, F.R.** (2007). Knee and hip loading patterns at different phases in the menstrual cycle: implications for the gender difference in anterior cruciate ligament injury rates. *Am J Sports Med*, 35(5), 793 - 800. doi: 10.1177/0363546506297537

**Coelingh Bennink, H.J.** (2004). Are all the estrogens the same? *Maturitas*, 47, 269 - 275.  
<http://dx.doi.org.proxy.kib.ki.se/10.1016/j.maturitas.2003.11.009>

**Compagnone, N.A., & Mellon, S.H.** (2000). Neurosteroids: Biosynthesis and Function of These Novel Neuromodulators. *Frontiers in Neuroendocrinology*, 21(1), 1 - 56.  
<http://dx.doi.org.proxy.kib.ki.se/10.1006/frne.1999.0188>

**Conneely, O., & Lydon, J.** (2000). Progesteron receptors in reproduction: functional impact of the A and B isoforms. *Steroids* 65; 571 - 577. doi: 10.1016/S0039-128X(00)00115-X

**Constantini, N.W. & Warren, M.P.** (1995). Menstrual dysfunction in swimmers: a distinct entity. *J Clin Endocrinol Metab*, 80, 2740 - 2744. doi: not found.

**Constantini, N.W., Dubnov, G., & Lebrun, C.M.** (2005). The menstrual cycle and sports performance. *Clin Sports Med*, 24, 51 – 82. doi: 10.1016/j.csm.2005.01.003

**Copas, P., Bukovsky, A., Asbury, B., Elder, R.F. & Caudle, M.R.** (2001). Estrogen, progesterone, and androgen receptor expression in levator ani muscle and fascia. *J Womens Health Gen Based Med* 10, 785 - 795. doi:10.1089/15246090152636541

**Crewther, B.T., Cook, C., Cardinale, M., Weatherby, R.P. & Lowe, T.** 2011 *review*. Two emerging concepts for elite athletes: the short-term effects of testosterone and cortisol on the neuromuscular system and the dose-response training role of these endogenous hormones. *Sports Med*, 41, 103 - 123. doi:10.2165/11539170

**Cureton KJ, Collins MA, Hill DW, & et al.** (1998). Muscle hypertrophy in men and women. *Med Sci Sports Exerc*, 20, 338 - 344. doi: not found.

**Darlington, C.L., Ross, A., King, J. & Smith, P.F.** 2001. Menstrual cycle effects on postural stability but not optokinetic function. *Neurosci Lett* 307, 147 - 150.  
[http://dx.doi.org.proxy.kib.ki.se/10.1016/S0304-3940\(01\)01933-4](http://dx.doi.org.proxy.kib.ki.se/10.1016/S0304-3940(01)01933-4)

**Davies, B.N., Elford, J.C. & Jamieson, K.F.** (1991). Variations in performance in simple muscle tests at different phases of the menstrual cycle. *J Sports Med Phys Fitness* 31, 532 - 537.

<https://www.ncbi.nlm.nih.gov/pubmed/1806730>

**Dieli-Conwright, C.M., Spektor, T.M., Rice, J.C., & Schroeder, T.E.** (2009). Hormone therapy attenuates exercise-induced skeletal muscle damage in postmenopausal women. *J Appl Physiol*, 107, 853–858. doi: 10.1152/jappphysiol.00404.2009

**Dimmock, P., Wyatt, K., Jones, P., & O'Brien, S.** (2000). Efficacy of selective serotonin-reuptake inhibitors in premenstrual syndrome: a systematic review. *Lancet*, 30, 1131- 1136.

[http://dx.doi.org.proxy.kib.ki.se/10.1016/S0140-6736\(00\)02754-9](http://dx.doi.org.proxy.kib.ki.se/10.1016/S0140-6736(00)02754-9)

**Drinkwater, Barbara L.** (2008). *Women in Sport*, edited by Barbara L. Drinkwater, John Wiley & Sons, Incorporated. Pro Quest Ebook Central,

<http://ebookcentral.proquest.com.proxy.kib.ki.se/lib/ki/detail.action?docID=351678>.

**Drouin, J.M., Valovich-McLeod, T.C., Shultz, S.J., Gansneder, B.M., & Perrin, D.H.** (2004) Reliability and validity of the Biodex system 3 pro isokinetic dynamometer velocity, torque and positions measurements. *Eur J Appl Physiol* 91:22-29. doi: 10.1007/s00421-003-0933-0

**Enns, D.L., & Tiidus, P.M.** (2010). The Influence of Estrogen on Skeletal Muscle. *Sports Med*, 40: 41 - 58. doi:10.2165/11319760-000000000-00000

**Eiling, E., Bryant, A.L., Petersen, W., Murphy, A. & Hohmann, E.**(2007). Effects of menstrual-cycle hormone fluctuations on musculotendinous stiffness and knee joint laxity. *Knee Surg Sports Traumatol Arthrosc* 15, 126–132. doi: 10.1007/s00167-006-0143-5

**Ekdahl, C., Jarnlo, G.B., & Andersson, S.I.**, (1989). Standing balance in healthy subjects. Evaluation of a quantitative test battery on a force platform. *Scand J Rehabil Med*, 21, 187–95. <https://www.ncbi.nlm.nih.gov.proxy.kib.ki.se/pubmed/2631193>

**Ekenros, L., Papoutsis, Z., Fridén, C., Dahlman Wright, K. & Hirschberg, A.L.** (2017). Expression of sex steroid hormone receptors in human skeletal muscle during the menstrual cycle. *Acta Physiol*, 219: 486–493. doi:10.1111/apha.12757

**ELISA encyclopedia** (2017). Retrieved 2017 April 3<sup>rd</sup> from: <http://www.elisa-antibody.com/ELISA-Introduction/ELISA-types/sandwich-elisa>

**Elliot, K.J., Cable, N.T., & Reilly, T.** (2005). Does oral contraceptive use affect maximum force production in women? *Br J Sports Med*, 39, 15 - 19. doi: 10.1136/bjism.2003.009886

**Endrikat, J., James, M.A., Mayerhofer, M., Pelissier, C., Müller, U., & Düsterberg, B.** (1995). A twelve-month comparative clinical investigation of two low-dose oral contraceptives containing 20 µg ethinylestradiol/75 µg gestordene and 20 µg ethinylestradiol/150 µg gestordene, with respect to efficacy, cycle control and tolerance. *Contraception*, 52, 229 - 235. [http://dx.doi.org.proxy.kib.ki.se/10.1016/0010-7824\(95\)00191-C](http://dx.doi.org.proxy.kib.ki.se/10.1016/0010-7824(95)00191-C)

**Enns, D.L., & Tiidus, P.M.** (2010). The influence of estrogen on skeletal muscle: sex matters. *Sports Med* 40, 41- 58. doi:10.2165/11319760

**Eriksen, H., & Gribble, P.A.** (2012). Sex differences, hormone fluctuations, ankle stability, and dynamic postural control. *J Athl Train*, 47(2), 143–148.  
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3418125/>

**Freemann, E.W., Halbreich, U., Grubb, G.S, Rapkin, A.J., Skouby, S.O., Smith, L., & Constantine, G.D.** (2012). An overview of four studies of a continuous oral contraceptive (levonorgestrel 90 mcg/ethinyl estradiol 20 mcg) on premenstrual dysphoric disorder and premenstrual syndrome. *Contraception*, 85(5), 437 – 445.

