

# Training and hormones in physically active women with and without oral contraceptive use

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*To my family*

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# Abstract

**Background:** In recent decades, the number of women participating in sports has increased dramatically, though research in sports are often performed on men. Physical exercise is known to increase physical performance and improve well-being. Although exercise has beneficial health effects for most of the women, it is known that strenuous exercise may also have negative health consequences that may lead to the female athlete triad. Common negative consequences among female athletes are menstrual dysfunctions and the medical effects of a long-standing amenorrhea are serious, including increased bone loss. Moreover a strenuous exercise without adequate recovery may lead to overreaching and overtraining syndrome. An improved muscle strength are of great importance in many sports, hence an increased understanding on how to generate optimal strength training programs in women without negative side effects are essential.

The aims of this work were to investigate the effects on strength and power of high frequency periodised leg resistance training to evaluate a training regime. Further to investigate the tolerance of the training, i.e. if the training was well-accepted and without potential exercise-related negative consequences on components in the female athlete triad (sex hormones, growth hormone, cortisol, total body fat mass and bone mineral density in the spine). Moreover, to provide normative data on oxytocin and cortisol to elucidate if these hormones could be one diagnostic marker in combination with others to monitor and diagnose female athletes that may be at risk to develop overreaching and overtraining syndrome

**Methods:** Fifty-nine women, participated in the four months intervention study. Two groups performed high frequency leg resistance training for two weeks of each menstrual/oral contraceptive cycle. The remaining part of the cycle they performed the leg training once a week. Group 1 (n=19) trained with high frequency (5 times·w<sup>-1</sup>) during the first two weeks of each cycle, and group 2 (n=19) during the last two weeks of each cycle. A control group (n=21) performed regular (3 times·w<sup>-1</sup>) leg resistance training. Another 33 women participated in the observational study. The oral contraceptive users (n=15) and non-users (n=18), were followed over a nine-month period with monthly blood sampling of oxytocin and cortisol, and also Profile of Mood State (POMS) as a subjective measure of overreaching and overtraining syndrome.

**Results:** The women who performed high frequency leg resistance training, 5 times·w<sup>-1</sup>, during the first two weeks of each cycle showed significant increase in squat and countermovement jump, peak torque values in

hamstrings, increased lean body mass of the legs, and their experiences of the training were positive. These results were not found when the periodised training was performed during the last two weeks of each cycle. In the control group an increase in squat jump, countermovement jump, and peak torque (left hamstring) was observed. There were no evident differences in the training effects between women with or without oral contraceptive use. Moreover, no significant negative impact on sex and growth hormones, cortisol, total body fat mass and bone mineral density in the spine, was detected in any of the three training groups. The women in the observational study showed seasonal variations in oxytocin and cortisol levels, with different pattern in oral contraceptive users to non-users. No convincing relationships to POMS were found.

**Conclusions:** The high frequency periodised leg resistance training during the first two weeks of the menstrual cycle is more beneficial to optimise resistance training, than the last two weeks. Resistance training during the first two weeks of the menstrual cycle even resulted in a larger gain of lean body mass than regular training. The high frequency periodised leg resistance training was not associated with exercise-related negative consequences on components in the female athlete triad. Moreover, the training was well accepted when performed during the first two weeks of each cycle. Due to seasonality, impact of oral contraceptive use, methodological considerations, and no convincing relationship to POMS, oxytocin and cortisol is not suggested to be optimal, diagnostic markers alone or in combination with others to detect overreaching and overtraining syndrome in physically active women.

# Abbreviations

ACSM	American College of Sports Medicine
ACTH	Adeno-corticotrophic hormone
ANOVA	Analysis of variance
BMD	Bone mineral density
CBG	Corticosteroid-binding globulin
CMJ	Countermovement jump
CRH	Corticotropin-releasing hormone
CV	Coefficient of variation
DHEA	Dehydroepiandrosterone
DEXA	Dual energy X-ray absorptiometry
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
ER	Estrogen receptor
FSH	Follicle-stimulating hormone
FTCR	Free testosterone cortisol ratio
GH	Growth hormone
GnRH	Gonadotropin-releasing hormone
HPA	Hypothalamus-pituitary-adrenal
HPG	Hypothalamus-pituitary-gonadal
HRT	Hormone replacement therapy
LBM	Lean body mass
LH	Luteinizing hormone
MTDS	Multi Component Training Distress Scale
NOC	No oral contraceptive
OC	Oral contraceptive
OD	Optical density
OR	Overreaching (non-functional)
OT	Oxytocin
OTR	Oxytocin receptors
OTS	Overtraining syndrome
PCOS	Polycystic ovary syndrome
POMS-	Profile of Mood State
PT	Isokinetic peak torque
REST-Q Sport	Recovery Stress Questionnaire for Athletes
RPE	Ratings of perceived exertion
SHBG	Sex hormone-binding globulin
SPSS	Statistical package for the social science
ST	Squat jump
V/F	Vigour/Fatigue



$\text{VCO}_2$	Carbon dioxide output
VE	Ventilation
$\text{VO}_{2\text{max}}$	Maximal oxygen uptake
$\text{VO}_{2\text{peak}}$	Peak oxygen uptake
W	Watts

# Sammanfattning på svenska

## Introduktion

Under de senaste decennierna har antalet idrottande kvinnor ökat mycket både inom motionsidrott och elitidrott. Trots ökningen är fortfarande övervägande idrottsforskning genomförd på män, och därför finns det idag endast en begränsad kunskap om hur idrottande kvinnor ska träna. De generella rekommendationerna för optimering av styrketräning är att periodisera träningen genom variationer i volym, intensitet och antalet träningstillfällen utan hänsyn till hormonvariationer som i kvinnors fall varierar cykliskt. Idag vet vi väldigt lite om hur kvinnliga könshormoner kan påverka träning och ingen hänsyn tas till var i menstruationscykeln kvinnor befinner sig vid träningsplaneringen. En ytterligare faktor att ta hänsyn till vid träningsplanering är att många idrottande kvinnor använder hormoninnehållande preventivmedel (p-piller). Vi vet således väldigt lite om hur man skulle kunna optimera träning för kvinnor genom att ta hänsyn till kvinnlig könshormoner. Vi saknar även full kunskap kring eventuella kvinnospecifika hälsorisker som en alltför högintensiv och för ofta bedriven träning utan tillräcklig återhämtning kan medföra såsom t.ex. menstruationsstörningar som i vissa fall kan leda till förlust av benmassa. Genom att det är en gradvis utveckling av negativa träningsrelaterade besvär bör fokus vara på förebyggande insatser och tidig diagnostisering. Kan man genom att periodisera sin träning efter menstruationscykeln/p-piller cykeln erhålla bättre träningseffekter utan att öka antalet träningstillfällen innebär det en positiv vinst och eventuellt bidrar det till att motverka utvecklingen av den kvinnliga idrottstriaden.

Träning är en typ av fysisk stress vilket medför en ökad produktion av stresshormoner. Blir kroppen utsatt för alltför mycket stress stimuli orsakat av för mycket eller för intensiv träning med för lite återhämtning i träningsplaneringen, och oftast ihop med andra psykiska/sociala stressfaktorer kan överträning/överträningssyndrom utvecklas. Genom att detta kan medföra allvarliga hälsoproblem är det viktigt att förebygga eller att tidigt diagnostisera, men idag finns inga pålitliga och enkla markörer. Mätning av hormonnivåer som markörer förekommer, t.ex. kortisol är använt inom idrotten för att studera stress och förebygga överträning. Hormonet oxytocin anses vara ett välmående hormon som inte är lika studerat på människa, men skulle också kunna vara en markör.

Syftet med interventionsstudien (delarbete I och II) var att undersöka om variationen i könshormoner över menstruationscykeln/p-piller cykeln kan

nyttjas för att ytterligare optimera träningsmodeller för idrottande kvinnor. Vidare undersöka om träningen är väl tolererad, dvs. utan träningsrelaterade besvär. Syftet med observationsstudien (delarbete III och IV) var att studera årstidsvariationer samt inverkan av p-piller på cortisol och oxytocin nivåerna för att undersöka om hormonerna ska kunna användas enskilt eller tillsammans med andra markörer för diagnostisering av överträning/överträningssyndrom.

## **Resultat och relevans för idrotten**

Fynden i interventionsstudien visade att högfrekvent benstyrketräning under de två första veckorna av menstruations/p-piller cykeln resulterade i signifikant ökning av hopphöjd och muskelstyrka samt muskelmassa i benen. I gruppen med högfrekvent träning de sista två veckorna av menstruations/p-piller cykeln påvisades inga signifikanta ökningar. Även gruppen som tränade regelbundet ökade sin hopphöjd samt muskelstyrka i ett av benen, men ingen ökning i muskelmassa. Vid jämförelse mellan träningsgrupperna visade träning de första två veckorna av menstruations/p-piller cykeln samt regelbunden träning en signifikant större styrkeökning i ben jämfört med träning de två sista veckorna av cykeln. Inga betydande skillnader hittades mellan kvinnor med och utan p-piller. Inga negativa effekter relaterade till den kvinnliga idrottstriaden uppmättes av den periodiserade högfrekventa träningen. Vidare upplevdes högfrekventa träningen under de första två veckorna av menstruations/p-piller cykeln som mer positiv. Således är det fördelaktigt att bedriva periodiserad styrketräning för kvinnor i relation till menstruations/p-piller cykeln då den är förlagd till de första två veckorna av cyklarna.

Fynden i observationsstudien (delarbete III och IV) visade att nivåerna av cortisol och oxytocin varierar under olika årstider och påverkas av p-piller användning. Detta medför att dessa hormoner inte kan rekommenderas som pålitliga markörer ensamma eller tillsammans med andra markörer för att diagnostisera överträning/överträningssyndrom.

# Original papers

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

- I. Effects on power, strength and lean body mass of menstrual/oral contraceptive cycle based resistance training.**  
Lisbeth Wikström-Frisén, Carl-Johan, Boraxbekk, Karin Henriksson-Larsén  
*Accepted*
- II. Increasing training load without risking the female athlete triad: Menstrual cycle based periodized training may be an answer?**  
Lisbeth Wikström-Frisén, Carl-Johan, Boraxbekk, Karin Henriksson-Larsén  
*Accepted*
- III. Mood, and oxytocin blood levels in physically active women with and without oral contraceptive use in relation to seasonal daylight variation.**  
Lisbeth Wikström-Frisén, Peter Larsson, Lucia Mincheva-  
Nilsson, Karin Henriksson-Larsén  
*Submitted*
- IV. Impact of season and oral contraceptive use on cortisol levels in physically active women.**  
Lisbeth Wikström-Frisén, Anna Nordström, Lucia Mincheva-  
Nilsson, Karin Larsén  
*Submitted*

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# Introduction

## **Training and hormones in physically active women**

In recent decades, the numbers of women participating in recreational exercise and elite athletic training and competition have increased dramatically (Greydanus and Patel 2002); (Goodman and Warren 2005); (Javed et al. 2013); (Costello, Bieuzen, and Bleakley 2014). Due to this growth in sport activities, a set of health problems unique to female athletes has emerged (Jenna C. Gibbs, Williams, and De Souza 2013). Although exercise has beneficial health effects for most women, strenuous exercise is known to have potential health consequences. Some well-known health benefits of increased strength and endurance are improved cognitive function, neuromuscular co-ordination, management of body composition, and improved mental well-being. However, female athletes may be at risk for the development of exercise-related disorders; for example, menstrual disturbances, common among elite athletes, could have serious medical consequences (M P Warren and Shantha 2000); (Goodman and Warren 2005); (Javed et al. 2013).

Moreover, strenuous exercise without adequate recovery may lead to non-functional overreaching (OR) and overtraining syndrome (OTS), due to imbalances among the strain of training, the time allowed for recovery, and individual tolerance of stress (Raglin, Morgan, and O'Connor 1991); (M. Lehmann, Foster, and Keul 1993); (Kentta and Hassmen 1998); (Kenttä, Hassmén, and Raglin 2001); (Halsen and Jeukendrup 2004); (Faude et al. 2009); (Meeusen et al. 2013). Athletes diagnosed with OR/OTS usually show the same symptoms of fatigue, performance decline, and mood disturbances. OTS has been suggested to be more severe than OR, due to more negative effects on the body mechanisms, and because a longer time is required for the restoration of performance capacity (Meeusen et al. 2013). Because of the gradual onset of OR and the gradual transition from OR to OTS, early detection of symptoms is important (Kuipers 1998); (Meeusen et al. 2013). Research has shown that the emphasis needs to be on the prevention of OR and OTS development, mostly by the appropriate periodisation of training with the inclusion of appropriate recovery time in training programmes. Athletes, coaches, and physicians would benefit greatly from a sensitive and simple diagnostic test for OR and OTS, which enables distinction of OTS from OR and other potential causes of underperformance (Meeusen et al. 2013).

As female athletes have become a common part of sport environments (Greydanus and Patel 2002) and most research in sports has been performed

with male athletes, studies of female athletes are warranted (Costello, Bieuzen, and Bleakley 2014); (Bruinvels et al. 2016); (Sheel 2016). Frequently cited reasons for the exclusion of women from sport and exercise research are monthly hormonal variations and the complexities of the menstrual cycle. The ways in which the menstrual cycle affects physical performance are not fully understood (Bruinvels et al. 2016). Various endogenous hormones fluctuate between the follicular and luteal phases of the cycle in women who do not take oral contraceptives (OCs). With the intake of OCs, biosynthesis and secretion of the endogenous hormones oestrogen and progesterone are suppressed and other sex steroids are altered in different ways. Variations in these hormonal milieus may influence the trainability of strength performance differently between menstrual cycle phases and/or between OC users and non-users. These actions of the female sex hormones during exercise may have implications in recreational exercise and elite athletic training (Oosthuyse and Bosch 2010); (Bruinvels et al. 2016). Hence, a better understanding of female physiology and definition of the effects of cyclic variations in hormones and their impacts on exercise are needed (Sheel 2016).

The anabolic effect of the male steroid testosterone is well established (William J Kraemer and Ratamess 2005), but the influences of the female sex steroids oestrogen and progesterone on muscle strength and performance are less studied and not completely clear. Oestrogen has been suggested to have an anabolic effect on muscle, whereas progesterone may have a catabolic effect (Reis, Frick, and Schmidtbleicher 1995); (Oosthuyse and Bosch 2010); (Sung et al. 2014). Fridén et al. (2003) concluded that muscle strength and endurance are not influenced by hormonal variations during the menstrual cycle, but they did not exclude the potential use of these fluctuations to optimise training programmes for female athletes (Friden, Hirschberg, and Saartok 2003). Oestrogen, progesterone, and other sex steroids are known to be important factors for strength capacity, and they may have as-yet unknown differential influences on strength training adaptation in OC users and non-users. Today, the use of OCs is prevalent among female athletes (Greydanus and Patel 2002); (Rechichi, Dawson, and Goodman 2009), and the impact of OC use on hormone levels in athletes has not been not well studied. Hence, the influences exogenous (OCs) and endogenous (menstrual cycle) hormonal profiles on athletic performance should be investigated more thoroughly (Rechichi, Dawson, and Goodman 2009).

Considering these aspects of female recreational exercise, elite athletic training, and sport performance, the development of innovative strategies to improve training effects while preventing exercise-related health consequences is an important issue (C. M. Lebrun and Rumball 2001); (M. P. Warren and Perlroth 2001); (Michelle P. Warren and Goodman 2003);

(Maïmoun, Georgopoulos, and Sultan 2014). Studies of resistance training are especially warranted, as most previous research has focused on endurance activities and negative exercise-related health effects (Meeusen et al. 2013). Resistance training is a large component of many training programmes, and chronic resistance exercise can result in differential responses to overtraining, depending on whether the training volume or intensity is excessive (A. C. Fry and Kraemer 1997). Hence, more studies are warranted to develop innovative strategies to improve resistance training effects in women while avoiding exercise-related health consequences.

## **Distinction between training for performance and exercise for recreation**

There are many female elite athletes who plan their training to improve performance and to participate in sport competitions. However, far more physically active women are not competitive, and perform regular exercise for recreational purposes. This thesis addresses both of these categories of physically active women, and the terms ‘training’ for performance and ‘exercise’ for recreation are used. The term ‘training’ is used when the physical activity is performed mainly for the purpose of satisfying a long-term performance goal, and thus is about the process, instead of the workouts themselves. As the process must generate a specific result, it must be planned to continually increase strength and/or endurance to meet the goal of increased performance. The term ‘exercise’ is used when the physical activity is performed mainly for recreational purposes, not to achieve a specific performance goal; it is also used to refer to workouts themselves. In numerous sports, endurance, strength, and power training are required to improve performance in competitions. These types of training are also required in recreational exercise to improve and maintain health and fitness. Through resistance training, athletes can firm, strengthen, and tone muscles, and recreational and elite athletic training can be performed in a periodised way. This thesis focus on the effects of menstrual/OC cycle-based periodised resistance training and women’s tolerance of this training. Moreover, it explores whether the hormones cortisol and oxytocin (OT) could serve as markers to detect negative health effects associated with training in physically active OC users and non-users.

## **Resistance training**

Resistance training is well known to increase muscle mass and strength, and it is used by elite athletes to improve sport performance and by the general population as recreational exercise. Resistance can be produced by free weights, machines, or the athlete’s own body weight. This type of training is

generally performed as concentric and eccentric muscle actions, executed over several sets and repetitions, and performed a few to several times per week, depending on the athlete's goal. An acute resistance exercise stimulus is used to overload the neuromuscular system and thereby develop muscle strength (Deschenes and Kraemer 2002); (Rhea, Alvar, and Burkett 2002) (Ratamess et al. 2009) due to muscle hypertrophy, nervous system adaptations, and increased lean mass (Chilibeck et al. 1996); (Chilibeck et al. 1998). This type of stimulus is affected by the mode, intensity, frequency, and volume of training (Rhea et al. 2003).