**Fridén, C., Hirschberg, A.L., Saartok, T., Bäckström, T., Leanderson, J. & Renström, P.** (2003a). The influence of premenstrual symptoms on postural balance and kinesthesia during the menstrual cycle. *Gynecol Endocrinol*, 17(6),433–439. doi: 10.1080/09513590312331290358

**Fridén, C., Hirschberg, A.L., & Saartok, T.** (2003b). Muscle strength and endurance do not significantly vary across 3 phases of the menstrual cycle in moderately active premenopausal women. *Clin J Sport Med*, 13, 238–241. doi: 10:00042752-200307000-00007

**Fridén, C., Ramsey, D.K., Bäckström, T., Benoit, D.L., Saartok, T. & Hirschberg, A.L.** (2005). Altered postural control during the luteal phase in women with premenstrual symptoms. *Neuroendocrinology*, 81(3), 150–157. doi:10.1159/000086592

**Fortney, J.A., Harper, J.M., & Potts, M.** (1986). Oral contraceptives life expectancy. *Stud Fam Plann*. 17: 117-125. doi: not found

**Fritz, M.A., & Speroff, L.** (2011). *Clinical gynecologic endocrinology and infertility* 8th ed Philadelphia.

**Galambos, S.A., Terry, P.C., Moyle, G.M., Locke, S.A.** (2005). Psychological predictors of injuries among elite athletes. *Br J Sports Med* 39: 351-354. doi: 10.1136/bjism.2005.018440

**Gastin, P.B.** (2001). Energy system interaction and relative contribution during maximal exercise. *Sports Med*, 31(10), 725-741. doi: 10.2165/00007256-200131100-00003

**Gee, K.W., Chang, W.C., Brinton, R.E., & McEwen, B.S.** (1987). GABA- dependent modulation of the Cl<sup>-</sup> ionophore by steroids in rat brain. *European Journal of Pharmacology*, 136, 419 – 423.  
doi: 10.1016/0014-2999(87)90317-7

**Giacomini, M., Bernard, T., Gavarry, O., Altare, S., & Falgairette, G.** (2000). Influence of the menstrual cycle phase and menstrual symptoms on maximal anaerobic performance. *Med Sci Sports Exerc*, 32 (2), 486- 492. doi: 00005768-200002000-00034

**Goldie, P.A., Bach, T.M., & Evans, O.M.** (1989). Force platform measures for evaluating postural control: reliability and validity. *Arch Phys Med Rehabil*, 70 (7), 510 - 517.

**Graham, C.A., & Sherwin, B.B.** (1992). A prospective treatment study of premenstrual symptoms using a triphasic oral contraceptive. *J Psychosom Res*, 36, 257 – 266. doi:10.1016/0022-3999(92)90090-O

**Gray, A.M., Gugala, Z., & Baillargeon, G.J.** (2016). Effects of oral contraceptive use on anterior cruciate ligament injury epidemiology. *Med Sci Sports Exerc*, 48 (4),648-654. doi: 10.1249/MSS.0000000000000806

**Gür, H.** (1997). Concentric and eccentric isokinetic measurements in knee muscles during the menstrual cycle: a special reference to reciprocal moment ratios. *Arch Phys Med Rehabil* 78, 501–505.  
[http://dx.doi.org.proxy.kib.ki.se/10.1016/S0003-9993\(97\)90164-7](http://dx.doi.org.proxy.kib.ki.se/10.1016/S0003-9993(97)90164-7)

**Gür, H., Akova, B. & Küçükoglu, S.** (1999). Continuous versus separate isokinetic test protocol: the effect of estradiol on the reproducibility of concentric and eccentric isokinetic measurements in knee muscles. *Arch Phys Med Rehabil* 80,1024–1029. doi: 10.1016/S0003-9993(99)90055-2

**Hagmar, M., Berglund, B., Brismar, K., & Hirschberg, A.L.** (2009). Hyperandrogenism May Explain Reproductive Dysfunction in Olympic Athletes. *Med. Sci. Sports Exerc*, 41(6), 1241 – 1248. doi: 10.1249/MSS.0b013e318195a21a

**Hammarbäck, S., & Bäckström, T.** (1988). Induced anovulation as treatment of premenstrual tension syndrome. A double-blind cross-over study with GnRH-agonist versus placebo. *Acta Obstet Gynecol Scand* 67:159 - 166.

**Hammarbäck, S., Bäckström, T., & MacGibbon-Taylor, B.** (1989). Diagnosis of premenstrual syndrome: Description and evaluation of a procedure for diagnosis and differential diagnosis. *J Psychosom Obstet Gynec*, 10, 25-42. doi:10.3109/01674828909016676

**Hammarbäck, S., Ekholm U-B. & Bäckström, T.** (1991). Spontaneous anovulation causing disappearance of symptom cyclicity in women with premenstrual syndrome. *Acta Endocrinologica*, 125, 132 – 137.

**Hansen, M., Koskinen, S.O., Petersen, G.S., Doessing, S., Frystyk, J., Flyvbjerg, A., & Langberg, H.** (2008). Ethinyl oestradiol administration in women suppresses synthesis of collagen in tendon in response to exercise *J Physiol*, 15(586), 3005 - 3016. doi: 10.1113/jphysiol.2007.147348

**Hansen, M., Miller, B.F., Holm, L., Doessing, S., Petersen, G., Skovgaard, D., Langberg, H.** (2009). Effect of administration of oral contraceptives in vivo on collagen synthesis in tendon and muscle connective tissue in young women. *J Appl Physiol*, 106, 1435 – 1443. doi:10.1152/jappphysiol.90933.2008

**Hansen, M., Coupe, C., Hansen, C., Skovgaard, D. Kovanen, V ; Larsen, J., & Michael, K.** (2013). Impact of oral contraceptive use and menstrual phases on patellar tendon morphology, biochemical composition, and biomechanical properties in female athletes. *J Appl Physiol* 114: 998-1008.

**Harriell, K., Jacobs, K.A., Kaplan, L.D., Perry, A.C., Candela, D., & Signorile, J.F.** (2010). The influence of the menstrual cycle on joint position sense, joint kinesthesia, and dynamic balance. *Med Sci Sports Exerc*, 42(10), 37. doi: 10.1249/01.MSS.0000389566.11981.f0

**Hartgens, F. & Kuipers, H.** (2004). Effects on androgenic – Anabolic steroids in athletes. *Sports Med* 34: 513 - 554. doi:10.2165/00007256-200434080-00003

**Heitz, N.A., Eisenman, P.A., Beck, C.L., & Walker, J.A.** (1999). *J Athl Train*. 1999 Apr-Jun; 34(2): 144–149. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1322903/>

**Hertel, J., Williams, N.I., Olmsted-Kramer, L., Leidy, H.J., & Putukian, M.** (2006). Neuromuscular performance and knee laxity do not change across the menstrual cycle in female athletes. *Knee Surg Sports Traumatol Arthrosc*, 14(9), 817-822. doi: 10.1007/s00167-006-0047-4

**Hewett, T.E.**, (2000). Review. Neuromuscular and hormonal factors associated with knee injuries in female athletes. *Sports Med*, 29, 313 - 327. doi:10.2165/00007256-200029050-00003

**Hewett, T.E., Myer, G.D., Ford, K.R., Heidt, R.S., Colosimo, A.J., McLean, S.G., van den Bogert, A.J., Paterno, M.V., & Succop, P.** (2005). Biomechanical measures of neuromuscular control and valgus loading of the knee predict anterior cruciate ligament injury risk in female athletes: a prospective study. *Am J Sports Med*, (33), 492 - 501. doi: <https://doi-org.proxy.kib.ki.se/10.1177/0363546504269591>

**Hewett, T.E., Myer, G.D., & Ford, K.R.** (2006). Anterior cruciate ligament injuries in female athletes. Part 1, Mechanisms and Risk Factors *The American Journal of Sports Medicine*, Vol. 34, No. 2 doi: 10.1177/0363546505284183

**Holbrook, A.M., Crowther, R., Lotter, A., Cheng, C., & King, D.** (2000). Meta-analysis of benzodiazepine use in the treatment of insomnia. *CMAJ* 162, 225 - 233.  
[https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1232276/pdf/cmaj\\_162\\_2\\_225.pdf](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1232276/pdf/cmaj_162_2_225.pdf)

**Horak, F., & Macpherson, J.** (1996). Postural orientation and equilibrium. In *Handbook References of physiology. exercise: Regulation and integration of multiple systems.* Oxford University Press, New-York, 255- 292.

**Horak, F.** (2006). Postural orientation and equilibrium: what do we need to know about neural control of balance to prevent falls? *Age and Ageing*, 35-S2: ii7–ii11. <https://doi-org.proxy.kib.ki.se/10.1093/ageing/af1077>

**Horak, F.B., Wrisley, D.M., & Frank, J.** (2009). The Balance Evaluation Systems Test (BESTest) to differentiate balance deficits. *Phys Ther* 89, 484 - 498. doi: 10.2522/ptj.20080071

**Huston, L.J., Greenfield, M.L., & Wojtys, E.M.** (2000) review. Anterior cruciate ligament injuries in the female athlete. Potential risk factors. *Clin Orthop*, 372, 50-63. doi: 00003086-200003000-000007.pdf

**Ismaili, E., Walsh, S., O'Brien, P.M.S., Bäckström, T., Brown, C., Dennerstein, L., & Yonkers, K.** (2016). Fourth consensus of the International Society for Premenstrual Disorders (ISPM): auditable standards for diagnosis and management of premenstrual disorder. *Arch Womens Ment Health*, 19(6), 953-958. doi: 10.1007/s00737-016-0631-7

**Janse de Jonge, X. A. K., Boot, C. R. L., Thom, J. M., Ruell, P. A. & Thompson, M. W.** (2001). The influence of menstrual cycle phase on skeletal muscle contractile characteristics in humans. *The Journal of Physiology*, 530, 161 - 166. doi:10.1111/j.1469-7793.2001.0161m.x

**Janse de Jonge, X.A.K.** (2003). *Sports Med*, 33, 833 - 851. doi:10.2165/00007256-200333110-00004

**Janse de Jonge, X. A.K., Han, A., & Thompson, B.** (2015). Oral contraception and the menstrual cycle in exercise science and sports medicine research – Should it be considered? A review of menstrual cycle verification methods used in research investigating exercise performance over the menstrual cycle. *J Sci Med Sports*, 195, 2 - 32. <http://dx.doi.org/10.1016/j.jsams.2015.12.446>

**Joels, M.** (1997). Steroid hormones and excitability in the mammalian brain. *Front Neuroendocrinol*, 18, 2 – 48. doi: 10.1006/frne.1996.0144

**Kadi, F., Bonnerud, P., Eriksson, A., & Thornell, L.E.** (2000). The expression of androgen receptors in human neck and limb muscles: effects of training and self-administration of androgenic-anabolic steroids. *Histochem Cell Biol*, 113, 25–29. doi: <https://www.ncbi.nlm.nih.gov.proxy.kib.ki.se/pubmed/10664066?dopt=Abstract>

**Karageanes, S.J., Blackburn, K., & Vangelos, Z.A.** (2000). The Association of the Menstrual Cycle with the Laxity of the Anterior Cruciate Ligament in Adolescent Female Athletes. *Clinical Journal of Sport Medicine*, 10, 162 – 168. Doi: 00042752-200007000-000003.pdf

**Kitaoka, K., Ito, R., Araki, H., Sei, H., & Morita, Y.** (2004). Effect of mood state on anticipatory postural adjustments. *Neurosci Lett*, 370, 65 - 68. doi:10.1016/j.neulet.2004.07.088

**Kurshan, N., & Epperson, C.N.** (2006) *review*. Oral contraceptives and mood in women with and without premenstrual dysphoria: a theoretic model. *Arch Womens Ment Health* 9, 1. doi:10.1007/s00737-005-0102-z

**Lackie J.**, (2015) A dictionary of biomedicine. Oxford University Press. Print ISBN-13: 9780199549351.

**Larsson, G., Blohm, F., Sundell, G., Andersch, B., & Milsom, I.** (1997). A longitudinal study of birth control and pregnancy outcome among women in a Swedish population. *Contraception*, 57, 9 - 16. [https://doi.org/10.1016/S0010-7824\(97\)00068-1](https://doi.org/10.1016/S0010-7824(97)00068-1)

**Lee, H., Petrofsky, J.S., Daher, N., Berk, L., & Laymon, M.** (2014). Differences in anterior cruciate ligament elasticity and force for knee flexion in women: oral contraceptive users and non-oral contraceptive users. *Eur J Appl Physiol*, 114, 285-294. doi:10.1007/s00421-013-2771-z

**Lebrun, C.M.** (1994). The effect of the phase of menstrual cycle and the birth control pill in athletic performance. *Clin Sports Med*, 13,419–441. (doi: no found)