Women are known to have the same physiological abilities as males to tolerate and adapt to heavy resistance training (Wang et al. 1993). Many guidelines for the progress of resistance training are available, but they are derived mainly from research on men (Rhea and Alderman 2004). Few scientific publications address the strength and power effects of resistance training guided by hormonal fluctuations during several consecutive menstrual/OC cycles in trained women. Hence, an increased understanding of how to generate resistance training programmes guided by hormonal fluctuations in women are warranted.

## **Periodised resistance training and in relation to the menstrual cycle**

To achieve greater gains in strength, the periodisation of training programmes by planned variations in training volume, intensity, frequency, and/or exercises performed is recommended (W J Kraemer et al. 2002); (Kok, Hamer, and Bishop 2009); (Ratamess et al. 2009); (Rhea and Alderman 2004). Notably, in one study (Kell 2011), women were found to achieve greater relative increases in strength and hence appeared to be more responsive than men to an identical 12-week periodised resistance training programme. Nevertheless, research has generally suggested that men and women of all ages and training experience levels can achieve greater strength when following a periodised training programme compared with a non-periodised programme. The periodisation of training allows sufficient regeneration time within the training programme, and the use of regenerative techniques, such as relaxation and high fluid and carbohydrate intakes, may enable athletes to cope with high training loads (Rhea and Alderman 2004). Hence, sport training is performed in a periodic manner to enhance its effects by using the supercompensation model, and to inhibit the development of OR/OTS. That is, the training effect is actually gained during the relative resting period, and not during active training (Meeusen et al. 2013). Most periodisation utilises 4-week intervals, with all athletes on a team following the same schedule. Thus, female athletes train under different hormonal influences due to



fluctuations during their menstrual cycles. Oestrogen and progesterone are known to influence substrate metabolism during exercise performance and the trainability of muscle strength (C. Lebrun 1994); (Janse de Jonge 2003); (Constantini, Dubnov, and Lebrun 2005). Hence, training programmes used by eumenorrheic women could possibly be timed in accordance with the menstrual cycle to maximise anabolic effects.

In an attempt to clarify the different effects of oestrogen and progesterone, Reis et al. (1995) studied how the frequency of training sessions during different phases of the menstrual cycle influenced strength gain by training each leg differently. That study and a more recent study by Sung et al. (2014) demonstrated that follicular phase–based leg resistance training had a greater effect on muscle strength, compared with luteal phase–based strength training. This research suggests that the fluctuation of hormones during the menstrual cycle influences the trainability of muscle strength, with more strength adaptation in skeletal muscle in the follicular phase. These two studies, however, were performed during rather short time periods (two and three menstrual cycles) and included untrained or moderately trained subjects, with no control group and no OC users. Moreover, they did not control for possible health consequences due to effects of the training regime. Hence, more research is warranted to investigate how to periodise resistance training in relation to the menstrual/OC cycle in trained female athletes without negative health consequences.

## **Female sex hormones, the menstrual cycle, and oral contraceptive use**

The menstrual cycle, with its monthly hormonal fluctuations, is one of the most basic differences between men and women; hence, the possible effects of these fluctuations on training is of interest. The anabolic effect of the male steroid testosterone is well known (William J Kraemer and Ratamess 2005), but the influences of the female sex steroids oestrogen and progesterone on muscle strength and performance are less studied and not completely clear (Bruinvels et al. 2016); (Sheel 2016). Hence, a better understanding of the effects of cyclic variations in hormones and their impact on training in both eumenorrheic women and OC users is warranted. Despite the decreasing gap between men and women in exercise participation, women continue to be under-represented in sport and exercise research (Costello, Bieuzen, and Bleakley 2014); (Bruinvels et al. 2016); (Sheel 2016). A possible explanation for the restriction of study inclusion to men is that the male physiology remains relatively consistent from day to day. When women are included as participants, they are often tested when their hormone levels are lowest, in the early follicular phase, to minimise the possible impacts of oestrogen and

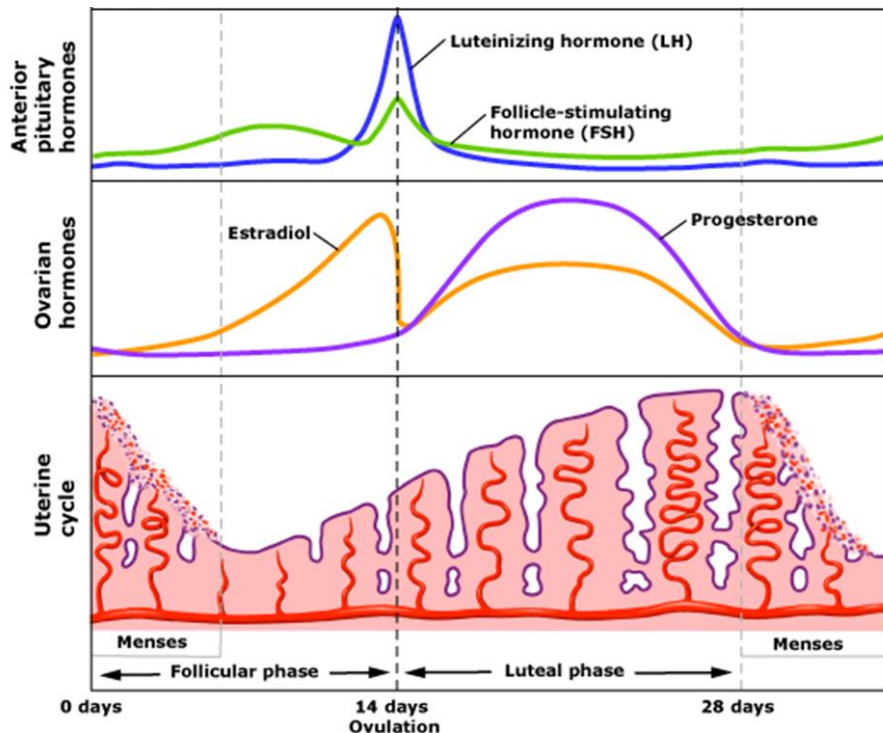
progesterone on study outcomes. However, women exercise and participate in sport competitions at all stages of the menstrual/OC cycle (Oosthuyse and Bosch 2010).

### ***The normal menstrual cycle and exercise***

Women between the ages of approximately 13 and 50 years experience a circamensal rhythm referred to as the menstrual cycle, during which the ovarian hormones fluctuate predictably (Figure 1). The menstrual cycle usually comprises 28 days, but it can vary within the typical range of 21–35 days (Keizer and Rogol 1990); (Diaz, Laufer, and Breech 2006). It is regulated by the hypothalamus, which produces gonadotrophin-releasing hormone (GnRH) to stimulate the pituitary hormones, follicle-stimulating hormone (FSH), and luteinising hormone (LH). This regulation system is called the hypothalamic–pituitary–gonadal (HPG) axis (A.B. Loucks and Horvath 1989); (Brukner and Khan 2010); (Oosthuyse and Bosch 2010). In women who ovulate, the menstrual cycle is regulated and defined by changes in the gonadotropic hormones FSH and LH and the sex steroid hormones estradiol (the most potent oestrogen) and progesterone (Shechter and Boivin 2010). The menstrual cycle is divided broadly into two phases, the follicular phase and the luteal phase, separated by ovulation. The first day of menstrual bleeding is referred to as cycle day 1; it is the first day of the follicular phase, which lasts approximately 14 days, until ovulation. The luteal phase, which also lasts about 14 days, until the next onset of menstrual bleeding, follows ovulation (Brukner and Khan 2010); (Oosthuyse and Bosch 2010).

The levels of female sex hormones are low during the first days of the follicular phase. The FSH concentration begins to increase in the early follicular phase, reaching a maximum during the first half of this phase. FSH stimulates the growth and development of a group of follicles, one of which usually matures fully and ruptures at the time of ovulation. The growing follicle begins to produce estradiol, the level of which increases on about cycle day 7 and peaks on the day before ovulation (Keizer and Rogol 1990); (Drinkwater 2000). The increase in estradiol has a negative feedback effect on FSH secretion and a positive feedback effect on LH secretion from the pituitary gland. At mid-cycle, a large peak in LH concentration, called the LH surge, and an increase in progesterone stimulate the maturation of a follicle and the release of an oocyte at ovulation, about 12 hours later. The luteal phase is marked by this LH surge, after which estradiol levels fall for several days. After ovulation, the empty follicle creates the corpus luteum during the luteal phase. LH stimulates the production of progesterone from the corpus luteum and the progesterone level peak in the middle of the luteal phase. Estradiol is also secreted from the corpus luteum, with a secondary increase in the mid-luteal

phase. When fertilisation does not occur, production of the female sex hormones declines, and the withdrawal of estradiol and progesterone initiates the breakdown of the endometrium, which leads to the next menstruation (Drinkwater 2000); (Brukner and Khan 2010); (Shechter and Boivin 2010).



**Figure 1.** The normal menstrual cycle, usually 28 days, divided into the follicular phase (ca. 14 days from menses onset to ovulation) and the luteal phase (ca. 14 days after ovulation to menses onset), graphs show approximate values, adapted from <http://biology.stackexchange.com/questions/48007/>

In men and women, androgens are produced by the reproductive organs and the adrenals. The most important androgen secreted is testosterone. The production of androgens, such as androstenedione and testosterone, from the ovaries is stimulated by LH and peaks prior to ovulation, during the mid-cycle LH surge, or at the time of ovulation. Androgen production enhances the degeneration of non-dominant follicles (Longcope 1986). The adrenal glands (in both sexes) and the ovaries produce very little testosterone, but secrete weaker androgens. In particular, dehydroepiandrosterone (DHEA; and its sulfo-conjugate), secreted by the adrenals, and androstenedione, secreted by

the adrenals and ovaries, are of physiological importance in women because they can undergo peripheral conversion to more potent androgens, such as testosterone. Androgens are precursors for endogenous oestrogen synthesis and play a key role in female physiology. Although produced in small amounts in women, androgens may have direct and crucial effects on physiology, e.g. bone density, muscle growth, and erythrocyte production (C. Enea et al. 2008); (Carina Enea et al. 2011).

Exercise can induce increases in circulating androgen levels; such increases have been observed after acute resistance exercises, but corresponding effects of chronic exercise are less clear. The free, bound, and total fractions of circulating androgens may also be influenced by the menstrual cycle, OC use, diet, and acute and/or chronic physical activity and training (C. Enea et al. 2008). Steroid hormones, such as androgens, are bound to sex hormone-binding globulin (SHBG) or albumin, and the free fractions are considered to be biologically active (Elliott et al. 2003); (Rosner 2006). Although this free hormone hypothesis has recently been challenged, circulating bound hormones may play significant biological roles and could be important for subjects with low circulating concentrations of androgens, such as women (Carina Enea et al. 2011). In addition to those of estradiol and progesterone, the levels of androgens fluctuate over the menstrual cycle, and androstenedione and testosterone levels peak prior to, or at the time of, ovulation (Longcope 1986). These fluctuations may influence exercise performance and the trainability of muscle strength (C. Lebrun 1994); (Janse de Jonge 2003); (Constantini, Dubnov, and Lebrun 2005); (Carina Enea et al. 2011).

Oestrogens and gestagens are important modulators of muscle physiology that impact the trainability of muscle strength; a striking decline in muscle strength occurs during the perimenopausal and postmenopausal periods, and can be reversed by hormone replacement therapy (HRT), especially that containing oestrogens (Barros and Gustafsson 2011). Hence, OC-induced alterations in blood concentrations of exogenous sex hormones may also effect training adaptation in OC users compared with non-users.

### ***Menstrual dysfunctions associated with exercise***

The female reproductive system is sensitive to physiological stress, and health problems associated with exercise, considered to be a form of physiological stress, have been recognised. Menstrual dysfunctions associated with exercise have been reported in women who participate in recreational exercise and strenuous training (Anne B. Loucks 2003). Exercise can impact the menstrual cycle in many ways, from delaying menarche, i.e. primary amenorrhea, to

inducing subclinical menstrual disorders, such as luteal phase defects, oligomenorrhea, and amenorrhea, described as existing along a continuum of menstrual disturbances (M P Warren and Shantha 2000); (De Souza 2003); (Goodman and Warren 2005).

Luteal phase defects, common among athletes, imply a shortened luteal phase (fewer than 10 days) and are usually associated with lower than normal levels of progesterone during the luteal phase and with anovulatory cycles. Women with such defects can still menstruate regularly and the follicular phase may be prolonged, resulting in a normal cycle length of 28 days (De Souza 2003). Luteal phase defects and anovulatory cycles are asymptomatic and difficult to detect clinically (M P Warren and Shantha 2000). Luteal suppression may be an intermediate condition between menstrual regularity and amenorrhea in athletes, or it may be the endpoint of successful acclimation to training. Oligomenorrhea is defined as the lengthening of the menstrual cycle to more than 35 days, and secondary amenorrhea is defined as the absence of the menstrual cycle for more than 3 months in previously menstruating women or one/no menstrual cycle per year, according to the International Olympic Committee (Goodman and Warren 2005); (Javed et al. 2013).

Menstrual cycle alterations, including delayed menarche, secondary amenorrhea, and luteal suppression, are more prevalent in athletes than in sedentary control subjects. Menstrual dysfunctions in physically active females are complex and appear to have multicausal aetiologies. Dissociation of the effects of physical exercise from those of other predisposing factors on the menstrual cycle is thus extremely difficult (Anne B. Loucks 2003); (Goodman and Warren 2005). Apart from a certain predisposition, athletes with training-induced altered menstrual cycles have been hypothesised to be affected by OR, which could lead to negative health consequences. Moreover, an imbalance between energy expenditure and energy intake, i.e. an energy deficit, changes hypothalamic–pituitary function and allows physiological mechanisms that inhibit reproduction to continue (Ann B. Loucks 1990); (J.C. Gibbs et al. 2011). Research has shown that inadequate energy availability is an important cause of menstrual disturbances in athletes, which could lead to inhibition of the HPG axis and activation of the hypothalamic–pituitary–adrenal (HPA) axis, resulting in elevated cortisol levels that inhibit reproductive function (A.B. Loucks and Horvath 1989). Hypothalamic amenorrhea due to energy drain is usually a reversible condition and could be a natural short-term adaption to prevent reproduction during starvation. It may, however, have long-term health consequences, including reduced bone mass and fertility.

Menstrual cycle alterations are most likely caused by subtle changes in the episodic secretion pattern of LH, as found in sedentary women with hypothalamic amenorrhea and in athletes after very strenuous exercise (M P Warren and Shantha 2000). These changes increase the secretion of anti-reproductive hormones, which can inhibit the normal pulsatile secretion patterns of the gonadotrophins FSH and LH. Altered LH secretion may be caused by increased secretion of corticotrophin-releasing hormone (CRH), which inhibits GnRH release. Continuous activation of the adrenals results in greater production of catecholamine, which may be converted to catechol oestrogens, a major group of active natural oestrogen metabolites formed by aromatic hydroxylation of primary oestrogens. These compounds are known to be potent inhibitors of GnRH secretion (Keizer and Rogol 1990). Amenorrhea due to the inhibition of GnRH release by the hypothalamus, leading to reduced secretion of FSH and LH, also leads to oestrogen deficiency (M P Warren and Shantha 2000).

Several mechanisms seem to be involved in the inhibition of the HPG axis and activation of the HPA axis with increased release of CRH (A.B. Loucks and Horvath 1989). Some cross-sectional studies of amenorrheic athletes have revealed abnormal reproductive hormone patterns, suggesting that the GnRH pulse generator in the hypothalamus fails to initiate normal hypothalamic–pituitary–ovarian function. Aetiological factors of menstrual disturbances described in the literature include large amounts of training, especially endurance training, and/or insufficient dietary intake, low body fat, and physiological stress and psychological stress.

Reported incidences of menstrual dysfunctions vary widely, depending on their definition and the type of exercise/sport activity (De Souza 2003). The relative incidence of menstrual dysfunctions is higher in athletes who engage in endurance and aesthetic sports, in which low body weight and low total body fat mass are favourable. Menarche is suggested to occur when the amount of body fat reaches 17%, and this amount should not decrease below 22% for regular menstrual cycles after maturation. Some athletes, however, maintain regularly ovulatory cycles despite hard training and total body fat mass below 17%, which is difficult to explain (M. P. Warren and Perlroth 2001); (Anne B. Loucks 2003).

Moreover, in the case of menstrual disturbances with low levels of estradiol and progesterone, the growth hormone (GH) response to acute resistance exercise is attenuated, potentially reducing the anabolic effects of such exercise (Nakamura et al. 2011).

## ***Oral contraceptive use and exercise***

A factor to consider when studying the effects of training in women is OC use. OCs are the main form of birth control in the general population; with the introduction of low-dose OCs, their use has increased in athletic women to match the prevalence in the general population (Rechichi, Dawson, and Goodman 2009). Female athletes use OCs not only for birth control, but also for other reasons, such as menstrual cycle control, reduction of premenstrual and menstrual symptoms, dysmenorrhea, reduction of menstrual blood loss, polycystic ovary syndrome (PCOS), reduction of musculoskeletal injury risk, and occasional alteration of menstrual cycle timing to delay menses prior to a major competition (Wojtys et al. 2002); (Constantini, Dubnov, and Lebrun 2005). In addition, physicians sometimes recommend oestrogen replacement, including OC use, to treat or prevent exercise-associated amenorrhea, to avoid increased bone loss. Athletes with oestrogen deficit show not only increased bone resorption, but also decreased bone formation, and the role of OCs as treatment for this condition remains uncertain (Michelle P. Warren and Goodman 2003). OCs control the concentrations of endogenous sex hormones, reducing the natural production of oestrogen and progesterone through inhibition of the pituitary secretion of gonadotropins, thereby inhibiting ovulation and preventing pregnancy. The OC suppress the pre-ovulatory peaks of FSH and LH levels, preventing follicular maturation and ovulation. Gestagens/progestins render the cervical mucus impermeable to sperm and modify the endometrium so that it will no longer support implantation (Fruzzetti, Trémollières, and Bitzer 2012). Moreover, OCs reduce variability in cycle length, ensuring a consistent 28-day cycle by systematically suppressing the hypothalamic-pituitary system (Fotherby 1996).