**Lebrun, C.** (1995). Effects of menstrual cycle phase on athletic performance. *Med Sci Sports Exerc*, 27, 437 - 444. Lebrun CM, Petit MA, McKenzie DC, et al (2003). Decreased maximal aerobic capacity with use of a triphasic oral contraceptive in highly active women: a randomised controlled trial. *Br J Sports Med*, 37, 315-320. <http://bjsm.bmj.com.proxy.kib.ki.se/content/37/4/315.full.pdf>

**Lemoine, S., Grainer, P., Tiffoche, C., Francoise, R.B., Thieulant, M-L., & Delamarche, P.** (2003). Estrogen receptor alpha mRNA in human skeletal muscles. *Med. Sci. Sports Exerc*, 35(3), 439 – 443. doi: 10.1249/01.MSS.0000053654.14410.78

**Lephart, S., & Fu, T.** (2000). Proprioception and neuromuscular control in joint stability.

**Lindh, I., Ellström, F., Blohm, G., & Milsom, I.** (2010). A longitudinal study of contraception and pregnancies in the same women followed for a quarter of a century. *Hum Reprod*, 25, 1415 – 1422. doi: <https://doi-org.proxy.kib.ki.se/10.1093/humrep/deq095>

**Liu, S.H., al-Shaikh, R., Panossian, V., Yang, R.S., Nelson, S.D., Soleiman, N., & Lane, J.M.** (1996). Primary immunolocalization of estrogen and progesterone target cells in the human anterior cruciate ligament. *J Orthop Res*, 14, 526 - 533.

**Liu, S.H., Al-Shaikh, R.A., Panossian, V., Finerman, G.A. & Lane, J.M.** (1997). Estrogen affects the cellular metabolism of the anterior cruciate ligament. A potential explanation for female athletic injury. *Am J Sports Med*, 25, 704–709. doi: 10.1177/036354659702500521

**Longo, L.P., & Johnson, B.**(2000). Addiction: Part I. Benzodiazepines – side effects, abuse risk and alternatives. *Am Fam. Phys.* 61, 2121-2128. doi: not found.

**Lopez, L.M., Kaptein, A.A., & Helmerhorst, F.M.** (2012). Oral contraceptives containing drospirenone for premenstrual syndrome. *Cochrane Database of Systematic Reviews*, 2. Art. No.: CD006586. doi: 10.1002/14651858.CD006586.pub4

**Mandelbaum, B.R., Silvers, H. J., Watanabe, D.S., Knarr, J.F., Thomas, S.D., Griffin, L.Y., & Garrett, W.** (2005). Effectiveness of a neuromuscular and proprioceptive training program in preventing anterior cruciate ligament injuries in female athletes: 2-year follow-up. *The Am J Sports Med*, 33(7), 1003 – 1010. doi: 10.1177/0363546504272261

**Majewska, M.D., Harrison, N.L., Schwartz, R.D., Barker, J.L., & Paul, S.M.** (1986). Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science*, 232, 1004 -1007. doi: 10.1126/science.2422758

**Martineau, P.A., Al-Jassir, F., Lenczner, E., & Burman, M.L.** (2004). Effect of the oral contraceptive pill on ligamentous laxity. *Clin J Sport Med*, 14, 281 - 286. doi: 00042752-200409000-00006

**McEwen, B.** (2002). Estrogen actions throughout the brain. *Recent Prog Horm Res*, 57, 357 - 384.

**McEwen, B., Akama, K.T., Spencer-Segal, J.L., Milner, T.A., & Waters, E.M.** (2012) Review. Estrogen Effects on the Brain: Actions Beyond the Hypothalamus Via Novel Mechanisms. *Behavioral Neuroscience*, 126(1), 4 -16. doi: 10.1037/a0026708

**Mc Lean, S.G., Huang, X., Su, A., & van den Bogert, A.J.** (2004). Sagittal plane biomechanics cannot injure the ACL during sidestep cutting. *Clinical Biomechanics*, 19, 828 – 838. doi: <http://doi.org.proxy.kib.ki.se/10.1016/j.clinbiomech.2004.06.006>

- Mertens, H.J., Heineman, M.J., Theunissen, P.H., de Jong, F.H., & Evers, J.L.** (2001). Androgen, estrogen and progesterone receptor expression in the human uterus during the menstrual cycle. *European Journal of Obstetrics and Gynecology*, 98(1), 58 - 65. doi: 10.1016/S0301-2115(00)00554-6
- Metcalf, M.G., & Mackenzie, J.A.** (1980). Incidence of ovulation in young women. *J Biosoc Sci*, 12, 345 - 352. doi: <https://doi-org.proxy.kib.ki.se/10.1017/S002193200001289X>
- Miller, W.R.** (1991). Aromatase activity in breast tissue. *J Steroid Biochem Mol Biol*, 39, 783 – 790. doi: 10.1016/0960-0760(91)90026-2
- Mishell, Jr D., Kletzky, O., Brenner, P., Roy, S., & Nicoloff, J.** (1977). The effects of contraceptive steroids on the hypothalamic-pituitary function. 128: 60 - 74. doi: not found.
- Montgomery, M.M., & Shultz, S.J.** (2010). Isometric knee-extension and knee-flexion torque production during early follicular and postovulatory phases in recreationally active women. *J Athl Train* 45, 586–593. doi: 10.4085/1062-6050-45.6.586
- Myer, G.D., Ford K.R., Palumbo, J.P. & Hewett, T.E.** (2005). Neuromuscular training improves performance and lower-extremity biomechanics in female athletes. *Journal of strength and conditioning research* , 19(1), 51-60. doi: 10.1519/13643.1
- Myklebust, G., Maehlum, S., Engebretsen, L., Strand, T. & Solheim, E.** (1997), Registration of cruciate ligament injuries in Norwegian top level team handball. A prospective study covering two seasons. *Scandinavian Journal of Medicine & Science in Sports*, 7: 289–292. doi:10.1111/j.1600-0838.1997.tb00155.x
- Myklebust, G., Engelbretsen, L., Braekken, I., Skjølberg, A., Olsen, O., & Bahr, R.** (2003). Prevention of anterior cruciate ligament injuries in female team handball players: a prospective intervention study over three seasons. *Scand J Med Sci Sports*, 13, 272. doi: 10.1097/00042752-200303000-00002
- Myklebust G., Maehlum, S., Holm, I. & Bahr, R.** 1998. A prospective cohort study of anterior cruciate ligament injuries in elite Norwegian team handball. *Scand J Med Sci Sports*, 8, 149–153. 10.1111/j.1600-0838.1998.tb00185.x
- Möller-Nielsen, J. & Hammar, M.** (1989). Women's soccer injuries in relation to the menstrual cycle and oral contraceptive use. *Med Sci Sports Exerc* 21, 126–129.
- Möller-Nielson, J., Hammar, M.** (1991). Sports injuries and oral contraceptive use: is there a relationship? *Sports Med* 12:152– 160. doi:10.2165/00007256-199112030-00002