Combined OCs contain synthetic female sex hormones, such as ethinyestradiol and progestogen, corresponding to naturally occurring estradiol and progesterone in women. OCs may be used in the monophasic form as constant daily doses or in the triphasic form, with variable doses throughout the cycle (Brukner and Khan 2010); (Lawrie et al. 2011); (Fruzzetti, Trémollières, and Bitzer 2012). Exogenously administered ethinylestradiol is chemically identical to endogenous 17- $\beta$  estradiol, the most potent of the natural oestrogens. Ethinylestradiol has been used for many years as the oestrogen component of OCs due to its good oral bioavailability. The doses of ethinylestradiol in combined OCs are approximately the same, although differences exist due to the characteristics of the gestagens/progestogens used as the progesterone components. Various names are used for the exogenous progesterone components, including gestagens, progestins, and progestogens (Fotherby 1996); (Fruzzetti,

Trémollières, and Bitzer 2012). Ethinylestradiol prevents the symptoms of hypo-oestrogenism, enhances contraceptive efficacy, and helps to regulate bleeding (Fruzzetti, Trémollières, and Bitzer 2012). Among the numerous gestagens/progestins in use, the newer components have fewer androgenic and metabolic effects than do earlier forms. Moreover, the doses of the components are markedly reduced compared with earlier doses, although they retain 100% efficacy in preventing conception (Baird and Glasier 1993); (Fruzzetti, Trémollières, and Bitzer 2012). The administration of OC pills usually consists of 21 days of active treatment, followed by 7 days of placebo/no treatment (low levels of oestrogen and progesterone) to induce withdrawal bleeding (Lawrie et al. 2011); (Ekenros et al. 2012). The first day of bleeding is referred as OC cycle day 1 (Lawrie et al. 2011).

Sex steroid hormones play considerable roles in training adaptation, and OC-induced alterations in their blood concentrations may lead to differences in the amount of such adaptation between OC users and non-users. However, studies have failed to detect significant differences in muscle strength during OC phases (Wirth and Lohman 1982); (Sarwar, Niclos, and Rutherford 1996); (Peters and Burrows 2006), suggesting that the effects of OC use on muscle strength and performance are minimal. Ekenros et al. (2012) also found no significant influence of OC use on muscle strength and hop performance in moderately active women. Since OC use has no effect on absolute measured muscle strength or hop performance during the different phases of the OC cycles these findings suggest that OC user would have the same possibility of strength training effects as women without OC. Though, if the use of OC differently influence effects of strength training is still not clear.

Elevated plasma cortisol concentrations have been documented in women who use combined oestrogen-progesterone OCs (Crook and Group 1997); (Aden, Jung-Hoffmann, and Kuhl 1998). However, Brien et al. (1975) found no increase in the free cortisol index (cortisol/corticosteroid-binding globulin, CBG) in such women, which indicates that the use of this type of OC does not increase the amount of metabolically active cortisol. Previous studies have shown a pronounced rise in CBG during OC use (Brien 1988), surmised to reflect the marked stimulatory action of ethinyl estradiol on the hepatic synthesis of serum binding protein, which is counteracted only slightly by the progestogen components of OCs. The increase in the serum concentration of CBG may contribute to the rise in cortisol level, although the inhibition of hepatic metabolism of corticosteroids by contraceptive steroids may also be involved (Wiegratz and Kuhl 1995).

Few studies have investigated the effects of the hormonal milieu throughout the cycle in OC users, including adaptation to strength training. Data on the



effects of OC use on muscular strength and performance are minimal and inconclusive (Rechichi, Dawson, and Goodman 2009). To date, investigations of strength trainability during the menstrual cycle have not included OC users (Reis, Frick, and Schmidtbleicher 1995) (Sung et al. 2014). As the intake of fixed doses of synthetic estradiol and progesterone in OCs suppresses endogenous estradiol and progesterone the factors for strength capacity, strength training adaptation may differ between OC users and non-users in as-yet unrecognised ways.

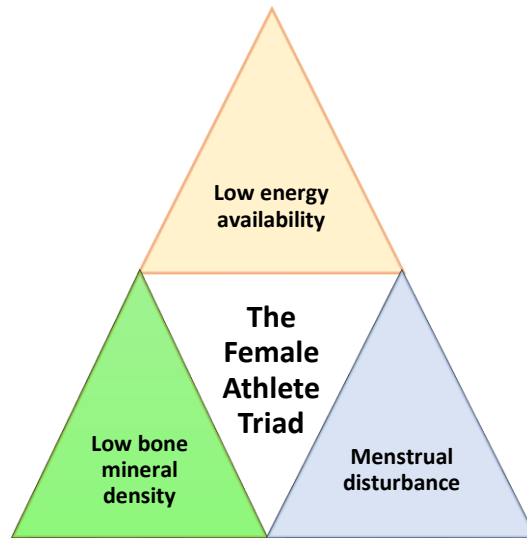
## **Consequences of training in female athletes**

Although exercise has beneficial health effects for most women, strenuous exercise may have health consequences. Negative long-term health effects are common in female athletes who are suffering from the ‘female athlete triad’, low energy availability, menstrual disturbance, and low bone mineral density (BMD), or some of its components, including an increased rate of musculoskeletal injuries, stress fractures, abnormal lipid profiles, endothelial dysfunction, cardiovascular effects, potential irreversible bone loss, depression, anxiety, low self-esteem, and increased mortality. These effects are particularly common in female athletes participating in aesthetic and endurance sports in which leanness is considered to be an advantage, such as ballet, other dance genres, figure skating, gymnastics, and cross-country running (Brukner and Khan 2010); (Barrack, Ackerman, and Gibbs 2013); (Javed et al. 2013); (Bertz and McCambridge 2016). The triad can occur, however, in females of all ages participating in any sport or in recreational exercise (Javed et al. 2013). Whether these negative effects are specific to endurance training, or also occur after increased resistance training load, remains unclear. Two major related problems – reduced fertility and reduced bone mass – are associated with menstrual dysfunctions (anovulatory cycles, amenorrhea, oligomenorrhea, or luteal phase defects) (M P Warren and Shantha 2000); (Brukner and Khan 2010). Hence, early recognition of the possible development of the female athlete triad and/or OR/OTS is important, and could enable the athlete and/or coach to take immediate action to avoid further progression.

### ***The female athlete triad***

At a consensus conference in 1992 organised by the American College of Sports Medicine (ACSM), the concept of the ‘female athlete triad’ was first defined as consisting of the interrelated conditions of disordered eating, amenorrhea, and osteoporosis (Yeager et al. 1993); (Javed et al. 2013). The ACSM began using the term in 1997 (Otis et al. 1997), and the definition was updated in 2007 (Nattiv et al. 2007) to include a spectrum of dysfunctions

related to energy availability, menstrual function, and BMD. The most recent model treats the female athlete triad as a syndrome, linking low energy availability (with or without disordered eating), menstrual disturbance, and low BMD across a continuum of symptoms ranging from healthy to unhealthy presentations (Figure 2) (Nattiv et al. 2007); (Javed et al. 2013); (Jenna C. Gibbs, Williams, and De Souza 2013).



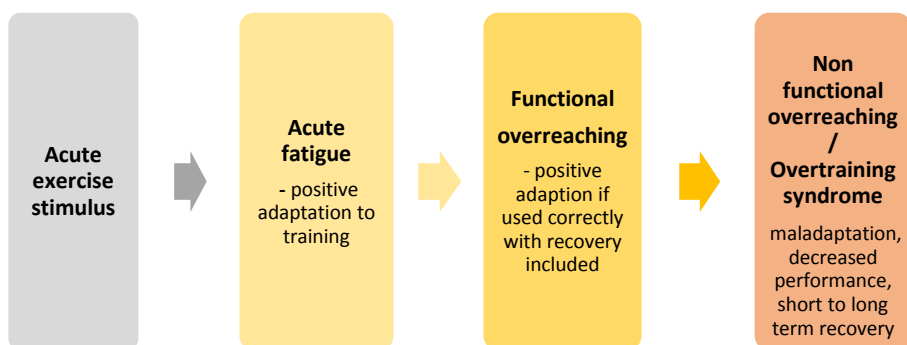
**Figure 2.** The female athlete triad and its components, low energy availability, menstrual disturbance, and low bone mineral density, as symptoms of the unhealthy presentation of each component.

Research has shown that few (0–16%) elite female athletes meet all criteria of the triad, although the prevalence of one or two components is 50–60% in certain athlete groups (Brukner and Khan 2010); (Barrack, Ackerman, and Gibbs 2013); (Javed et al. 2013). These findings imply that a significant proportion of female athletes, and not only elite athletes, suffer from components of the triad, rather than the complete syndrome (Brukner and Khan 2010); (Javed et al. 2013). Increases in the intensity and duration of the endurance training load increase the risk of developing one or more triad components (Arena et al. 1995); (C. M. Lebrun and Rumball 2001); (M. P. Warren and Perlroth 2001); (Michelle P. Warren and Goodman 2003); (Javed et al. 2013); (Maïmoun, Georgopoulos, and Sultan 2014); (Matzkin, Curry, and Whitlock 2015). Low energy availability, due to dietary restriction or increased exercise energy expenditure (i.e. failure to increase caloric intake to match increased exercise), contributes to the development of the triad. If

accompanied by weight loss and hypo-oestrogenic amenorrhea, it may lead to decreased BMD (Nattiv et al. 2007); (Javed et al. 2013); (Jenna C. Gibbs, Williams, and De Souza 2013); (Matzkin, Curry, and Whitlock 2015), especially in the spine (Prior et al. 1990); (Arena et al. 1995). Low BMD could lead to osteoporosis, a skeletal disorder characterised by compromised bone strength, and an increased risk of fracture (Nattiv et al. 2007); (Javed et al. 2013). Research has shown that a reduction in peak bone mass may not be compensated later in life when energy intake is normalised and the normal menstrual cycle returns (Nattiv et al. 2007); (Javed et al. 2013). Thus, the two major and severe problems associated with menstrual dysfunctions in the female athlete triad are reduced fertility and reduced bone mass (M P Warren and Shantha 2000); (Brukner and Khan 2010).

### ***Overreaching and overtraining syndrome***

Success in most sports today requires year-round training, with increasingly shorter off-seasons, and training and competitions throughout the year in some sports. Meeusen et al. (2013) concluded that researchers had reached consensus that excessively frequent or intense training without sufficient recovery may lead to physiological maladaptation and decreased performance. An imbalance can occur between the demands placed on athletes and their ability to meet those demands, which can lead to negative effects, such as OR and OTS. OR is defined as an overloading of the body beyond its ability to adapt, and the required recovery time is relatively short (several days to several weeks) (Kentta and Hassmen 1998); (Halsen and Jeukendrup 2004); (Meeusen et al. 2013). OTS is described as the result of an excessive training load with inadequate recovery between training sessions and periods (R W Fry, Morton, and Keast 1991); (M. Lehmann, Foster, and Keul 1993); (Kuipers 1998), requiring a longer recovery time (several weeks or months) (Halsen and Jeukendrup 2004); (Meeusen et al. 2013). Although a successful training programme must involve overload (functional overreaching) to improve performance, it must also include adequate recovery to avoid persistent fatigue, which could have negative effects. Functional overreaching causes a short-term performance decrement, but leads to improved performance after short-term recovery. However, if the athlete does not sufficiently respect the balance between training and recovery, non-functional overreaching, OR, may occur. Distinguishing between OR and OTS is very difficult, and depends on the clinical outcome and diagnosis. The terminology used in this thesis is based on the definitions used by Urhausen and Kindermann (2002), and Halsen and Jeukendrup (2004), and Meeusen et al. (2013) (Figure 3).



**Figure 3.** Illustration of the overtraining continuum as a progression from acute exercise stimuli with acute fatigue to non-functional overreaching/overtraining syndrome

The term ‘overtraining’ is used as a verb to refer to the process of intensified training, with possible positive training effects if adequate recovery is included, i.e. functional overreaching, which may have a super-compensation effect. This process is a planned phase of many training programmes, as it is believed to contribute to subsequent improved performance. The terms ‘OR’ or ‘OTS’ are used when extreme training is performed without sufficient recovery. The inclusion of the word ‘syndrome’ in OTS emphasises the multifactorial aetiology and the knowledge that training is not necessarily the only cause of this condition (Meeusen et al. 2013). Because of the gradual onset of OR and gradual transition from OR to OTS, early detection of symptoms is important (Kuipers 1998); (Meeusen et al. 2013), as OR/OTS may become a serious problem for the athlete. Symptoms of OTS, such as fatigue, performance decline, and mood disturbances, have been suggested to be more severe than those of OR, due to their impacts on several biological, neurological, and hormonal mechanisms and the very long rehabilitation period (Halsen and Jeukendrup 2004); (Meeusen et al. 2013). The definitive diagnosis of OTS requires the exclusion of organic diseases and infections or other factors, such as negative energy balance (Meeusen et al. 2013).

Two distinct types of OTS have been described: sympathetic and parasympathetic. Sympathetic OTS involves increased sympathetic activity at rest, whereas parasympathetic OTS involves decreased sympathetic activity, with parasympathetic activity predominating at rest and with exercise. All overtraining is believed to result ultimately in parasympathetic OTS.

However, few studies have addressed the topic of OR and OTS in athletes performing resistance exercise (A. C. Fry and Kraemer 1997). No single, specific, reliable parameter (i.e. performance or psychological test; biochemical, hormonal, or immune marker) for the diagnosis of OR or OTS at an early stage has been identified, and only little improvement has occurred in recent years in the development of suitable diagnostic tools (Kuipers 1998); (Urhausen and Kindermann 2002); (Halsen and Jeukendrup 2004); (Ackel-D'Elia et al. 2010); (Meeusen et al. 2013). Impairment of physical performance and disturbed mood profile, evaluated with psychological questionnaires, seem to be reliable markers for OR/OTS (Ackel-D'Elia et al. 2010); (Meeusen et al. 2013). In addition, the measurement of time to fatigue can show greater changes in exercise capacity as a result of OR/OTS (Halsen and Jeukendrup 2004). Psychological factors, such as stress, negative social environment, and personal and/or emotional problems, also seem to be important in the development of OTS. The psychological and behavioural variables often associated with OTS include mood state changes, as shown by the Profile of Mood States (POMS); these changes can be useful predictors of OR/OTS, but they cannot serve alone as a reliable diagnostic tool. The POMS is used to quantify total mood disturbance, including increased fatigue, confusion, tension, depression, and decreased vigour (McNair, Lorr, and Droppelmann 1971). Most studies of overtraining have been performed with men and endurance athletes, although overtraining can also occur in power athletes, such as weightlifters. Decreased muscle strength and endurance were seen in association with fatigue in a study of 17 weight-trained males who exercised on a squat machine daily for 2 weeks with a relative intensity of 100% of one repetition maximum (RM) (A. C. Fry et al. 1994). The effects in female weightlifters were not investigated, and remain unclear

### ***Monitoring of overreaching and overtraining syndrome***

Although performance optimisation is important in sports, finding a balance between training stress and recovery throughout the whole training period is critical; it could determine the difference between success and failure in competitions (Meeusen et al. 2013). Sufficient monitoring instruments are thus important to assess an athlete's mood, need for recovery, and current life circumstances. Combinations of some parameters (biochemical, hormonal, and self-reported measures) could be used for monitoring to prevent the development of OR and OTS and for early diagnosis (Kuipers 1998); (Urhausen and Kindermann 2002); (Halsen and Jeukendrup 2004); (Ackel-D'Elia et al. 2010); (Meeusen et al. 2013). The measurement of hormones associated with stress and/or well-being is important for OR/OTS monitoring (Leslie et al. 2002); (Maïmoun, Georgopoulos, and Sultan 2014). However, given the potential for seasonal periodicity of various endocrine functions;

(Nathorst-Böös, Stock, and von Schultz 1994); (Hansen et al. 2001) hormone concentrations should be measured repeatedly over a long period of time to provide normative data. As hormones can be modulated by ovarian steroids (Forsling 2000), the impact of menstrual and OC cycles must be considered when performing such assessments in physically active women. Psychological and behavioural variables are often associated with OR/OTS, and the POMS can successfully identify athletes predisposed to these conditions (Raglin, Morgan, and O'Connor 1991); (Berglund and Safstrom 1994). The use of a daily log book is also recommended as an effective self-analysis method in OR/OTS monitoring and to improve athletes' self-awareness. (Hooper and Mackinnon 1995). The use of self-reported monitoring as an additional tool in diagnosing OR/OTS is inexpensive, time efficient, non-invasive, and easily implemented by coaches or team personnel to monitor athletes' progress throughout the training and competitive year (Main et al. 2016).

### ***Hormonal monitoring***

#### *Cortisol*

In addition to oestrogen, other hormones may serve as markers of exercise-related disorders in female athletes (Leslie et al. 2002); (Maïmoun, Georgopoulos, and Sultan 2014). Cortisol could be used as such a marker to reflect long-term training stress resulting in catabolic metabolism (William J Kraemer and Ratamess 2005). The free testosterone/cortisol (FTCR) ratio can be used to detect imbalances between anabolic and catabolic metabolism (Urhausen, Gabriel, and Kindermann 1995); (Hug et al. 2003), although it may simply indicate actual physiological strain during training (Urhausen, Gabriel, and Kindermann 1995).

Cortisol is a steroid hormone produced in the adrenal cortex, regulated primarily by the hypothalamic–pituitary axis. Circulating levels of cortisol are detected by the hypothalamus, which secretes CRH into the portal circulation of the pituitary in response to low cortisol levels. In return, CRH signals the anterior pituitary to release the peptide adeno-corticotrophic hormone (ACTH), which stimulates the secretion of cortisol. One of cortisol's principal effects involves energy substrate availability, achieved by increasing gluconeogenic activity in the liver, decreasing glucose uptake and increasing glycogen synthesis in muscle, and mobilising amino acids from skeletal muscle. These effects result in decreased utilisation of glucose by muscle cells and increased substrate availability (A. C. Fry and Kraemer 1997).

The resting cortisol level has been suggested to play an interesting role in the detection of OR and OTS, as it is thought to indicate stress (R W Fry, Morton,

and Keast 1991); (M. Lehmann, Foster, and Keul 1993). However, increased, decreased, and unchanged levels of resting cortisol have been detected in overtrained athletes, and research has been performed mainly with male athletes (Adlercreutz et al. 1986); (Rod W. Fry et al. 1992); (M. Lehmann et al. 1992); (Urhausen, Gabriel, and Kindermann 1995); (M. J. Lehmann et al. 1997); (Uusitalo et al. 1998); (Hedelin et al. 2000); thus, research on and knowledge about this factor in female athletes are lacking. It is known that female athletes have become a common part of the sport environment (Greydanus and Patel 2002), and although OC use is prevalent among them (Greydanus and Patel 2002); (Rechichi, Dawson, and Goodman 2009), its impact on cortisol levels has not been well studied. Hence, the influences of both the exogenous and the endogenous hormonal profiles on athletic performance should be investigated (Rechichi, Dawson, and Goodman 2009). The administration of oestrogen, as with OCs, can increase the total plasma cortisol (Brien 1980); (Brien 1988) and free cortisol (Nolten et al. 1979) levels. Coolens et al. (1987) showed that total cortisol levels were elevated in oestrogen-treated women, but that unbound cortisol (the free fraction) levels were similar to the control group. The role of cortisol in indicating stress is multifaceted and complex, and as oestrogen may influence cortisol levels the impact of OC use on cortisol levels should be investigated. Testosterone and cortisol are thought to have opposing effects on muscle metabolism, protein synthesis, and growth. Thus, the FTCCR ratio has been suggested to indicate the balance between androgenic-anabolic activity (testosterone) and catabolic activity (cortisol), and its use as a diagnostic tool for OR/OTS has been proposed. A FTCCR below the reference value of 0.35 (10-3) and/or a decrease in the ratio of 30% or more has been suggested to be an indication of OR/OTS (Banfi and Dolci 2006). However, studies have failed to support the usefulness of the ratio, as it has been found to be unchanged in OR athletes. When measured over time in individual athletes, however, this ratio may provide an indication of athletes' adaptive responses to short-term physiological strain (Urhausen, Kullmer, and Kindermann 1987); (Urhausen, Gabriel, and Kindermann 1995). The FTCCR ratio is a commonly used marker in men, but it alone is not a suitable indicator of the anabolic/catabolic balance in females, as female free testosterone values are much lower than those of men and show a varying seasonal response (Vervoorn et al. 1992). The ratio decreases in relation to the intensity and duration of training, but it indicates only the actual physiological strain of training; hence, it is not a suitable marker of long-term negative training stress, as in OR and OTS (Urhausen, Gabriel, and Kindermann 1995).

## *Oxytocin*

Another hormone, besides cortisol, to identify physiological and psychosocial stress are oxytocin (OT), due to its relation to different forms of positive social interaction and stress management (C. S. Carter et al. 2007). OT is a peptide hormone, known primarily for its role in parturition and lactation and its peripheral roles in reproduction. Research in past decades has focused on the functions of OT in the brain in relation to social behaviours (Lee et al. 2009). However, most knowledge has been derived from animal studies (Gimpl and Fahrenholz 2001). In humans, OT improves positive communication, reduces stress during conflict, and may be involved in an important central nervous mechanism related to stress protection (Ditzen et al. 2009). OT may also suppress the 'classical' stress hormones of the HPA axis (M Heinrichs 2003); (Markus Heinrichs, von Dawans, and Domes 2009). In general, plasma concentrations and central release of OT are increased following physiological, psychological, and social stress (Lee et al. 2009). Moreover, OT is also thought to function as an anxiolytic, as it decreases stress hormones in humans and rats and modulates physiological and behavioural responses to stress. OT seems to have primarily parasympathetic actions; it has been shown to increase directly after endurance running (S. C. Carter 2003), and it facilitates the reduction of the heart rate in trained individuals (Micheline 2007); (Hew-Butler et al. 2008).

OT is synthesised in the hypothalamus, transported to the posterior pituitary gland, and stored and released into the circulation to act on target organs. OT is also released within the brain, where it acts at specific oxytocin receptors (OTRs) (Gutkowska et al. 2000); (Leng, Meddle, and Douglas 2008); (Viero et al. 2010). Recent studies have shown that OT is an omnipresent hormone that is synthesised at many sites in the body (Gutkowska et al. 2000). Differences between the sexes in OT and OTR distributions in the brain have been reported (C. S. Carter 2007). OT and OTR expression is usually higher in females than in males, and the central roles of OT on behaviour and physiology depend strongly on steroid hormones and sex. (C. S. Carter 2007). Following an appropriate stimulus, OT is released centrally from axon terminals as a rapid synaptic neurotransmitter (Dabrowska et al. 2011), or from dendrites, soma, or non-terminal axonal regions as a non-synaptic neuromodulator (Tobin, Leng, and Ludwig 2012). A neuromodulator is recognised by slow substance diffusion via the extracellular fluid, and binding to nearby or distant receptors in brain regions that are not necessarily connected via axonal projections. Interestingly, central and peripheral release of OT from neurohypophysial terminals into the blood stream can be independent or co-ordinated, depending on the type of stimulus (Neumann and Landgraf 2012). Early results showed that OT secretion varies over the



menstrual cycle, although this variation is minimal (Stock, Bremme, and Uvnas-Moberg 1991); (Hull et al. 1995); (Steinwall et al. 1998). The OT concentration has been shown to increase with estradiol administration (Nathorst-Böös, Stock, and von Schultz 1994), and evidence shows that the magnitude of changes in the circulating OT concentration in pregnancy is related to the estradiol/progesterone ratio (Fuchs, Behrens, and Liu 1992). OC use has also been shown to significantly increase the OT level (Silber et al. 1987); (Stock, Karlsson, and von Schoultz 1994). Previous studies have documented significant circadian and seasonal periodicity in various endocrine functions (Hansen AM et al. 2001), which must be taken into consideration when studying OT levels and periodicity. OT secretion increases during sleep in humans (Kostoglou-Athanassiou et al. 1998); (Forsling 2000), although Nathorst-Böös et al. (Nathorst-Böös, Stock, and von Schultz 1994) found minimal diurnal variation in OT (with a peak at 04:00 h), but substantial differences in basic levels among individuals. A location with large variation in daylight hours during the year would be favourable for studies of seasonal variation in this hormone. However, no study has examined the seasonal periodicity of OT in OC users and non-users. OT is also thought to work as an anxiolytic as it decreases stress hormones in both humans and rats, and to modulate the physiological and behavioral responses to stress. Physiological training stress can be used as a model to study the relationships among OT level, mood, and stress.

If a significant relationship exists between mood and OT levels in peripheral blood, OT could serve as a marker, alone or in combination with other markers, for the identification of physiological and psychosocial stress leading to OR and OTS. Whether OC use affects OT levels, and the possible seasonality of OT expression, in female athletes need to be clarified.

### ***Self-report monitoring tools***

The POMS questionnaire, a well-known, frequently applied self-report monitoring tool has been used to assess affective mood state fluctuations in male and female athletes. Other self-report monitoring tools, such as the Recovery Stress Questionnaire for Athletes (REST-Q Sport) (Kellmann 2010); (Otter et al. 2015) and the Multi Component Training Distress Scale (MTDS) (Main et al. 2016), were developed subsequent to the POMS. The REST-Q Sport assesses an athlete's perceived state of recovery, and the MTDS monitors psycho-behavioural responses to training stimuli in athletes. Findings reported by Main et al. (2016) support the utility of both of these self-report monitoring tools for athletes; the authors demonstrated their ability to predict athletic performance changes.

### *The Profile of Mood State questionnaire*

OTS is accompanied by negative disturbances in the total mood state, which can be determined by an increase in a total mood score (Global POMS), and a decrease in the vigour/fatigue (V/F) ratio (McNair, Lorr, and Dropplemann 1971); (M. Lehmann et al. 1992); (M. Lehmann, Foster, and Keul 1993); (M. J. Lehmann et al. 1997); (Kenttä, Hassmén, and Raglin 2001). The POMS a is an easy-to-use, inexpensive instrument developed originally for psychiatric patients to assess response to treatment (Lewis et al. 2005). It is also used frequently to assess affective mood state fluctuations in athletes (McNair, Lorr, and Dropplemann 1971); (Mendel 1989); (Hedelin et al. 2000); (Lewis et al. 2005); (Meeusen et al. 2013). To date, impaired physical performance and disturbed mood profile seem to be the most reliable markers of OR and OTS (Ackel-D'Elia et al. 2010). The 65-item POMS provides an index of total mood disturbance, Global POMS score, measured by six mood states (tension, depression, anger, fatigue, confusion, and vigour) on a five-point scale ranging from 'not at all' (0) to 'extremely' (4) (McNair, Lorr, and Dropplemann 1971). To avoid negative values, a constant of 100 is added when calculating Global POMS scores (Cramer, Nieman, and Lee 1991); (Raglin, Morgan, and O'Connor 1991); (Berglund and Safstrom 1994). A rating scale of estimated training intensity ranging from 2 ('very, very low') to 14 ('very, very high') is used to determine whether a high Global POMS score and decreased V/F ratio are connected to high training intensity and thus could be a sign of OR/OTS development due to training load.

Main et al. (2016) concluded that a combination of self-reported measurement with selected physical performance tests and/or biochemical and hormonal markers is a logical direction for future research to develop markers to diagnose OR/OTS. This combination may provide the clearest picture of an athlete's current training state, and enable the prediction of athletic performance changes and readiness for competition.

# Aims

The general aim of the thesis was to investigate effects of menstrual/oral contraceptive cycle based resistance training on strength and power in physically active women, and to detect if the training was well tolerated and without potential exercise-related negative consequences on components in the female athlete triad. Moreover, to provide normative data on oxytocin and cortisol to elucidate if these hormones could be one diagnostic marker in combination with others to monitor and diagnose female athletes that may be at risk to develop overreaching and overtraining syndrome.

## **The specific aims were:**

- To investigate the effects of high frequency periodised leg resistance training on, isokinetic peak torque in right and left knee flexor and extensor, squat jump, and countermovement jump, and lean body mass in oral contraceptive users and non-users (paper I).
- To investigate potential exercise related negative consequences of the high frequency periodised leg resistance training on sex hormones, growth hormone, cortisol, total body fat mass, and bone mineral density in the spine and moreover to investigate the experiences of the training (paper II).
- To provide normative data on oxytocin and cortisol, and elucidate its seasonality in users and non-users of oral contraceptives (papers III-IV).

# Materials and methods

## Subjects

Two main samples were included in this thesis. Fifty-nine women, participated in the intervention study (papers I-II) and another 33 women participated in the observational study (papers III-IV). The subject characteristic data at baseline (papers I-IV) are presented in Table 1. The women were recruited by flyers at local gym/sports facilities in Umeå, Sweden, and volunteered to take part in the studies. All subjects received written and oral information about the current study, including the measurement procedures, and all women gave their written consent for participation. The study procedures were in accordance with the ethical standards for human experimentation, established by the Declaration of Helsinki, and the Regional Ethical Review Board at Umeå University, Umeå, Sweden, approved the studies (papers I-II, Dnr 95-055 and papers III-IV, Dnr 05-148M).

### *General inclusion and exclusion criteria (papers I-IV)*

The general inclusion criteria were; age 18-35 years, non-smoker, physically active, healthy by own report, and a regular menstrual cycle of 28 days (acceptable 21-35 days) or an oral contraceptive (OC) cycle of 28 days. The participants were also medically screened to ensure that no medical problem, medication or sports related injury would contraindicate their participation (Fields and Delaney 1990); (Fields 1994). Exclusion criteria were irregular menstrual cycles during the study, change of contraceptive habits, not training according to the training regime, not participating at post-test.

**Table 1.** Subject characteristic data of the two samples (papers I-II and papers III-IV) at baseline. Data are presented as mean and standard deviation (SD) for women with and without oral contraceptive use.

	<b>Papers I and II (n = 59)</b>		<b>Papers III and IV (n = 33)</b>	
	OC (n = 32)	NOC (n = 27)	OC (n = 15)	NOC (n = 18)
Age (yrs.)	23 ± 2	25 ± 4	23 ± 3	26 ± 4
Height (cm)	167 ± 6	168 ± 8	166 ± 6	168 ± 7
Weight (kg)	64 ± 7	67 ± 10	61 ± 6	67 ± 13

*Notes; OC, oral contraceptive use; NOC, without oral contraceptive use*

The recruitment of participants was ongoing during the years 1999-2000 and 2004-2006, to the intervention study (papers I-II) and during the years 2006-2007, to the observational study (papers III-IV). Approximately 300 women participated in information sessions about the studies, including a general information about training, recovery, and the importance of sufficient calorie intake required during endurance and resistance training. After that, the women had to pass inclusion criteria, including a health formulary, before they signed the informed consent document to participate. Some women did not fulfil the inclusion criteria or they were not able to participate during the whole study period. In all, 134 subjects passed the inclusion criteria and participated in a pre-test, 86 subjects in the intervention study (papers I-II) and 48 subjects in the observational study (papers III-IV). At post-test, 59 subjects participated in the intervention study (papers I-II) and 33 subjects participated in the observational study (papers III-IV). The drop-out rate was explained by long study periods in these two prospective longitudinal studies and also by the young study population and their change of living conditions and contraceptive habits, which resulted in difficulties with high attendance. However, the discontinuers did not differ substantially from the study groups in subject characteristics at baseline in these studies. In the intervention study (papers I-II) the three training groups, as well as OC users and non-users, were comparable in subject characteristics and outcome variables at pre-test. In the observational study (papers III-IV) the two groups, OC users and non-users, were comparable in terms of subject characteristics for height and weight. OC users were a few years younger ( $23 \pm 3$  yrs. vs.  $26 \pm 4$  yrs.;  $p = 0.006$ ) and had a slightly higher  $\text{VO}_2$  max (ml/kg x min), both at inclusion

( $50.0 \pm 4.7$  ml/kg x min vs.  $46.4 \pm 4.6$  ml/kg x min;  $p = 0.037$ ) and at the end of the study ( $49.9 \pm 5.0$  ml/kg x min vs.  $45.3 \pm 6.1$  ml/kg x min;  $p = 0.040$ ). Mean estimated training intensity on the Borg RPE scale was equal in the two study groups, ( $15 \pm 2$ , vs.  $15 \pm 2$ ) during the study period.

### ***Inclusion criteria in the intervention study (papers I-II)***

The inclusion criteria concerning the physical activity in this study were experience of resistance training in specific machines for leg-press and leg curl, for a minimum of 2 months, 3 times·w<sup>-1</sup> prior the start of the study. The subjects were randomly allocated to one of two periodised training groups (group 1,  $n = 19$  and group 2,  $n = 19$ ) and to one control group ( $n = 21$ ) with regularly training. Subject characteristics in the three groups at pre-test are presented in Table 2. In all three groups the number of women, OC users and non-users, were balanced after being assigned group in consecutive order so that there were approximately equal numbers in each group. Subjects with OC use, combined hormones in monophasic or triphasic patterns, were randomly distributed in the three groups by being assigned to a training group in consecutive order.

**Table 2.** Subject characteristics in the three training groups at pre-test in the intervention study (papers I-II), including women without oral contraceptive use and women with oral contraceptive use, containing combined hormones in monophasic or triphasic patterns

	<b>Group 1 n = 19</b>	<b>Group 2 n = 19</b>	<b>Control group n = 21</b>
NOC use, n	8	9	10
OC use, n	11	10	11
	10 triphasic, 1 gestagen	3 triphasic, 7 monophasic	7 triphasic, 4 monophasic
Age (years)	25.0 ± 4.0	24.5 ± 2.6	24.5 ± 3.9
Height (cm)	164.5 ± 7.0	168.1 ± 7.2	169.5 ± 5.8
Weight (kg)	61.9 ± 9.5	66.7 ± 7.1	66.2 ± 8.5
Menstrual cycle length, (days)	29 ± 1.4	28 ± 0.3	27 ± 2.4

*Notes: OC; oral contraceptive use, NOC; no oral contraceptive use. Group 1; high frequency training during the first two weeks, Group 2; high frequency training during the last two weeks, Control group; regularly training during the entire menstrual/OC cycle.*

### ***Inclusion criteria in the observational study (papers III-IV)***

The inclusion criteria concerning physical activity in this study were defined as experience of endurance/aerobic training, including cycling sessions, at least 3 times a week, at a level not less than 13 on the Borg RPE scale (Borg 1970); (Borg, Hassmen, and Lagerstrom 1987) corresponding to 7 on the scale for training intensity in the POMS questionnaire, and two days of rest included every week, for a minimum of 3 months, prior the start of the study. Subject characteristics at baseline are presented in Table 1. Among OC users who completed the study (n = 15), 13 used combined estrogen-gestagen type of OC, one used combined anti-androgen and one used OC with only gestagen. In non-users who completed the study (n = 18), menstrual cycles longer than 35 days (samples, n = 8) during the study were excluded from the results.