- Nassaralla, C., Stanford, J., Daly, D., Schneider, M., Schliep, K.C., & Fehring, R.J.** (2011). Characteristics of the menstrual cycle after discontinuation of oral contraceptives. *J Women's health*, 20, 169 - 177. doi:10.1089/jwh.2010.2001
- Nevatte, T.O., Brien, P.M.S., Bäckström, T., Brown, C., Dennerstein, L., Eriksson, E., & Yonkers, K.** (2013). ISPMMD consensus on the management of premenstrual disorders, *Arch Womens Ment Health*, 16(4), 279-91. doi:10.1007/s00737-013-0346-y
- Nilsson, S., Makela, S., Treuter, E., Tujague, M., Thomsen, J., Andersson, G., & Pettersson, K.** (2001). Mechanisms of estrogen action. *Physiol Rev* 81:1535-1565.
- Oliveira, L.F., Simpson, D.M., & Nadal, J.** (1996). Calculation of area of stabilometric signals using principal component analysis. *Physiol Meas* 17: 205-312.
- Oosthuysen, T. & Bosch, A.N.**(2010). The effect of the menstrual cycle on exercise metabolism: implications for exercise performance in eumenorrhoeic women. *Sports Med* 40, 207–227. doi:10.2165/11317090-000000000-00000
- Papoulis A.** Probability, random variables and stochastic processes. 2<sup>nd</sup> ed. 1984, Auckland: McGraw-Hill.
- Peolsson, A., Hedlund, R., & Öberg, B.** (2001). Intra- and inter-tester reliability and reference values for hand strength. *J Rehabil Med* 33:36-41.
- Pearlstein, T., Yonkers, K., Fayyad, R., & Gillespie, J.A.** (2005). Pretreatment pattern of symptom expression in premenstrual dysphoric disorder. *Journal of Affective Disorders*, 85, 275 – 282. doi:10.1016/j.jad.2004.10.004
- Burrows, M., & Peters, C.E.** (2007). The influence of oral contraceptives on athletic performance in female athletes. *Sports Med*, 37, 557 - 575. doi:10.2165/00007256-200737070-00001
- Phillips, S.K., Sanderson, A.G., Birch, K., Bruce, S.A. & Woledge, R.C.** (1996). Changes in maximal voluntary force of human adductor pollicis muscle during the menstrual cycle. *J Physiol* 496(Pt 2), 551–557.
- Popat, Prodanov, Calis & Nelson** (2008) The menstrual cycle: A biological marker of general health in adolescents. *Ann. N.Y. Acad. Sci.* 1135: 43–51 DOI: 10.1196/annals.1429.040
- Posthuma, B.W., Bass, M.J., Bull, S.B & et al.**(1987). Detecting changes in functional ability in women with premenstrual syndrome. *Am J Obstet Gynecol*, 156(2), 275- 278.

**Prior, J.C., Vigna, Y., & et al.** (1986). Conditioning exercise decreases premenstrual symptoms. A prospective controlled three month trial. *Eur J Appl Physiol Occup Physiol* 55(4):349–355.  
doi:10.1007/BF00422732

Rahr-Wagner L, Muncholm Thillemann T et al, (2014)

**Rapkin A, Morgan M, Goldman L., Brann, D.W., Simone, D & Mahesh, V.B.** (1997) Progesterone metabolite allopregnanolone in women with premenstrual syndrome. *Obstet gynecol.* 1997, Vol.90(5), pp.709-714

**Rapkin, A.** (2003). A review of treatment of premenstrual syndrome & premenstrual dysphoric disorder. *Psychoneuroendocrinology*, 28, 39–53. [http://dx.doi.org.proxy.kib.ki.se/10.1016/S0306-4530\(03\)00096-9](http://dx.doi.org.proxy.kib.ki.se/10.1016/S0306-4530(03)00096-9)

**Rapkin, A., Morgan, M., Sogliano, C., Biggo, G., & Concas, A.** (2006). Decreased neuroactive steroids induced by combined oral contraceptive pills are not associated with mood changes. *Fertil Steril* 85: 1371- 1378.

**Rapkin, A.J., & Akopians, A.L.** (2012). Pathophysiology of premenstrual syndrome and premenstrual dysphoric disorder *Menopause International*, 18, 52–59. doi: 10.1258/mi.2012.012014

**Rechichi, C., & Dawson, B.** (2009). Effect on oral contraceptive cycle phase on performance in team sports players. *J Sci Med Sports*, 12(1), 190 - 195.  
<http://dx.doi.org.proxy.kib.ki.se/10.1016/j.jsams.2007.10.005>

**Rechichi, C., Dawson, B., & Goodman, C.** (2006). Oral contraceptive phase has no effect on endurance test. *Int J Sports Med*, 29(4), 277-281. doi: 10.1055/s-2007-965334

**Redman, L.M., & Weatherby R.P.**, (2004). Measuring performance during the menstrual cycle: A model using oral contraceptives. *Med. Sci. Sports Exerc*, 36(1), 130–136 doi: 10.1249/01.MSS.0000106181.52102.99

**Reid, R.** (2010). Oral contraceptives and the risk of venous thromboembolism: An update. *Int J Gynecol Obstet*, 112, 252 – 256. doi:10.1016/j.ijgo.2010.12.003

**Reis, E., Frick, U. & Schmidtbleicher, D.** (1995). Frequency variations of strength training sessions triggered by the phases of the menstrual cycle. *Int J Sports Med*, 16, 545 - 550. doi: 10.1055/s-2007-973052

**Renstrom, P., Ljungqvist, A., Arendt, E., Beynonn, B., Fukubayashi, T., Garrett, W., & Engebretsen, L.** (2008). Non-contact ACL injuries in female athletes: an International Olympic Committee current concepts statement. *Br J Sports Med*, 42, 394 - 412. doi:10.1136/bjism.2008.048934

- Reis, E., Frick, U., & Schmidtbleicher, D.** (1995). Frequency variations of strength training sessions triggered by the phases of the menstrual cycle. *Int J Sports Med*, 16, 545–550. doi: not found.
- Riemann, B.L., & Lephart, S.M.** (2002). The sensorimotor system, Part II: The role of proprioception in motor control and functional joint stability. *J Athl Train*, 37(1), 80 – 84.  
[https://www.ncbi.nlm.nih.gov/pmc/articles/PMC164312/pdf/attr\\_37\\_01\\_0080.pdf](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC164312/pdf/attr_37_01_0080.pdf)
- Rickenlund, A., Carlström, K., Ekholm, B., Brismar, T.B., von Schoultz, B., Hirschberg, A.L.** (2003). Hyperandrogenicity is an alternative mechanism underlying oligomenorrhea or amenorrhea in female athletes and may improve physical performance. *Fertil Steril*, 79, 967 - 955. doi:10.1016/S0015-0282(02) 04850-1
- Rickenlund, A., Carlström, K., Ekblom, B., Brismar, T.B., von Schoultz, B., & Hirschberg, A.L.** (2004). Effects of Oral Contraceptives on Body Composition and Physical Performance in Female Athletes. *J Clin Endocrinol Metab*, 89(9), 4364 - 4370. doi: 10.1210/jc.2003-031334
- Riemann, B.L.** (2002). Is there a link between chronic ankle instability and postural stability? *J Athl Train*, 37(4), 386 – 393. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC164370/>
- Rogind, H., Simonsen, H., Era, P., & Bliddal, H.** (2003). Comparison of Kistler 9861A force platform and Chattecx Balance system for measurement of postural sway: correlation and test-retest reliability. *Scand J Med Sci Sports*, 13, 106 - 114. doi: 10.1034/j.1600-0838.2003.01139.x
- Ronkainen, P.H.A, Kovanen, V., Alén, M., Pöllänen, E., Palonen, E.M., Ankarberg-Lindgren, C., & Sipilä, S.** (2009). Postmenopausal hormone replacement therapy modifies skeletal muscle composition and function: a study with monozygotic twin pairs. *J App Phys*, 107(1), 25 - 33 doi: 10.1152/jappphysiol.91518.2008
- Ross, S.E., & Guskiewicz, K.M.** (2003). Time to stabilization: a method for analyzing dynamic postural stability. *Athl Ther Today*, 8 (3), 37–39. doi: not found.
- Ross, K.G., Marshall, S.W., Kerr, Z.Y., Golightly, Y.M., Kucera, K.L., Myers, J.B., & Comstock, R.D.** (2015). Epidemiology of Overuse Injuries in Collegiate and High School Athletics in the United States. *Am J Sports Med*, 43(7), 1790 - 1797. doi: 10.1177/0363546515580790
- Rubinow, D.R., & Schmidt, P.J.** (1995). The neuroendocrinology of menstrual cycle mood disorders. *Annals of the New York Academy of Sciences*, 771, 648–659. doi:10.1111/j.1749-6632.1995.tb44717.x
- Rubinson, K., & Lang, E.J.** (2008). The nervous system. In B.M., Koeppen & B.A., Stanton (Eds.), *Berne & Levy Physiology*. (6.ed.,p. 233-285). Philadelphia: Mosby /Elsevier.

- Ruedl, G., Ploner, P., Linortner, I. Schranz, A., Fink, C., Sommersacher, R. Pocecco, E., Nachbauer, W., & Burtscher, M.** (2009). Are oral contraceptive use and menstrual cycle phase related to anterior cruciate ligament injury risk in female recreational skiers? *Knee Surg Sports Traumatol Arthrosc*, 17, 1065-1069. doi:10.1007/s00167-009-0786-0
- Russell, J.B., Mitchell, D., Musey, P.I., & Collins, D.C.** (1984). The relationship of exercise to anovulatory cycles in female athletes: hormonal and physical characteristics. *Obstet Gynecol*, 63,452 - 456. doi: 10. 00006250-198404000-000003.pdf
- Sell, T.** (2012). An examination, correlation, and comparison of static and dynamic measures of postural stability in healthy, physically active adults. *Physical Therapy in Sport* 13, 80-86. <http://dx.doi.org.proxy.kib.ki.se/10.1016/j.ptsp.2011.06.006>
- Sakamoto, H., Takahashi, H., Matsuda, K., Nishi, M., Takanami, K., Ogoshi, M., & Kawata, M.** (2012). Rapid signaling of steroid hormones in the vertebrate nervous system. *Front Biosci*, 17, 996–1019. <http://www.bioscience.org.proxy.kib.ki.se/2012/v17/af/3970/list.htm>
- Sarwar, R., Niclos, B.B. & Rutherford, O.M.** (1996). Changes in muscle strength, relaxation rate and fatiguability during the human menstrual cycle. *J Physiol*, 493(1), 267 - 272. doi: not found.
- Schaumberg, M., Jenkins, D., Janse De Jonge, X., Emmerton, L., & Skinner, T.** (2015). Oral contraception and the menstrual cycle in exercise science and sports medicine research – Should it be considered? A three step method for ovarian hormone phase verification. *J Sci Med Sports*, 195, 2 - 32. <http://dx.doi.org/10.1016/j.jsams.2015.12.447>
- M.D. Schmidt, P.J, Nieman, L.K., Danaceau, M.A., Adams, L.F., & Rubinow D.R.** (1998). Differential behavioural effects of gonadal steroids in women with and those without premenstrual symptoms. *New Eng J Med*, 338(4), 209 - 216. <http://www.nejm.org.proxy.kib.ki.se/doi/pdf/10.1056/NEJM199801223380401>
- Schroeder, A.N., Comstock, R.D., Collins, C.L., Everhart, J., Flanigan, D., & Best, T.M.** (2015). Epidemiology of Overuse Injuries among High-School Athletes in the United States. *J Pediatr*, 166, 600 - 606. doi:10.1016/j.jpeds.2014.09.037
- Segebladh, B., Borgström, A., Nyberg, S., Bixo, M., & Sundström-Poromaa, I.** (2009). Evaluation of different add-back estradiol and progesterone treatments to gonadotropin-releasing hormone agonist treatment in patients with premenstrual dysphoric disorder. *Am J Obstet Gynecol* 201(139), 1 - 8. 10.1016/j.ajog.2009.03.016

**Shultz, S.J., Wideman, L., Montgomery, L.L., & Levine, B.J.** (2011). Some sex hormone profiles are consistent over time in normal menstruating women: implication for sports injury epidemiology. *Br J Sports Med*, 45, 735 - 742 doi: 10.1136/bjism.2009.064931

**Shultz, S.J., Kirk, S.E., Johnsson, M.L., Sander, T.C., & Perrin, D.H.** (2004) Relationship between sex hormones and anterior knee laxity across the menstrual cycle. *Med. Sci. Sports Exerc*, 36(7), 1165 - 1174. doi: 10.1249/01.MSS.0000132270.43579.1A

**Shumway-Cook, A., & Wollacott, M.H.** (2012). *Motor control: Translating research into clinical practice*. (4.ed.) Philadelphia: Lippincott Williams & Wilkins.