The subjects were all examined at Sports Medicine Unit, Umeå University, at the start of the studies, pre-test, and at the end of the studies, post-test, and moreover in the observational study (papers III-IV) monthly during the 9 month study period. The subjects were all informed to avoid hard training two days before pre- and post-test to ensure no delayed onset muscle soreness at the test days. All measurements and blood sample collections were performed at approximately the same time of the day and by the same technical assistants, i.e. the tests were performed as identically as possible at the test occasions for each individual. Resting blood samples were always taken in the morning and the measurements were performed daytime, both without heavy activity and heavy meal before, thus helping to keep the possible confounding variables of diet and hydration status to a minimum. All subjects used a personal log book (self-report monitoring tool) during the study period to document all training, the perception of the training, the menstrual cycle length, and also note if any health problem occurred. The log books were inspected every month to evaluate that the training was continued as instructed and that no health problems or menstrual irregularities occurred during the study period. An illustration of the flowcharts of the studies, including blood samples, measurements and self-report monitoring tools, are illustrated in Figure 4, the intervention study, (papers I-II) and in Figure 5, the observational study (papers III-IV).

### ***The intervention study design (papers I-II)***

In this prospective longitudinal intervention study of four menstrual/OC cycles the training programs were performed periodised in relation to the menstrual/OC cycle. The periodised leg resistance training (group 1 and group 2) refers to high frequency leg training during two weeks of the menstrual/OC cycle (5 times·w<sup>-1</sup>). The remaining two weeks of respective cycle the women performed the leg resistance training once a week as part of the training program. The subjects in the control group trained regularly throughout the menstrual/OC cycle (3 times·w<sup>-1</sup>). The training programs for the three groups were planned to consist of the same amount of leg training sessions (48 sessions), besides their ordinary training. The pre-test and post-tests were performed at day 7 (SD ± 2 days) of the menstrual/OC. The training regimes are presented in Figure 6. The completed number of leg training sessions was logged and was equivalent in the three groups (group 1 = 41 ± 4.0, group 2 = 41 ± 4.8, control group = 42 ± 4.4). The leg resistance training consisted of two compulsive exercises, leg press and leg curls, and were started at an individual load. The participants performed double leg press and leg curls three sets of 8–12 repetition maximum (RM) with 1-2 minutes of rest between sets and with 2-10 % increase in load applied when the individual performed the current workload for one or two repetitions over the desired number,



according to recommendations to achieve strength gains (W J Kraemer et al. 2002); (Ratamess et al. 2009). The women also continued their ordinary training with the exception of leg exercise, which were exchanged to the leg exercise provided by us. The participants were instructed how to perform the leg resistance training in the machines prior the start of the training program, and throughout the study a gym instructor was available to guide the training at the gym. The subjects participated in identically performed pre and the post-tests, and additionally at the post-test, the subjects also reported their experiences of their leg training program during the four consecutive menstrual/OC cycles. Their experiences were categorized on a three-graded scale; 1 = good, 2 = neither good nor bad, 3 = bad, and were used in the analyses as dichotomized to 1 = good, and 2 = not good (paper II).

### ***The observational study design (papers III-IV)***

In this prospective longitudinal observational study over a nine month period, the subjects were informed to continue with their ordinary training and continue to keep the endurance/aerobic training at the intensity of 13 or more on the Borg RPE scale, at least 3 times weekly and assert two days of rest every week. The mean estimated training intensity on the Borg RPE scale was measured and found equal in OC users vs. non users, ( $15 \pm 2$ , vs.  $15 \pm 2$ ) during the study period. This study included a monthly blood sample collection and a POMS questionnaire performed in addition to; measurements, POMS, and blood sample collections at pre- and post-test (Figure 5). The blood samples were collected for each subject, with maximal one-week difference between calendar days at different days of the menstrual cycle to represent the whole menstrual cycle. This prospective longitudinal study, with two study groups, OC users and non-users, was performed in a setting with different daylight hours to investigate seasonality in OT levels (paper III) and in cortisol levels (paper IV), and moreover to elucidate if there was a different pattern in the hormone levels in OC users to non-users. The amount of hours from sunrise to sunset at this location at high latitude, was in average of 10h during the autumn, 6h during the winter, and 15h during the spring seasons (U.S. Naval Observatory Astronomical Applications Department/Sun rise sun set Sweden),([Http://www.sunrise-and-Sunset.com/sv/sun/sverige/umea](http://www.sunrise-and-Sunset.com/sv/sun/sverige/umea) n.d.).

### **Measurements and blood samples (papers I-IV)**

In the current studies (papers I-IV), at pre- and post-test, blood samples were collected, measurements were performed and day of menstrual cycle was documented. Moreover the log books were monthly checked. In the observational study (papers III-IV) also monthly blood samples and POMS

test were performed. An illustration of the flowcharts of the studies, including measurements, blood samples, and self-monitoring tools are presented in Figure 4, the intervention study (papers I-II) and in Figure 5, the observational study (papers III-IV).

Pre-test	Monthly four cycles	Post-test
<ul style="list-style-type: none"> <li>• Body weight, height</li> <li>• Menstrual cycle day</li> <li>• Blood samples; sex hormones, growth hormone and cortisol</li> <li>• Vertical Jump tests; squat jump and countermovement jump</li> <li>• Body composition; lean body mass, fat mass and bone mineral density in L2-L4</li> <li>• Isokinetic peak torque: hamstrings and quadriceps in right and left leg</li> </ul>	<ul style="list-style-type: none"> <li>• Personal log book; leg resistance training, ordinary training, menstrual cycle length ev medical problem</li> </ul>	<ul style="list-style-type: none"> <li>• Body weight</li> <li>• Menstrual cycle day</li> <li>• Blood samples; sex hormones, growth hormone and cortisol</li> <li>• Vertical Jump tests; squat jump and countermovement jump</li> <li>• Body composition; lean body mass, fat mass and bone mineral density in L2-L4</li> <li>• Isokinetic peak torque: hamstrings and quadriceps in right and left leg</li> <li>• Experiences of the leg resistance training regime</li> </ul>

**Figure 4.** An illustration of the flowchart of the intervention study, including measurements, blood samples, self-report monitoring tool (log book), during four consecutive, menstrual/OC cycles (papers I-II).

Pre-test	Monthly autumn, winter, spring	Post-test
<ul style="list-style-type: none"> <li>• Body weight, height</li> <li>• Menstrual cycle day</li> <li>• Blood samples; oxytocin, cortisol and CBG</li> <li>• Aerobic capacity test</li> <li>• POMS questionnaire</li> </ul>	<ul style="list-style-type: none"> <li>• Personal logbook; body weight, day of menstrual cycle, all training including aerobic training, rated perceived exhaustion of the training</li> <li>• Blood samples; cortisol, CBG and oxytocin</li> <li>• POMS questionnaire</li> </ul>	<ul style="list-style-type: none"> <li>• Body weight</li> <li>• Menstrual cycle day</li> <li>• Blood samples; oxytocin, cortisol and CBG</li> <li>• Aerobic capacity test</li> <li>• POMS questionnaire</li> </ul>

**Figure 5.** An illustration of the flowchart of the observational study, including measurements, blood samples, and self-monitoring tools (log book, POMS) during the nine month study period (papers III-IV)

Pre-test at day 7 in the first menstrual/OC cycle and start of the 16 week training period												Post-test at day 7 in the last menstrual/OC cycle and the training period ends											
↓												↓											
Day in the menstrual/OC cycle																							
1 7 15 22 1 7 15 22 1 7 15 22 1 7 15 22 1 7 15 22												1 7 15 22 1 7 15 22 1 7 15 22 1 7 15 22											
6 14 21 28 6 14 21 28 6 14 21 28 6 14 21 28 6 14 21 28												6 14 21 28 6 14 21 28 6 14 21 28 6 14 21 28											
Group 1: Weekly training sessions		5	1	1	5	5	1	1	5	5	1	1	5	5	1	1	5						
Group 2: Weekly training sessions		1	5	5	1	1	5	5	1	1	5	5	1	1	5	5	1						
Control group: Weekly training sessions		3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3						

**Figure 6.** The training regimes during four menstrual/OC cycles, day 1- day 28. The periodised leg training, 5 times·w<sup>-1</sup> vs. 1 times·w<sup>-1</sup> in group 1 and group 2, and the regularly performed training, 3 times·w<sup>-1</sup>, in the control group. In total, the training regimes included 48 leg training sessions in each group from pre-test to post-test

### ***Anthropometric measurement (papers I-IV)***

Body weight (kg) and height (cm) were measured with participants using similar training clothes without shoes at pre- and post-test. Weight and height were measured using standardised equipment

### ***Body composition measurement (papers I-II)***

Body composition was measured using a Dual energy X-ray Absorptiometry (DEXA), (Lunar DPX-IQ software version 4.7, Lunar Co, Wi, USA) to determine BMD and to distinguish soft tissue with a high precision in the whole body and parts of the body (Figure 7). The soft tissue was divided into fat-free mass (lean body mass, LBM) and fat mass (Mazess et al. 1990); (Kohrt 1995); (Albanese, Diessel, and Genant 2003); (Duren et al. 2008). This measurement is a valid and reliable method for measurement of bone and soft-tissue composition (Mazess et al. 1990); (Albanese, Diessel, and Genant 2003). The coefficient of variation (CV) for LBM has been reported to be 0.9 % in total body scans in our laboratory at the Sport Medicine Unit, Umeå, (Nordström et al. 1995). Every test day, The Lunar DPX-IQ was calibrated using a standardised phantom. Measurement of LBM (kg) in right and left leg, and in total body are presented in paper I. BMD (g/cm<sup>2</sup>) in the spine (L2-L4) and body fat mass (kg) are presented in paper II.



**Figure 7.** Measurements of body composition using a Dual energy X-ray Absorptiometry (DEXA). Total body acquisition (left) and the spine (right), (papers I-II)

### ***Measurement of power using vertical jump tests (paper I)***

Vertical jump height was measured with standard vertical jump tests, squat jump (ST) and countermovement jump (CMJ) using a contact mat; Bosco mat and a Newtest 300 Powertimer mat (Oulu, Finland) (Figure 8). Each individual used the same equipment at pre- and post-test. These systems measures jump height (in cm) converted from flight time. Three trials, were permitted with the highest jump being used for statistical analysis. The reliability of vertical jump height measurements, ST and CMJ, with a contact mat connected to a digital timer is a reliable and valid test for estimation of explosive power of the lower limbs, Cronbach  $\alpha$ , ST = 0.97 and CMJ = 0.98 (Markovic et al. 2004), with no difference between men and women (Ortega et al. 2008).

### ***Isokinetic muscle strength measurement (paper I)***

Measurements of maximal muscle torque (Nm) of the knee flexors and knee extensors were performed, measured as isokinetic peak torque (PT) with a standard Biodex isokinetic dynamometer, Biodex Co, New York, USA, and Biodex system 3, rev 3.30 02/14/2003, Biodex Co, New York, USA. Each individual used the same equipment at pre- and post-test (Figure 8). After a 5-min warm-up period on a cycle ergometer some test-specific repetitions were performed to familiarize with the dynamometer followed by five maximal consecutive contractions at angular velocity at  $90^{\circ} \cdot s^{-1}$ . The PT of the highest contraction in quadriceps (PTq) and hamstrings (PTh) muscles of the right (r) and left (l) leg respectively was noted and used in the analyses (Drouin et al. 2004) The Biodex isokinetic dynamometer was calibrated according to the manufacturer's manual. The test re-test reliability of the Biodex isokinetic dynamometer has been shown to range from 0.93-0.99 for knee extension/flexion peak torque (Biodex multi-joint system manual). The mechanical reliability and validity in measurements of the Biodex System 3 isokinetic dynamometer, are considered acceptable for research purposes (Drouin et al. 2004).



**Figure 8.** Measurement of power with vertical jump test on a contact mat (left) and measurement of muscle strength using Biodex isokinetic dynamometer (right) (paper I).

### ***Aerobic capacity measurement (papers III-IV)***

In the observational study, aerobic capacity was measured in an incremental test (Figure 9) on an electronically braked bicycle (Monark, 839 E, Sweden) with a metabolic gas measurement system (MetaMax II, CORTEX, Biophysik GmbH, Leipzig, Germany). The work load at the start of this tests was 30 Watts (W) and with an increase in the work load every three minutes by 30 W. The test continued until exhaustion, i.e. when the subject was unable to maintain the cadence of 60 pedal stroke/per minute (rpm). Heart rate was monitored with Polar chest transmitter (Polar Electro, Kempele, Finland) and transmitted to the MetaMax II. During the incremental cycle ergometer test a metabolic gas measurement system (MetaMax II, CORTEX, Biophysik GmbH, Leipzig, Germany) was used to measure the subject's ventilation (VE), oxygen uptake ( $\text{VO}_2$ ) and carbon dioxide output ( $\text{VCO}_2$ ). The subjects breathed through a mask that was placed over the mouth and nose. A small sample of expired air was drawn into a mixing chamber from which  $\text{O}_2\%$  and  $\text{CO}_2\%$  were measured twice every second. The test procedures have been described elsewhere (P. Larsson and Henriksson-Larsén 2005) and the MetaMax II has been found valid and reliable for metabolic gas measurements (P. U. Larsson et al. 2004). The MetaMax II was calibrated every test day for measurements of gas contents and volume (P. Larsson and Henriksson-Larsén 2005). The highest oxygen uptake measured during the incremental cycle ergometer test was determined, the peak oxygen uptake, ( $\text{VO}_{2\text{ peak}}$ , ml/kg x min) close to, or equaling, the maximum oxygen uptake  $\text{VO}_{2\text{ max}}$ .



**Figure 9.** Measurement of the peak oxygen uptake, ( $\text{VO}_2$  peak,  $\text{ml/kg} \times \text{min}$ ) during an incremental cycle ergometer test (papers III-IV).

### ***Blood sample collections (papers II-IV)***

Resting blood samples for analysis of hormone levels (Nilsson-Ehle 2012) were drawn from an antecubital vein into plastic tubes, in a seated position (Figure 10) in the morning at 0730h-0830h about 45-60 minutes after a light breakfast without any heavy physical activity or stress before the sampling. The condition of blood sampling was strictly standardized. The analysis of serum hormone levels were performed with standard laboratory analysis at the clinical chemical laboratory, an accredited clinical laboratory, of the university hospital in Umeå (papers II and IV). The reference ranges for the serum blood samples were given by the laboratory. Analyses of OT (paper III) and CBG levels (paper IV) were not standard analyses at the clinical chemical laboratory, hence samples were stored until all analysis could be performed at the same time at an accredited laboratory, Clinical Microbiology/Clinical Immunology, Umeå University, to assert standardized assay with same analyse kit and the same calibration.



**Figure 10.** Blood sample collection in a seated position (papers II-IV).



**Figure 11.** The OT analyses were performed by a competitive EIA methodology and CBG analyses by ELISA methodology (papers III-IV).



**Figure 12.** The POMS questionnaire was filled out in a calm environment (papers III-IV).

## ***Hormonal assays***

***(papers II-IV)***

### ***Sex hormones, growth hormone and cortisol assays (paper II)***

Blood serum samples were used for determination of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, testosterone, sex hormone-binding globulin (SHBG), cortisol, and growth hormone (GH). To investigate free sex hormones, the ratios were calculated for the sex hormones and their binding protein; estradiol/SHBG, progesterone/SHBG and testosterone/SHBG. The free testosterone/cortisol ratio (FTCR) was calculated and used to investigate the anabolic/catabolic balance. These ratios were used in the statistical analyses.

### ***Oxytocin, cortisol and CBG assays (papers III and IV)***

Blood samples for detection of OT (paper III), and cortisol and CBG (paper IV) were collected once a month in September to May, giving a total of 9 samples for each subject allocated into three seasons, the autumn (September, October, November), the winter (December, January, February) and the spring season (March, April, May). The blood samples were collected for each subject, with maximal one-week difference between calendar days at different days of the menstrual cycle to represent the whole menstrual cycle. Samples



of serum-cortisol were collected into plastic tubes and standard laboratory analyses at the clinical chemical laboratory of the university hospital were performed by Roche Elecsys reagents on Modular E170 analysers. Samples of OT and CBG were collected into chilled EDTA-containing plastic tubes, put on ice directly and immediately centrifuged at 1,600 x g for 15 minutes at 0° C. The plasma was then stored at -80°C until laboratory analyses at the Department of Clinical Microbiology/Clinical Immunology, Umeå University, were performed at same time to assert standardized assay with same analyse kit and same calibration (Figure 11). The CBG levels were analysed by ELISA methodology using a commercial ELISA kit from USCN Life Science Inc and following the manufacturer's instructions. The cortisol-to-CBG ratio was used to estimate the free cortisol level, modified Bonte et al (Bonte et al. 1999). OT was analyzed by a competitive EIA methodology with a commercial Oxytocin Enzyme Immunoassay Kit (Assay Designs, Ann Arbor, MI, U.S.A.) according to the manufacturer's instructions. The lower limits of detection of OT were 11 pg/mL, and samples with values below limits of detection were given the lowest value for detection (samples, n = 2 in women with OC and samples, n = 2 without OC). The highest limits of detection were 1,000 pg/mL. Samples with values over the highest limit were given the highest value for detection (samples, n = 9 in women with OC use and samples, n = 2 without OC use).

### ***Self-report monitoring tools (papers I-IV)***

#### *Personal log book (I-IV)*

All subjects used a personal log book during the study period to document all training, the perception of the training, the menstrual cycle length, and also noted if any health problem occurred. The log books were inspected every month to evaluate that the training was performed as instructed and that no health problems or menstrual irregularities occurred during the study period.