**Skelton, D.A., Phillips, S.K., Bruce, S.A., Naylor, C.H. & Woledge, R.C.** (1999). Hormone replacement therapy increases isometric muscle strength of adductor pollicis in post-menopausal women. *Clin Sci*, 96, 357 - 364. doi: 10.1042/cs0960357

**Skouby, S.** (2010). Contraceptive use and behavior in the 21st century: a comprehensive study across five European countries. *Eur J Contracept Reprod Health Care*, 15, 42 – 53. doi: 10.3109/13625187.2010.533002

**Slauterbeck, J.R., Fuzie, S.F., Smith, M.P., Clark, R.J., Xu, K., Starch, D.W., & Hardy, D.M.** (2002). The menstrual cycle, sex hormones, and anterior cruciate ligament injury. *J Athl Train*, 37, 275 - 278. [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC164356/pdf/attr\\_37\\_03\\_0275.pdf](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC164356/pdf/attr_37_03_0275.pdf)

**Stanczyk, F.Z.**, (2003). All progestins are not created equal. *Steroids*, 68, 879 – 890. <https://www.ncbi.nlm.nih.gov.proxy.kib.ki.se/pubmed/14667980?dopt=Abstract>

**Steege, J.F., & Blumenthal, J.A.** (1993). The effects of aerobic exercise on premenstrual symptoms in middle-aged women: a preliminary study. *J Psychosom Res.* 37(2), 127 - 133. <https://www.ncbi.nlm.nih.gov.proxy.kib.ki.se/pubmed/8463989?dopt=Abstract>

**Steffen K, Nilstad A, Krosshaug, T., Pasanen, K., Killingmo, A & Bahr, R.** (2017). No association between static and dynamic postural control and ACL injury risk among female elite handball and football players: a prospective study of 838 players *Br J Sports Med*, 51, 253 - 259. doi: 10.1136/bjsports-2016-097068

**Stoddard, J.L., Dent, C.W., Shames, L., & Bernstein, L.** (2007). Exercise training effects on premenstrual distress and ovarian steroid hormones. *Eur J Appl Physiol*, 99: 27-37. doi:10.1007/s00421-006-0313-7

- Sundström, I., Bäckström, T., Wang Olsson, T., Seippel, L., & Bixo, M.** (1999). Premenstrual syndrome, neuroactive steroids and the brain. *Gynecol. Endocrinol*, 13(3), 206 - 220.  
<http://dx.doi.org.proxy.kib.ki.se/10.3109/09513599909167557>
- Sung, E., Han, A., Hinrichs, T., Vorgerd, M., Manchado, C., & Platen, P.** (2014). Effects of follicular versus luteal phase-based strength training in young women. *Springerplus* 3, 668. doi: 10.1186/2193-1801-3-668
- Sveindottir, H., & Bäckström, T.** (2000). Prevalence of menstrual cycle symptom cyclicality and premenstrual dysphoric disorder in a random sample of women using and not using oral contraceptives. *Acta Obstet Gynecol Scand*, 79(5), 405 - 413 doi: 10.1034/j.1600-0412.2000.079005405.x
- Södergård, R., Bäckström, T., Shanbhag, V. & Carstensen, H.** (1982). Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. *J Steroid Biochem*, 16, 801–810. doi: 10.1016/0022-4731(82)90038-3
- Söderman, K., Alfredsson, H., Pietilä, T., & Werner, S.** (2001). Risk factors for leg injuries in female soccer players: a prospective investigation during one out-door season. *Knee Surg Sports Traumatol Arthrosc*, 9, 313–321. doi:10.1007/s001670100228
- Söderpalm, A.H., Lindsey, S., Purdy, R.H., Hauger, R. & Wit, d.H.** (2004). Administration of progesterone produces mild sedative-like effects in men and women. *Psychoneuroendocrinology* 29, 339–354. [http://dx.doi.org.proxy.kib.ki.se/10.1016/S0306-4530\(03\)00033-7](http://dx.doi.org.proxy.kib.ki.se/10.1016/S0306-4530(03)00033-7)
- Tamarova, Z.A., Sirota, M.G., Orlovsky, G.N., Deliagina, T.G., & Beloozerova, I.N.** (2007). Role of GABA-A inhibition in modulation of pyramidal tract neuron activity during postural corrections. *European Journal of Neuroscience*, 25(5), 1484-1491. doi: 10.1111/j.1460-9568.2007.05413.x
- Thompson, H.S., Hyatt, J-P., De Souza, M.J., & Clarkson, P.M.** (1997). The effects of oral contraceptives on delayed onset muscle soreness following exercise. *Contraception*, 56, 59-65. doi: 10.1016/S0010-7824(97)00093-0
- To, W.W., & Wong, M.W.** (2000). The relationship of physical trauma and surgical stress to menstrual dysfunction. *Aust NZ J Obstet Gynaecol*, 40, 48–53. doi: 10.1111/j.1479-828X.2000.tb03166.x
- Toffoletto, S., Lanzenberger, R., Gingnell, M., Sundström-Poromaa, I., & Comasco, E.** (2014). Emotional and cognitive functional imaging of estrogen and progesterone effects in the female human brain: A systematic review. *Psychoneuroendocrinology*, 50, 28-52.  
<http://dx.doi.org.proxy.kib.ki.se/10.1016/j.psyneuen.2014.07.025>

- Tozzi, L., Doolin, K., Carballedo, A., Lavelle, G., Doyle, M., McCarthy, H., & Frodl, T.** (2015). P.1.i.021 Improvement of well-being measures after regular physical exercise correlates with resting state connectivity in attentional and default mode network. *European Neuropsychopharmacology*, 25, S311 - S312. doi: 10.1016/S0924-977X(15)30380-1
- Tropp, H., Ekstrand, J., & Gillquist, J.** (1984). Stabilometry in functional instability of the ankle and its value in predicting injury. *Med Sci Sport Exerc*, 2(16), 64 - 66. ISSN: 0195-913
- van Emmerik, R.E.A., & van Wegen, E.E.H.** (2002) Review. On the functional aspects of variability in postural control. *Exerc. Sport Sci. Rev*, 30(4), 177–183, doi:10: 0091-6631/3004/177–183
- Waynman, S. & Gomez-Pinilla, F.** (2005) Review. License to run: exercise impacts functional plasticity in the intact and injured CNS by using neurotrophins. *Neurorehabil Neural Repair*, 19, 283 - 295. doi: 10.1177/1545968305280753
- Vescovi, J.D.** (2011). The menstrual cycle and anterior cruciate ligament injury risk implications of menstrual cycle variability. *Sports Med*, 41, 91 - 101. doi:10.2165/11538570
- Wada, M., Sunaga, N., & Nagai, M.** (2001) *Neuroscience Letters*, 302(2), 157-159 . doi:10.1016/S0304-3940(01)01662-7
- van Rooijen, M., von Schultz, B., Silveira, A., Hamsten, A., & Bremme, K.** (2002). Different effects of oral contraceptives containing levonorgestrel or desogestrel on plasma lipoproteins and coagulation factor VII. (2002). *Am J Obstet Gynecol*, 186(1), 44 – 48. <http://dx.doi.org.proxy.kib.ki.se/10.1067/mob.2002.119179>
- Watras, J.M.** (2008). Skeletal muscle physiology. In B.M., Koeppen & B.A., Stanton (Eds.), *Berne & Levy Physiology*. (6.ed.,p. 233-285). Philadelphia: Mosby /Elsevier.
- Wedderkopp, M., Kalfot, M., Lundgaard, B., Rosendahl, M., & Froberg, K.** (1999). Prevention of injuries in young female players in European team handball. A prospective intervention study. *Scand J Med Sci Sport*, 9, 41 - 47. doi: 10.1111/j.1600-0838.1999.tb00205.x
- Wester, J.U., Jespersen, S.M., Nielsen, K.D., & Neumann, L.** (1996). Wobble board training after partial sprains of the lateral ligaments of the ankle: A prospective randomized study. *J Orthop Sport Phys Ther*, 23, 332 - 336. <http://www.jospt.org.proxy.kib.ki.se/>
- Wiik A., Glenmark, B., Ekman, M., Esbjörnsson-Liljedahl, M., Johansson, O., Bodin, K., & Jansson, E.** (2003). Oestrogen receptor beta is expressed in adult human skeletal muscle both at the mRNA and protein level. *Acta Physiol Scand*, 179, 381 - 387. doi:10.1046/j.0001-6772.2003.01186.x

- Wiik, A., Ekman, M., Morgan, G., Johansson, O., Jansson, E., & Esbjörnsson, M.** (2005). Oestrogen receptor beta is present in both muscle fibres and endothelial cells within human skeletal muscle tissue. *Histochem Cell Biol*, 124, 161 - 165. doi:10.1007/s00418-005-0030-z
- Wiik, A., Ekman, M., Johansson, O., Jansson, E., & Esbjörnsson, M.** (2009). Expression of both oestrogen receptor alpha and beta in human skeletal muscle tissue. *Histochem Cell Biol*, 131, 181 - 189. doi: 10.1007/s00418-008-0512-x
- Wikström-Frisén, L., Boraxbekk, C-J., & Henriksson-Larsén, K.** (2015). Effects on power, strength and lean body mass of menstrual/oral contraceptive cycle based resistance training. *J Sports Med Phys Fitness*, 11. [Epub ahead of print] <http://www.minervamedica.it>
- Wikström, E.A., Powers, M., & Tillman, M.** (2004). Dynamic stabilization time after isokinetic and functional fatigue. *J Athl Train*, 39(3), 247 - 253. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC522147/>
- Wikström, E.A., Tillman, M.D., Kline, K., & Borsa, P.A.** (2006). Gender and limb differences in dynamic stability and energy absorption during landing. *Clin J Sports Med*, 16, 311–315. <https://www.ncbi.nlm.nih.gov.proxy.kib.ki.se/pubmed/16858214>
- Wikstrom, E.A., Tillman, M.D., Chmielewski, T.L., & Borsa P.A.** (2006). Measurement and evaluation of dynamic joint stability of the knee and ankle after injury. *Sports Med*, 36, 393 - 399. doi:10.2165/00007256-200636050-00003
- Wikström, E.A., Tillman, M.D., Chmielewski, T.L., Cauraugh, J.H., Naugle, K.E., & Borsa, P.A.** (2010). Dynamic postural control but not mechanical stability differs among those with and without chronic ankle instability. *Scand J Med Sci Sports*, 20 (1), 137 - 144. doi: 10.1111/j.1600-0838.2009.00929.x
- Williford, H., Scharff-Olson, M., & Blessing, D.** (1993). Exercise prescription for women. *Sports Med*, 15, 299 – 311.
- Winter, D.A.** (1995). A.B.C. of balance during standing and walking, Waterloo Biomechanics, Waterloo.
- Winter, D.A., Patla, A.E., Prince, F., Ishac, M., & Gielo-Periczak, K.** (1998). Stiffness control of balance in quiet standing. *J Neurophysiol*, 80(3), 1211 - 1221. <http://jn.physiology.org.proxy.kib.ki.se/content/80/3/1211>

- Wojtys, E.M., Huston, L.J., Lindenfeld, T.N., Hewett, T.E. & Greenfield, M.L.** (1998). Association between the menstrual cycle and anterior cruciate ligament injuries in female athletes. *Am J Sports Med*, 26, 614 - 619. doi: 10.1177/03635465980260050301
- Wojtys, E.M., Huston, L.J., Boynton, M.D., Spindler, K.P. & Lindenfeld, T.N.** (2002). The effect of the menstrual cycle on anterior cruciate ligament injuries in women as determined by hormone levels. *Am J Sports Med*, 30(2), 182 - 188. doi: 10.1177/03635465020300020601
- World Health Organization.** The ICD-10 classification of mental, behavioral and developmental disorders. 10th revision. Geneva , Switzerland , 1996.
- Wyatt, K.M., Dimmock, P.W., Ismail, K.M., Jones, P.W., & O'Brien, P.M.S.** (2004). The effectiveness of GnRH with and without 'add-back' therapy in treating premenstrual syndrome: a meta-analysis. *BJOG*, 111(6), 585 - 593. doi: 10.1111/j.1471-0528.2004.00135.x
- Yonkers, K.A., Brown, C., Pearlstein, T.B., Foegh, M., Sampson-Landers, C., & Rapkin, A.** (2005). Efficacy of a new low-dose oral contraceptive with drospirenone in premenstrual dysphoric disorder. *Obstetrics and gynecology*, 106(3), 492 - 501.
- Yonkers, K.A., O'Brien, P.M.S., & Eriksson, E.** (2008). Premenstrual symptoms. *Lancet*, 371, 1200 - 1210. doi: 10.1016/S0140-6736(08)60527-9
- Yu, W.D., Liu, S.H., Hatch, J.D., Panossian, V. & Finerman, G.A.** (1999). Effect of estrogen on cellular metabolism of the human anterior cruciate ligament. *Clin Orthop Relat Res*, 366, 229–238. doi: 10.1097/00003086-199909000-00030
- Zazulak, B.T., Paterno, M., Myer, G.D., Romani, W.A. & Hewett, T.E.** (2006). The effects of the menstrual cycle on anterior knee laxity: a systematic review. *Sports Med*, 36, 847 - 862. doi: 10.2165/00007256-200636100-00004
- Zeller, B.L., McCrory, J.L., Kibler, W.B., & Uhl, T.L.** (2003). Differences in kinematics and electromyographic activity between men and women during the single-legged squat. *Am J Sports Med*, 31(3), 449 - 456. doi: 10.1177/03635465030310032101
- Åman, M., Forssblad, M., Henriksson-Larsén, K.** (2016). Incidence and severity of reported acute sports injuries in 35 sports using insurance registry data. *Scand J Med Sci Sports*, 26, 451 - 462. doi: 10.1111/sms.12462