#### *The Profile of Mood State (III-IV)*

At pre and post-test and also once a month during the observational study the Swedish POMS questionnaire (licensed Data Medic AB & Melebo AB, copyright EdITS) was filled out in a separate and calm environment (Figure 12). The POMS test, provided an index of total mood disturbance (Global POMS), measured by six mood states (tension, depression, anger, fatigue, confusion and vigour) on a five-point scale from *not at all* (0) to *extremely* (4) (McNair, Lorr, and Dropplemann 1971). Adding a constant of 100 prevented the occurrence of negative score in Global POMS. (McNair, Lorr, and Dropplemann 1971); (Cramer, Nieman, and Lee 1991); (Raglin, Morgan, and O'Connor 1991); (Berglund and Safstrom 1994). The standard instruction of

the POMS questionnaire was used, i.e. the subjects answered the question, “How have you been feeling during the past week including today?” (McNair, Lorr, and Dropplemann 1971). A rating scale from 2 (very, very low) to 14 (very, very high) was also used to estimate training intensity, to elucidate if a high training intensity was connected to high Global POMS score and to decreased Vigour/Fatigue ratio (V/F ratio).

## **Statistics**

Data (papers I-IV) were analysed with the Statistical Package for the Social Sciences (SPSS v19 and v22). Conventional methods were used to calculate mean (m) and standard deviation (SD) and the level of statistical significance was set at  $p < 0.05$ . Normality of distribution was investigated in subject characteristics and outcome variables at baseline.

In the intervention study (papers I and II) the Independent Samples T-tests were used to compare discontinuers with completers and further OC users with non-users within the three groups at baseline. All variables were found normally distributed except GH, hence LnGH was used in the analysis in paper II. Analysis of variance, One-way ANOVAs, were used to compare the variables in the three training groups at pre-test.

In paper I, Paired Samples T-Test was used to analyse training effects in the outcome variables, in each training group separately. One-way ANOVAs was used to analyse group differences between pre- to post-test in the outcome variables (ST, CMJ, PTqr, PTql, PThr, PThl, LBM legs, LBM total body). Bonferroni corrections were made to reduce multiple testing issues including the post hoc testing following significant ANOVAs. Due to small groups, the Wilcoxon Signed Rank Test (within groups) and the Kruskal-Wallis test (between groups) were used to analyse differences in OC users and non-users respectively. When significant differences were found between the training groups with the Kruskal-Wallis test, the MannWhitney U-test was used.

In paper II, repeated measures analysis of variance was used to evaluate changes in hormone levels and body composition parameters between the three groups over time, with pre- and post- test as a within-subject variable and group as a between-subject variable. Bonferroni corrected post hoc tests were used to reveal group differences. OC was added as covariate in the analysis. Significant main and interaction effects were further qualified with pair-wise comparisons. Chi-square was used to analyse the participant's experience of their training on a dichotomised scale and also used to analyse the participant's FTCD decrease on a dichotomised scale. The Pearson product-moment correlation coefficient was used to investigate the

relationship between the significant improvements in ST, CMJ, PThr, PThl, LBM legs (paper I), sex hormones, LnGH and cortisol (paper II) following high frequency periodised resistance training the first two weeks of the menstrual/OC cycle. Correlation analyses, between changes in performance and changes in these hormones as well as baseline hormonal values, were performed.

In papers III and IV, statistical analyses of OT, cortisol, and CBG levels and Global POMS scores showed normal distribution in OC users and non-users respectively. No extreme outliers were identified that interfered substantially with the analysis. Paired Samples T-Test was used to compare means within groups and Independent Samples Test was used to compare means between groups. To evaluate seasonal variations in the hormones and Global POMS, repeated measures analysis of variance was used, with season as a within-subject variable and group as a between-subject variable. When the sphericity assumption was violated based on Mauchly's test, the Huynh-Feldt adjustment was used. If there was a significant interaction between season and group, the data were split by the group variable and the seasonal variable, respectively. When data were split by group, a repeated measures analysis of variance with season as a within-subject variable and no between-subject variable was used. If it was found significant, contrast analysis were used to establish which season that differed. When data was split by season Independent Samples Test was used to compare groups for each season.

To clarify the relationship between day in the menstrual/OC cycle, and OT level (paper III), and cortisol level (paper IV) respectively, the Linear regression was used, and  $R^2$  was calculated. In paper III, to clarify the relationship between OT levels, Global POMS scores, and hours of daylight respectively, each woman was analysed separately with the non-parametric test, Spearman's rank correlation coefficient (in paper IV between cortisol and hours of daylight). Due to related observations, with each individual represented multiple times during this observational study (paper III and IV), the p-value could not be calculated for the whole study group. Instead analyses were performed for each individual separately and the numbers of significant correlations in OC users and non-users respectively, were summarised.

# Results

## **Menstrual/oral contraceptive cycle based high frequency periodised leg resistance training in physically active women (papers I-II).**

### ***Effects on squat jump, countermovement jump and isokinetic peak torque (paper I)***

In the within group comparisons we found significantly increased jump height (ST and CMJ) and increased PT in the knee flexor in both the right and the left leg (PThr and PThl) in the group with high frequency (5 times·w<sup>-1</sup>) leg resistance training during the first two weeks of the menstrual/OC cycle (group 1). We also found significantly increased jump height (ST and CMJ) and increased PT in the left knee flexor (PThl) in the group with regularly training (3 times·w<sup>-1</sup>) throughout the menstrual/OC cycle (control group). Further, in group 1 we found increased PT in the knee extensor in right leg (PTqr;  $p = 0.009$ ), and in the control group, we found increased PT in the knee flexor in right leg (PThr;  $p = 0.009$ ), but these analyses did not survive Bonferroni correction. No significant increase was found in high frequency (5 times·w<sup>-1</sup>) leg training during the last two weeks of the menstrual/OC cycle (group 2). In the between group comparisons regarding changes in ST, CMJ and PT, significant differences were found in PT of the knee extensor in right leg (PTqr;  $F(2,53) = 10.407$ ,  $p < 0.001$ ), and the knee flexor in right leg (PThr;  $F(2,53) = 5.464$ ,  $p = 0.007$ ). Post hoc analysis revealed significantly higher increase in PTqr in both group 1,  $p < 0.001$  and in the control group,  $p = 0.001$ , compared to group 2. Further, post hoc analysis showed significantly higher increase in PThr in group 1,  $p = 0.005$ , compared to group 2. We also found in the between group comparison a tendency towards a difference in CMJ changes,  $F(2,54) = 3.643$ ,  $p = 0.033$ , and post hoc analyse showed higher increase in jump height in group 1 compared to group 2,  $p = 0.035$ . We did not find any difference in ST changes,  $F(2,54) = 0.888$ ,  $p = 0.417$ , in between group comparisons. Data are presented in Table 3.

In within group comparisons, in OC users and non-users respectively, we found significant increased PThr, in OC users, and no significant increase for non-users, in group 1. No significant increase were found neither in group 2 nor in the control group. In between group comparisons, (OC users and non-users respectively) we found a significant difference in PTqr for non-users between the three groups ( $p = 0.003$ ) and further a higher increase in non-users in PTqr in group 1 ( $p = 0.004$ ) and in PTqr in the control group ( $p = 0.004$ ) compared to group 2. Data are presented in Table 4.

**Table 3.** Outcome measures; ST, CMJ, PT and LBM at pre-test and post-test, in the three training groups, Group 1, group 2, and the control group. Number of training sessions, mean and standard deviation, in each group respectively.

	Group 1 (n = 18)				Group 2 (n = 19)				Control group (n = 20)			
	TS 41 ± 4.0				TS 41 ± 4.8				TS 42 ± 4.4			
Outcome measures	pre-test mean ± SD	post-test mean± SD	p-value t-value		pre-test mean ± SD	post-test mean± SD	p-value t-value		pre-test mean ± SD	post-test mean± SD	p-value t-value	
ST (cm)	27.7 ± 3.71	29.1 ± 3.52	*p < 0.001 t (17) = -4.437		25.6 ± 3.74	26.3 ± 3.02	p = 0.165 t (18) = -1.446		26.4 ± 3.75	27.6 ± 4.05	*p = 0.005 t (19) = -3.199	
CMJ (cm)	29.7 ± 3.44	31.5 ± 4.35	*p = 0.002 t (17) = -3.552		27.8 ± 3.9	28.0 ± 3.57	p = 0.589 t (18) = -0.550		28.4 ± 4.03	29.8 ± 4.15	*p = 0.003 t (19) = -3.361	
PTqr (N·m) # <sup>†</sup>	137 ± 30.3	143 ± 28.7	p = 0.009 t (17) = -2.971		142 ± 22.9	135 ± 21.4	p = 0.017 t (18) = 2.629		144 ± 23.9	148.2 ± 24.5	p = 0.028 t (18) = -2.389	
PTql (N·m)	142 ± 29.7	145 ± 28.5	p = 0.113 t (17) = -1.673		140 ± 22.1	137 ± 23.0	p = 0.343 t (18) = 0.973		146 ± 22.6	151 ± 25.1	p = 0.031 t (18) = -3.344	
PTthr (N·m) #	67.9 ± 13.1	75.8 ± 14.8	*p < 0.001 t (17) = -5.151		70.5 ± 10.0	71.6 ± 8.4	p = 0.445 t (18) = -0.781		76.1 ± 11.3	80.0 ± 11.2	p = 0.009 t (18) = -2.923	
PThl (N·m)	72.4 ± 15.6	77.5 ± 16.5	*p = 0.003 t (17) = -3.470		69.2 ± 9.7	73.7 ± 8.1	p = 0.025 t (18) = -2.449		76.0 ± 12.2	81.4 ± 11.1	*p = 0.001 t (18) = -3.958	
LBM l (kg)	15.1 ± 2.6	15.3 ± 2.7	*p < 0.001 t(18) = -4.765		15.7 ± 2.2	15.6 ± 2.0	p = 0.598 t(18) = 0.536		16.2 ± 1.6	16.3 ± 1.5	p = 0.403 t(20) = -0.855	
LBM t (kg) #	42.1 ± 6.0	42.5 ± 6.1	p = 0.009 t(18) = -2.952		43.5 ± 4.9	43.2 ± 5.0	p = 0.166 t(18) = 1.444		44.1 ± 3.7	44.3 ± 3.7	p = 0.281 t (20) = -1.109	

**Table 4.** - Outcome measures in women *with and without OC respectively*; ST, CMJ, PT and LBM, at pre-test and post-test in the three training groups; group 1, group2 and the control group. Number of training sessions, mean and standard deviation, in each group respectively.

		Group 1, (NOC n = 8, OC n = 11), TS 41 ± 4.0			Group 2, (NOC n = 9, OC n = 10), TS 41 ± 4.8			Control group C, (NOC n = 10, OC n = 11), TS 42 ± 4.4		
Outcome measures		pre-test mean ± SD	post-test mean ± SD	p-value	pre-test mean ± SD	post-test mean ± SD	p-value	pre-test mean ± SD	post-test mean ± SD	p-value
ST (cm) NOC	ST (cm) NOC	25.8 ± 2.59	27.3 ± 1.45	p = 0.025	25.6 ± 4.33	26.2 ± 3.24	p = 0.477	24.9 ± 3.59	25.5 ± 3.54	p = 0.553
	ST (cm) OC	28.6 ± 4.27	30.6 ± 4.07	p = 0.021	25.6 ± 3.35	26.3 ± 2.98	p = 0.092	27.6 ± 3.57	29.4 ± 3.48	p = 0.011
CMJ (cm) NOC	CMJ (cm) NOC	28.3 ± 2.44	29.0 ± 2.23	p = 0.261	27.3 ± 4.15	27.8 ± 3.91	p = 0.312	26.5 ± 3.79	27.9 ± 4.48	p = 0.043
	CMJ (cm) OC	30.9 ± 3.80	33.5 ± 4.68	p = 0.008	28.3 ± 3.88	28.2 ± 3.44	p = 0.959	30.0 ± 3.67	31.3 ± 3.28	p = 0.074
PTqr (N·m) NOC #, †	PTqr (N·m) NOC #, †	132 ± 37.8	135 ± 33.5	p = 0.674	143 ± 18.2	130 ± 15.9	p = 0.028	139 ± 22.1	146 ± 27.0	p = 0.022
	PTqr (N·m) OC	142 ± 24.0	149 ± 24.0	p = 0.028	142 ± 27.5	139 ± 25.4	p = 0.386	149 ± 26.2	151 ± 22.5	p = 0.575
PTql (N·m) NOC	PTql (N·m) NOC	139 ± 39.3	137 ± 36.1	p = 0.401	139 ± 17.9	132 ± 18.2	p = 0.441	140 ± 21.4	147 ± 28.0	p = 0.093
	PTql (N·m) OC	144 ± 21.2	152 ± 20.1	p = 0.009	141 ± 26.2	141 ± 26.9	p = 0.838	153 ± 23.3	156 ± 22.2	p = 0.214
PThr (N·m) NOC	PThr (N·m) NOC	67.0 ± 16.2	74.6 ± 19.1	p = 0.025	69.7 ± 7.13	70.0 ± 7.53	p = 0.767	75.5 ± 11.3	78.6 ± 13.3	p = 0.173
	PThr (N·m) OC	68.6 ± 10.8	76.8 ± 11.3	*p = 0.005	71.1 ± 12.4	73.3 ± 9.23	p = 0.359	76.7 ± 11.8	81.5 ± 8.86	p = 0.021
PThl (N·m) NOC	PThl (N·m) NOC	68.7 ± 21.5	71.3 ± 19.4	p = 0.362	71.9 ± 3.35	74.2 ± 11.2	p = 0.314	75.8 ± 10.8	80.9 ± 12.9	p = 0.025
	PThl (N·m) OC	75.4 ± 8.95	82.5 ± 12.5	p = 0.013	66.7 ± 10.7	73.2 ± 4.13	p = 0.074	76.1 ± 14.3	81.9 ± 9.55	p = 0.051
LBM 1 (kg) NOC	LBM 1 (kg) NOC	45.1 ± 3.83	45.40 ± 4.02	p = 0.012	46.1 ± 2.46	45.8 ± 1.77	p = 0.515	46.0 ± 1.76	46.2 ± 1.59	p = 0.074
	LBM1 (kg) OC	45.0 ± 1.35	45.3 ± 1.26	p = 0.013	45.3 ± 2.04	45.4 ± 2.35	p = 0.508	46.4 ± 1.57	46.4 ± 1.53	p = 0.534
LBM t (kg) NOC	LBM t (kg) NOC	42.0 ± 8.83	42.5 ± 9.00	p = 0.263	44.7 ± 4.85	44.2 ± 4.65	p = 0.374	43.6 ± 3.51	43.7 ± 3.65	p = 0.074
	LBM t (kg) OC	42.2 ± 3.33	42.5 ± 3.23	p = 0.058	42.5 ± 5.03	42.3 ± 5.41	p = 0.445	45.0 ± 3.82	44.9 ± 3.82	p = 0.657

*Table 3. Notes:* static jump (ST), countermovement jump (CMJ), peak torque quadriceps right leg (PTqr), peak torque quadriceps left leg (PTql), peak torque hamstrings right leg (PThr), peak torque hamstrings left leg (PThl), lean mass legs (LBM l), lean total body mass (LBM t), women with oral contraceptive (OC) use and women without oral contraceptive (NOC) use, number of training sessions (TS), high frequency training in the follicular phase (group 1), high frequency training in the luteal phase (group 2) and regularly training in both phases as a control group. \* = these tests within groups survived Bonferroni corrections, # = significantly higher increase in PTqr ( $p < 0.001$ ), PThr ( $p = 0.005$ ) and LBM t in group 1 compared to group 2 (post hoc Bonferroni corrected), † = significantly higher increase in PTqr ( $p = 0.001$ ) in the control group compared to group 2 (post hoc Bonferroni corrected).

*Table 4. Notes:* static jump (ST), countermovement jump (CMJ), peak torque quadriceps right leg (PTqr), peak torque quadriceps left leg (PTql), peak torque hamstrings right leg (PThr), peak torque hamstrings left leg (PThl), lean mass legs (LBM l), lean total body mass (LBM t), women with oral contraceptive (OC) use and women without oral contraceptive (NOC) use, number of training sessions (TS), high frequency training in the follicular phase (group 1), high frequency training in the luteal phase (group 2) and regularly training in both phases as a control group. \* = these tests within groups survived Bonferroni corrections. NOC: # = significant higher increase in PTqr ( $p = 0.004$ ) in group 1 compared to group 2. NOC † = significantly higher increase in PTqr ( $p = 0.004$ ) in the control group compared to group 2.

### ***Effects on Lean Body Mass (paper I)***

In the within group comparisons we found a significant increase in mean values of LBM in legs ( $p < 0.001$ ) in group 1, but not in group 2 or in the control group. In the between group comparisons we found a tendency towards a difference in total body LBM ( $p = 0.022$ ), and a higher increase (post hoc,  $p = 0.023$ ) in group 1 compared with group 2 (Table 3). No significant changes within or between groups were found when analyzing LBM (legs and total body) in OC users and non-users separately. (Table 4).

### ***Effects on sex hormone, growth hormone and cortisol levels (paper II)***

No significant group interactions in hormone values and ratios were found; cortisol,  $p = 0.225$ , LnGH,  $p = 0.239$ , estradiol/SHBG,  $p = 0.400$ , progesterone/SHBG,  $p = 0.272$ , testosterone/SHBG,  $p = 0.510$ , FTCR,  $p = 0.978$ , FSH,  $p = 0.834$ , and LH,  $p = 0.069$ . Controlling for OC did not alter the results. No significant differences were found between mean values of the outcome measures at pre- and post-test in; FSH, LH, cortisol, LnGH and the ratios of estradiol/SHBG, progesterone/SHBG, testosterone/SHBG and FTCR, within the groups (Table 5). We did not find any of the non OC users who lowered their level of FSH, LH, estradiol or progesteron below the lowest

reference value for each test after training. We found four non OC user, who lowered their testosterone level below the lowest reference value after training. We did not find a significant increase in the number of subjects with high cortisol levels, neither in OC users or non-users. We did not find any FTCD decrease of 30% or more in OC users and non-users, and no significant differences between the groups,  $p = 0.131$ . No participant with FTCD below the reference value of 0.35 (10-3) (Banfi and Dolci 2006) at pre- or post-test were found.

There were no significant correlations between performance improvements (ST, CMJ, PThr, PThl and LBM legs) and change in hormonal levels nor baseline values observed (all  $p$ 's  $> 0.05$ ) following high frequency periodised resistance training during the first two weeks of the menstrual/OC cycle.

### ***Effects on total body fat mass and bone mineral density in the spine (paper II)***

In the between group analysis we did not find any significant interaction between the groups over time in fat mass,  $p = 0.378$  and BMD L2-L4,  $p = 0.999$ . Controlling for OC did not alter the results. No significant differences in mean values between pre- and post-test in body fat mass and BMD L2-L4 within the groups were found (Table 5).

### ***Experiences of the leg resistance training programs (paper II)***

We found a significant difference in experience of the training,  $\chi^2(2) = 11.552$ ,  $p = 0.003$ , and more women experienced the leg training program as positive compared to negative in group 1,  $\chi^2(1) = 11.842$ ,  $p = 0.001$ , but not in group 2 or in the control group.



**Table 5.** Hormone levels, hormone ratios and body composition (mean  $\pm$  SD ) at pre- and post-test, in the three training groups

Hormones	Group 1 (n = 18)				Group 2 (n = 19)				Control group (n = 21)			
	pre-test mean $\pm$ SD	post-test mean $\pm$ SD	Statistics <sup>a</sup>	pre-test mean $\pm$ SD	post-test mean $\pm$ SD	Statistics <sup>a</sup>	pre-test mean $\pm$ SD	post-test mean $\pm$ SD	Statistics <sup>a</sup>	pre-test mean $\pm$ SD	post-test mean $\pm$ SD	Statistics <sup>a</sup>
FSH (IU/L)	5.42 $\pm$ 2.11	4.89 $\pm$ 1.93	t (17) = 1.340, p = 0.198	5.91 $\pm$ 2.17	5.69 $\pm$ 1.86	p = 0.436 t (18) = 0.796	5.78 $\pm$ 1.53	5.44 $\pm$ 1.86	p = 0.401 t (20) = 0.859			
LH ( $\mu$ U/L)	5.69 $\pm$ 3.76	4.78 $\pm$ 2.33	p = 0.073 t (17) = 1.912	4.72 $\pm$ 1.75	4.95 $\pm$ 2.35	p = 0.594 t (18) = -0.543	6.77 $\pm$ 4.00	4.94 $\pm$ 3.50	p = 0.037 t (20) = 2.229			
Estradiol/SHBG	1.88 $\pm$ 1.55	1.77 $\pm$ 1.22	p = 0.750 t (17) = 0.324	1.82 $\pm$ 1.65	1.97 $\pm$ 1.44	p = 0.605 t (18) = -0.526	3.08 $\pm$ 5.32	1.91 $\pm$ 1.52	p = 0.307 t (20) = 1.047			
Progesterone/SHBG	0.05 $\pm$ 0.03	0.03 $\pm$ 0.01	p = 0.098 t (17) = 1.752	0.04 $\pm$ 0.03	0.05 $\pm$ 0.04	p = 0.380 t (18) = -0.899	0.05 $\pm$ 0.05	0.04 $\pm$ 0.03	p = 0.476 t (20) = 0.726			
Testosterone/SHBG	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	p = 0.095 t (15) = 1.779	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	p = 0.805 t (18) = 0.250	0.12 $\pm$ 0.01	0.01 $\pm$ 0.01	p = 0.377 t (20) = 0.904			
Cortisol (nmol/L)	688 $\pm$ 223	648 $\pm$ 239	p = 0.330 t (17) = 1.004	639 $\pm$ 190	696 $\pm$ 203	p = 0.263 t (17) = -1.157	605 $\pm$ 189	628 $\pm$ 218	p = 0.417 t (20) = -0.828			
FTCR	0.02 $\pm$ 0.02	0.02 $\pm$ 0.01	p = 0.209 t (15) = 1.314	0.02 $\pm$ 0.02	0.02 $\pm$ 0.02	p = 0.594 t (17) = 0.543	0.02 $\pm$ 0.02	0.02 $\pm$ 0.02	p = 0.588 t ( 20) = 0.551			
LnGH	0.19 $\pm$ 1.51	0.43 $\pm$ 1.70	p = 0.634 t (17) = -0.485	0.14 $\pm$ 1.56	0.54 $\pm$ 1.65	p = 0.376 t(18) = -0.908	0.58 $\pm$ 1.77	0.06 $\pm$ 1.33	p = 0.110 t (20) = 1.675			
BMD L2-L4 (g/cm <sup>2</sup> )	1.26 $\pm$ 0.11	1.27 $\pm$ 0.11	p = 0.383 t(18) = -0.895	1.32 $\pm$ 0.15	1.32 $\pm$ 0.15	p = 0.407 t(18) = -0.849	1.26 $\pm$ 0.14	1.26 $\pm$ 0.14	p = 0.263 t (20) = -1.152			
Fat mass (kg)	16.4 $\pm$ 4.6	16.3 $\pm$ 4.4	p = 0.865 t(18) = 0.173	20.2 $\pm$ 5.3	19.2 $\pm$ 5.3	p = 0.157 t(18) 1.478	18.8 $\pm$ 6.6	18.6 $\pm$ 6.3	p = 0.609 t(20) = 0.519			

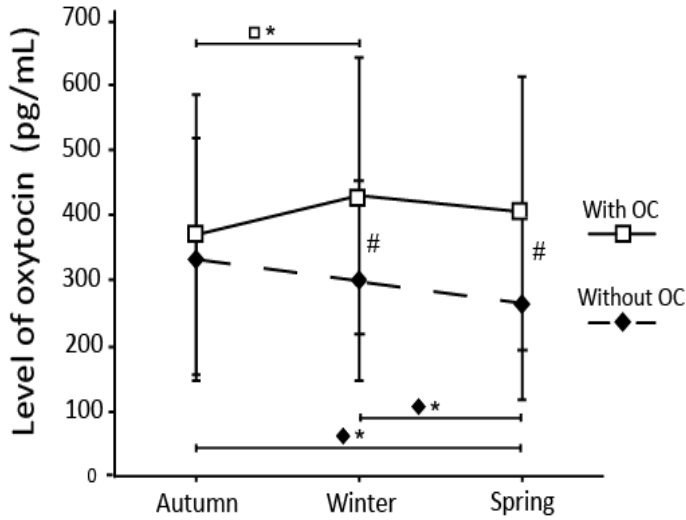
*Table 5. Notes:* FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone-binding globulin; FTCR, free testosterone/cortisol ratio; LnGH, Ln growth hormone; BMD L2-L4, bone mineral density in lumbar spine; TS, Training sessions. Group 1 indicates high frequency training during the first two week; group 2, high frequency training during the last two weeks of the menstrual/OC cycle; control group, regularly training; TS, training sessions. Interaction effects are shown in the text and the significant main and interaction effects are qualified with this pair-wise comparisons. No significant differences within or between groups survived the Bonferroni correction.

## **Impact of season and oral contraceptive use on oxytocin and cortisol levels in physically active women (papers III-IV)**

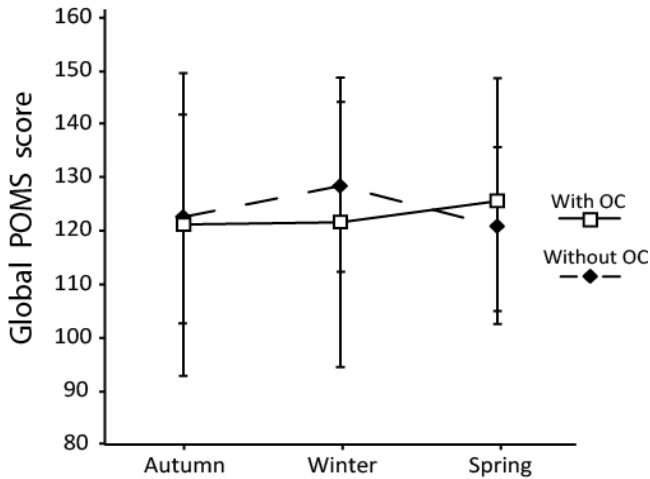
### ***Impact of season and oral contraceptive use on oxytocin levels, and on Global POMS score (paper III)***

We found a significant interaction in OT levels between season and group ( $p = 0.001$ ), and further a significant seasonal variation in OT levels for each group separately (OC users,  $p = 0.027$ ; non-users,  $p = 0.003$ ). A significant difference in OC users between autumn and winter ( $p = 0.022$ ) and in non-users between autumn and spring ( $p = 0.003$ ) as well as between winter and spring ( $p = 0.032$ ) were found (Figure 13). We found significantly higher mean levels of OT in OC users both during the winter ( $p = 0.048$ ) and during the spring ( $p = 0.026$ ), but not during the autumn (Figure 13). OC users peaked in the winter season ( $430 \text{ pg/mL} \pm 212$ ), and the lowest mean value was detected during the autumn ( $370 \text{ pg/mL} \pm 215$ ). Non-users peaked during the autumn ( $332 \pm 186 \text{ pg/mL}$ ), and the lowest mean value was detected in the spring season ( $263 \text{ pg/mL} \pm 138$ ), also reflected by ratios  $> 1$  of mean OT levels in OC users/mean OT levels in non-users (Table 6).

We found no significant seasonal variation in Global POMS scores within OC users and non-users respectively, and no significant differences in Global POMS scores between the two groups (Figure 14).



**Figure 13.** Average concentration of oxytocin levels in women with and without oral contraceptive (OC) use. In autumn, winter, and spring (mean  $\pm$  1SD). Statistically significant seasonal variations are shown in women with OC ( $\square^*$   $p < 0.05$ ) and woman without OC use ( $\diamond^*$   $p < 0.05$ ). Significant differences in oxytocin levels between the groups are shown ( $\#$   $p < 0.05$ ).



**Figure 14.** Average Global POMS score in women with and without oral contraceptive (OC) use. In autumn, winter, and spring (mean  $\pm$  1SD). No significant seasonal variation was found in Global POMS score within women with OC use ( $\square$ ) and without OC use ( $\diamond$ ) and no significant differences in levels between the groups were detected.

**Table 6.** Levels of oxytocin, seasonal daylight hours and ratios of oxytocin levels in oral contraceptive users and non-users.

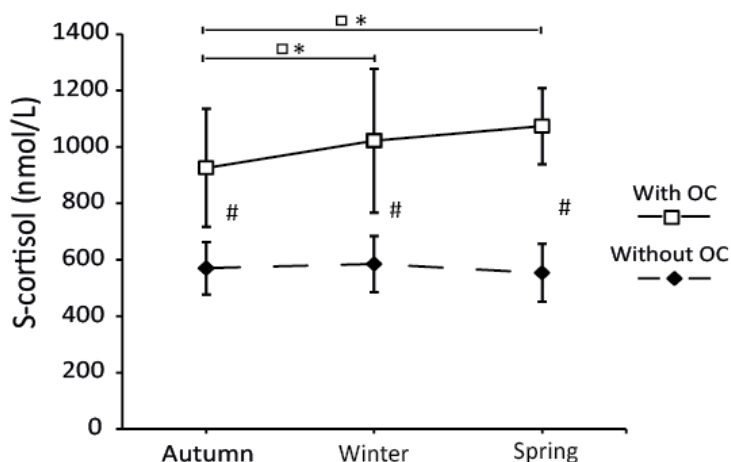
Mean Seasonal Daylight Hours	6 (Winter)	10 (Autumn)	15 (Spring)
Study groups	Oxytocin Level (pg/ml)		
Women with OC use	430 ± 212	370 ± 215	405 ± 207
Women without OC use	301 ± 149	332 ± 186	263 ± 138
Mean OT Level with OC use/without OC use	1.4	1.1	1.5

*Notes; Oxytocin (OT), oral contraceptive (OC)*

Relationships were investigated for each woman separately (papers III-IV). We found no tendency for the Global POMS scores to either increase or decrease with changes in OT levels during the study period. We found a few significant relationships between OT levels, Global POMS scores, the six mood states, and estimated training intensity, respectively (in OC users and non-users). We found no clear relationship between OT levels, and hours of daylight, and Global POMS respectively. No clear relationship between day of menstrual/OC cycle and OT level.

### ***Impact of season and oral contraceptive use on cortisol levels, and Global POMS (paper IV)***

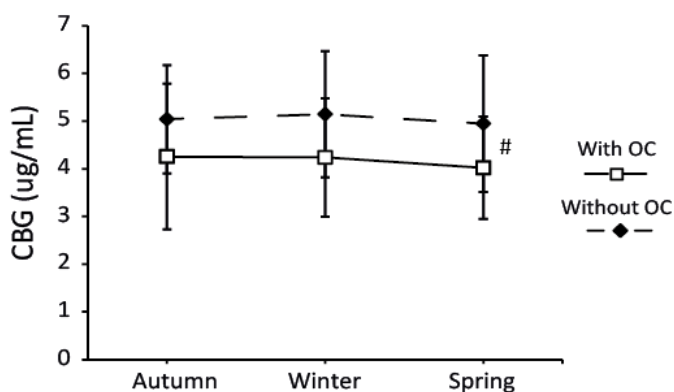
We found a seasonal variation in cortisol levels in the whole study group ( $p = 0.001$ ) and moreover significant seasonal variations in cortisol level in OC users ( $p = 0.008$ ), found between the autumn and the winter seasons ( $p = 0.001$ ) and between the autumn and the spring seasons ( $p = 0.005$ ). We found no significant seasonal variation in cortisol levels in non-users (Figure 15). We found significantly higher mean levels of cortisol in OC users vs non OC users both in the autumn ( $p < 0.001$ ), the winter ( $p < 0.001$ ) and the spring seasons ( $p < 0.001$ ) (Figure 15), (Table 7). We found no clear relationship between cortisol levels, and hours of daylight and Global POMS respectively. No clear relationship between day in the menstrual/OC cycle and cortisol level.



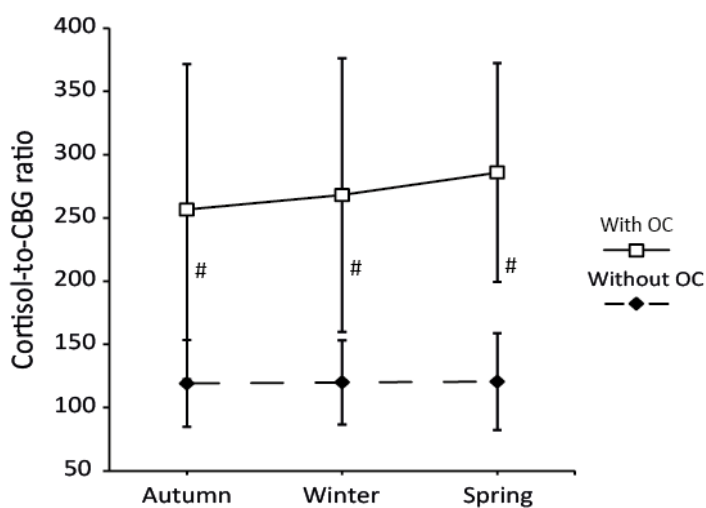
**Figure 15.** Average concentration of cortisol levels in women with and without oral contraceptive (OC) use in the autumn, winter and spring seasons (mean  $\pm$  1SD). Statistically significant seasonal variations are shown in women with OC use ( $\square^*$   $p < 0.05$ ). Statistically significant differences in cortisol levels between the groups are shown ( $\#$   $p < 0.05$ ).

### ***Impact of season and oral contraceptive use on CBG levels and cortisol/CBG ratios, and Global POMS (paper IV)***

We did not find any significant seasonal variation in CBG levels in the whole study group, and we found that CBG levels in non-users were significantly higher in the spring season than OC users ( $p = 0.048$ ) (Figure 16). We did not find any significant seasonal variation in cortisol-to-CBG ratio (Figure 17). The cortisol-to-CBG ratios were significantly higher in OC users in the autumn ( $p = 0.001$ ), the winter ( $p < 0.001$ ) and the spring seasons ( $p < 0.001$ ) compared to non OC users (Figure 17), (Table 7). We found no clear relationship between cortisol-to CBG ratio, and hours of daylight and Global POMS respectively.



**Figure 16.** Average concentration of corticosteroid-binding-globulin (CBG) levels ( $\mu\text{g/mL}$ ) in women with and without oral contraceptive (OC) use in the autumn, winter and spring seasons (mean  $\pm$  1SD). No statistically significant seasonal variations are found in CBG within women with and without OC use respectively. Statistically significant differences in levels between the groups are shown (#  $p < 0.05$ )



**Figure 17.** Average concentration of cortisol-to-corticosteroid-binding globulin ratio (cortisol-to CBG ratio) in women with and without oral contraceptive (OC) use in the autumn, the winter and the spring seasons (mean  $\pm$  1SD). No statistically significant seasonal variations are shown in women with and without OC use respectively. Statistically significant differences in levels between the groups are shown (#  $p < 0.001$ ).

**Table 7** Mean levels and SD of cortisol (nmol/L), cortisol-to-CBG ratios and mean hours of daylight hours in women with and without OC use.

Mean seasonal daylight hours	6 (Winter)	10 (Autumn)	15 (Spring)
Women without OC use, n=18			
cortisol level	585 ± 99	570 ± 93	554 ± 102
cortisol-to-CBG ratio	120 ± 33	119 ± 34	121 ± 38
Women with OC use, n=15			
cortisol level	1022 ± 254	926 ± 210	1074 ± 135
cortisol-to-CBG ratio	268 ± 108	257 ± 125	286 ± 86

*Notes: Corticosteroid-binding globulin (CBG), oral contraceptive (OC)*

# Discussion

An important issue in female sport performance is to develop innovative strategies to improve training effects without exercise-related negative health consequences. (C. M. Lebrun and Rumball 2001); (M. P. Warren and Perlroth 2001); (Michelle P. Warren and Goodman 2003); (Maïmoun, Georgopoulos, and Sultan 2014). It is well known that an improved muscle strength are of great importance in many sports and to achieve an optimal athletic performance, hard training is necessary. Periodisation and avoidance of overtraining are critical to an athlete's success in performance. In females, due to fluctuations of steroid hormones during the menstrual/OC cycle, the training could to be timed in accordance with the cycles to maximise anabolic effects (C. Lebrun 1994); (Janse de Jonge 2003); (Constantini, Dubnov, and Lebrun 2005); (Oosthuyse and Bosch 2010). It is known that training too often and without recovery can be a serious problem for the athletes, with decreased performance and exercise-related negative consequences (Lawrie et al. 2011). The central topics investigated in this thesis were; the effects of menstrual/OC cycle based resistance training on strength and power in physically active women (paper I), potential exercise-related negative consequences on components in the female athlete triad, due to that training (paper II), normative data on OT (paper III) and cortisol (paper IV) to elucidate if these hormones could be used as biological diagnostic markers in combination with others to monitor and diagnose female athletes that may be at risk to develop OR and OTS. In these longitudinal studies (papers I-IV) physically active women with and without OC use were included in all groups.

## **Menstrual/oral contraceptive cycle based high frequency periodised leg resistance training (papers I-II)**

Based on the results from the intervention study it appears as if the known different levels of steroid hormones in menstrual/OC cycles can be utilised in order to maximise the effects of resistance training (paper I). Our results indicates that high frequency periodised leg resistance training performed during the first two weeks of the menstrual/OC cycle is more beneficial to gain power, strength and to increase LBM, than high frequency periodised leg resistance training performed the last two weeks. A control group of women with regularly performed leg resistance training during the menstrual/OC cycle achieved a slight increase in strength and power. However, high frequency periodised training during the first two weeks of the menstrual cycle also resulted in a larger gain of lean body mass compared to the regular training. When we compared OC users to non-users, in the three training groups respectively, we could not detect any clear differences in strength gain,



improved jump height, and increased LBM (paper I). Earlier studies have failed to detect significant differences in muscle strength during OC phases (Wirth and Lohman 1982); (Sarwar, Niclos, and Rutherford 1996); (Peters and Burrows 2006), suggesting that the effects of OC use on muscle strength and performance are minimal. Ekenros et al. (2012) also found no significant influence of OC use on muscle strength and hop performance in moderately active women. These findings suggest that OC user could have the same possibility of strength training effects as women without OC. Moreover we found that the high frequency periodised leg resistance training was well tolerated, hence no exercise-related negative consequences on components in the female athlete triad (sex hormones, growth hormone, cortisol, total body fat mass, and bone mineral density in the spine) were detected, irrespectively if the periodised leg resistance training was performed in the first two weeks or in the last two weeks of the menstrual/OC cycle. Further, if the high frequency periodised leg resistance training was performed during the first two weeks the women had a more positive experience of the training, which not was evident in the other two groups (paper II). These results (papers I-II) indicates that high frequency periodised leg resistance training during the first two weeks of the menstrual/OC cycle is favourable for female athletes.

Our findings, that strength training during the first two weeks of the menstrual cycle, the follicular phase, is beneficial, due to the potentially anabolic effect of oestrogen (Reis, Frick, and Schmidtbleicher 1995), are supported by Reis et al (1995) and Sung et al (2014). Their studies demonstrated that follicular phase-based strength training induced a greater effect on muscle strength, compared to a luteal phase-based strength training. In contrast to these studies by Reis et al. (1995) and Sung et al (2014), we analysed the effects of a longer-lasting training period, four menstrual cycles compared to two/three menstrual cycles. Furthermore, we clearly varied the strength training periodisation between the first two weeks, follicular phase, and last two weeks, luteal phase, and we used a control group with regularly training during the whole cycles. Reis et al. (1995) focused on a periodisation between a follicular phase-based training versus a “regular training” every third day throughout the whole menstrual cycle and in the study by Sung et al. (2014) one leg was mainly trained in the follicular phase and the other leg was mainly trained in the luteal phase. Compared to these two studies we included both OC users and non-users in our study.

These findings, that strength training during the first two weeks of the menstrual/OC cycle is beneficial is further supported by results of studies on postmenopausal women and ovariectomized rodents indicating that oestrogen replacement is favourable for muscle strength (Lowe, Baltgalvis, and Greising 2010). A second potential explanation may be related to muscle

damage and recovery. Markofski and Braun (2014) found less muscle damage, and better strength recovery when women performed one training session in the follicular phase compared to the luteal phase, providing a further possible explanation to the results in our study. In addition, oestrogen may also influence post-damage repair processes through activation and proliferation of satellite cells (Enns and Tiidus 2010). Furthermore, it has recently been postulated that the beneficial effect of oestrogen on muscle strength is accomplished by improving the intrinsic quality of the skeletal muscle, whereby fibers are enabled to generate force, i.e., myosin strongly binds to actin during contraction (Lowe, Baltgalvis, and Greising 2010). The discovery of three types of oestrogen receptors (ERs) has led to the findings that oestrogen may regulate a number of downstream genes and molecular targets (Enns and Tiidus 2010); (Lowe, Baltgalvis, and Greising 2010). Since surge of oestrogen is the highest around the ovulation without the antagonistic effects of progesterone (Bunt 1990); strength training might be optimised in the late follicular phase and around the ovulation. The responses may be dependent on the concentrations of the respective ovarian hormones and the metabolic demand. Though the menstrual phase-associated changes to the various metabolic measurements are not today consistently identified and the potential mechanism underlying oestrogenic action remain subtle (Oosthuyse and Bosch 2010).

Only very few data exist on the physiological effects of progesterone on the female skeletal muscle cell. Recent studies have consistently found amino acid oxidation and protein degradation to be greater in the luteal phase compared with the follicular phase at rest and during exercise. It appears that progesterone is responsible for increased protein catabolism in the luteal phase, while oestrogen may reduce protein catabolism (Oosthuyse and Bosch 2010).

In a review of physical exercise-induced changes in the concentration of circulating androgens in women, the authors concluded that studies regarding the effect of resistance exercise on circulating androgens in women are still contradictory (Carina Enea et al. 2011). Apart from the effects of androgens, the more pronounced increase in muscle strength and muscle diameter in follicular phase compared to luteal phase may be explained by the alterations of ovarian hormones throughout the menstrual cycle (Oosthuyse and Bosch 2010); (Carina Enea et al. 2011). Though, the role of oestrogen in mediating the acute and chronic effects of training is unclear and warrants further investigations (William J Kraemer and Ratamess 2005). The effects of oestrogens on the human muscle have mainly been investigated in peri- and postmenopausal women. Results have shown that the striking decline in muscle strength occurring during the perimenopausal and postmenopausal

period can be reversed by hormone replacement therapy (HRT) (Pöllänen et al. 2007). In post-menopausal women, muscle performance, muscle mass, and muscle composition are improved by HRT. Though, the beneficial effects of HRT combined with high impact physical training may exceed those of HRT alone (Sipilä et al. 2001). A possible link between menstrual cycle based training and induced increases in muscle mass, shown in our study, may be the fluctuation of steroid hormones throughout the cycle and their possible effects on protein synthesis, which is supported by Sung et al. (2014).

We could not detect any gain in strength, power or lean body mass in women who performed high frequency leg resistance training during the last two weeks of the menstrual/OC cycle. It is plausible that these women could have attained more muscle damage and needed more time for recovery than women who trained the first two weeks of the cycles. This is supported by Markofski and Braun (2014) who found less muscle damage, and better strength recovery when the training session was performed in the follicular phase compared to the luteal phase. Women who trained in the luteal phase recovered strength significantly slower and had a larger increase in creatine kinase (a marker of muscle damage), indicating that the women experienced less muscle damage and recovered quicker during the follicular phase. The results from our study showed that potentially the last two weeks in the cycles could instead be used for other kinds of training or for recovery from the performed high frequency resistance training.

However, regular training 3 times per week (the control group) also showed a significant increase in jump height and PT of the knee flexors in the left leg (PThl). Though no significant increase in LBM (total body and legs) was observed. Potentially the observed gains in this group were due to the amount of training performed during the first two weeks of the cycles. Six training sessions during the first two weeks of the menstrual/OC cycle during the regularly training compared to 10 training sessions the same period during the high frequency periodised training period. This reflects that low frequency resistance training regularly in the menstrual/OC cycles may be beneficial to achieve a slight increase in strength and power but not to increase muscle mass. A possible explanation could also be that less muscle damage occurred, and better strength recovery was achieved compared to women training periodised the last two weeks of the cycles. Based on the results in the intervention study (paper I) it seems that it is not the high frequency training that is most important to gain strength and power but rather to perform the high frequency training during the first two weeks of the menstrual cycle, which also is supported in earlier studies (Reis, Frick, and Schmidtbleicher 1995); (Sung et al. 2014).

When we compared OC users to non-users in the three training groups respectively we could not detect any clear differences in strength gains, improved jump heights, and increased LBM between OC users and non-users. This is in line with earlier studies showing no support for any significant influence of OC use on muscle strength and hop performance (Lawrie et al. 2011); (Ekenros et al. 2012). We therefore conclude that trained female OC users with combined OC, mimicking the menstrual cycle, could perform their periodised strength training in the same manner as the non OC users.

It is well known that intense endurance training in women may effect the female reproductive system which is sensitive to physiological training stress. When increasing the training load in endurance training, negative exercise-related consequences are common (Lawrie et al. 2011) . This may result in menstrual irregularities; oligomenorrhea, hypo-oestrogenic amenorrhea, or luteal suppression, especially when accompanied with an inadequate caloric intake (William J Kraemer and Ratamess 2005); (Diaz, Laufer, and Breech 2006); (Kell 2011); (Lawrie et al. 2011); (Ekenros et al. 2012). Female athletes, who's bodies are under physical training stress and not receiving enough calories to compensate for the exercise load, with an energy drain, may lead to low fat mass and menstrual dysfunction (M P Warren and Shantha 2000). Multiple factors should be considered in the prevention of menstrual disorders, including duration and intensity of training, prevention of negative caloric balance that could lead to a negative weight loss, possibly leading to a low oestrogen status and further to low BMD, which are components in the female athlete triad (Matzkin, Curry, and Whitlock 2015). Hence we investigated if the high frequency periodised leg resistance training was well tolerated and without such negative exercise-related consequences (paper II). We observed no evidence that the high frequency periodised menstrual/OC cycle based resistance training resulted in exercise-related negative consequences which could contribute to a suppression of LH, FSH, and further to decrease of the estradiol production (Keizer and Rogol 1990). The fat mass was unchanged and no decrease in BMD in the spine was detected in the two training groups with high frequency training or in the control group.

The periodised high frequency leg resistance training, did not negatively influence the sex hormone levels in women without OC. Hence it is possible for eumenorrheic women to periodise the training in accordance with the menstrual cycle without any negative consequences related to the female athlete triad.

## **Impact of season and oral contraceptive use on oxytocin and cortisol levels in physically active women (papers III-IV)**

If an imbalance occur between physiological and psychological demands on athletes and their ability to meet those demands, it could lead to OR/OTS. Hence, monitoring instruments, for example hormones associated to stress and/or well-being (Leslie et al. 2002); (Maïmoun, Georgopoulos, and Sultan 2014) and POMS, are important markers to assess the risk of OR and OTS (R W Fry, Morton, and Keast 1991); (Raglin, Morgan, and O`Connor 1991); (Kentta and Hassmen 1998); (Kellmann 2010). In our research (papers III-IV) the levels of OT and cortisol were measured to provide normative data and in order to evaluate if these hormones could be used as additional diagnostic markers in physically active women with and without OC use. Since a seasonal periodicity in various endocrine functions could occur (Nathorst-Böös, Stock, and von Schultz 1994); (Hansen et al. 2001) the cortisol and OT levels were repeatedly measured during the autumn, winter and spring seasons in order to evaluate their possible seasonality. These hormones related to stress and wellbeing can also be modulated by ovarian steroids (Forsling 2000) therefore the impact of both menstrual cycles and OC cycles were considered when investigating normative data of these hormones.

Based on the results from our study, an impact of season and OC use on OT levels (paper III) and cortisol levels (paper IV) indicated that these hormones are not optimal biological diagnostic markers to detect OR and OTS in physically active women. A disturbed mood profile, evaluated through psychological questionnaires, are suggested to be a reliable marker for OR/OTS (Ackel-D'Elia et al. 2010); (Meeusen et al. 2013). In paper III and IV, POMS was used (Kenttä, Hassmén, and Raglin 2001). Our results indicated that the seasonal Global POMS scores neither resembled the seasonal pattern of cortisol, nor corresponded to the increased levels of cortisol in OC users, and further no high rated training load was observed, we could not suggest that the high cortisol levels were related to OR and OTS in this study. Moreover, cortisol levels, both total and free cortisol, were found to be higher in OC users vs. non-users irrespective of season. These findings makes it important to consider OC use in studies of cortisol, regardless of cortisol is measured as total or free cortisol. Our results of markedly increased cortisol levels in OC users have also been demonstrated in other non-sporting female cohorts when women received oestrogen or were pregnant; (Brien 1980); (Brien 1988); (Vervoorn et al. 1992) (Aden, Jung-Hoffmann, and Kuhl 1998) or used OC (Wiegratz and Kuhl 1995); (Crook and Group 1997); (Aden, Jung-Hoffmann, and Kuhl 1998).

We also detected significant seasonal variations in OT levels within OC users and non-users, respectively, and significant differences in OT levels between the OC users and non-users at diverse seasons.

Further, no clear relationship between OT levels, cortisol levels, Global POMS scores, and hours of daylight respectively could be detected. Thus, the seasonality in OT and cortisol levels, are probably not influenced by the number of daylight hours at the investigated location. Even though the geographic location was suitable for studies of seasonal variations in hormones.

Due to the results from the observational study confident normative data on OT and cortisol levels in physically active women could not be provided. These findings (papers III-IV) implies, when designing studies, analysing and interpreting results of cortisol and OT levels, that both time of year for measurements and use of OC must be considered.

## **Perspectives and relevance in sports**

The relevance in sports of this thesis is focused on providing new knowledge to optimise training for women, a field with few studies, due to that research in sports are often performed on men (Costello, Bieuzen, and Bleakley 2014); (Bruinvels et al. 2016); (Sheel 2016). We found that to optimise resistance training in female athletes to gain power, strength and lean body mass, a high frequency resistance training should preferably be periodised to the first two weeks of the menstrual/OC cycle. Notably, this training could be performed without any negative health consequences on components in the female athlete triad and also be experienced as positive. The high frequency resistance training should not be concentrated to the last two weeks of the menstrual/OC cycle. To determine the optimal amount of training to optimise performance of individual athletes would be important, though it is difficult. Optimal training for one athlete may result in overtraining or insufficient training to others, though both may result in performance plateaus (C. M. Lebrun and Rumball 2001); (M. P. Warren and Perlroth 2001); (Michelle P. Warren and Goodman 2003); (Maïmoun, Georgopoulos, and Sultan 2014). Hence, reliable diagnostic markers are warranted (Meeusen et al. 2013). Physicians, coaches and trainers, who diagnose and monitor female athletes that may be at risk, should be aware of the results from this thesis that the hormones OT and cortisol are unreliable diagnostic markers in female athletes, due to seasonal variation and impact of OC use on OT and cortisol levels.

## Methodological aspects

Some limitations of this thesis (papers I-IV), should be addressed, see Material and methods section. There were some difficulties in the recruitment of subjects to these two studies due to the inclusion criteria. There were also some difficulties to retain the subjects throughout the long periods of time in these longitudinal studies. The subjects were followed during 4 months (papers I-II) and 9 months (papers III-IV) respectively, and the young study population sometimes changed their living conditions and contraceptive habits, which resulted in difficulties with high attendance. Both the long study period and the young study population most likely influenced the dropout rate and the total number of subjects was therefore limited. Still, these studies (papers I-IV) comprised a well-defined material of physically active women with regular menstrual cycles of 28 days  $\pm$  2 days (accepted 21-35 days), and OC cycles of 28 days. It should be noted that when dividing the subjects into subgroups, OC users and non-users, the number in each group was rather small, which may have influenced the power to detect differences between the groups for some of the variables investigated. Therefore comparing OC users to non-users needs to be confirmed in larger studies. Though, the strength of these present studies was the prospective longitudinal designs in which the subjects were followed during long periods. In the intervention study, the subjects were followed during four consecutive cycles, to investigate effects of menstrual/OC cycle based periodised resistance training and in the observational study the subjects were followed during the autumn, winter and spring seasons, to investigate impact of season and OC use on OT and cortisol levels.

The regularly menstruating women in these studies (paper I-IV) were not allowed to have a history of menstrual dysfunction (see inclusion criteria in Materials and methods), and were thus considered to have stable menstrual cycles. Additionally, the monthly checked personal log books gave information on, if menstrual irregularities occurred and these women were excluded (papers I-IV) or some of their menstrual cycles were excluded from the analysis (papers III-IV, women without OC use, samples;  $n = 127$ , missing samples  $n = 8$ ).

To make the training programs in the intervention study (papers I-II) practically implementable in an ordinary life, the women counted days of the menstrual/OC cycles as guidance of the training instead of measuring the LH-surge directly. We therefore did not ensure ovulation, in these regularly menstruating women. Hence, occasional anovulation or luteal phase defects in these women cannot be excluded. Though, we showed that good periodisation effects were reached by counting the days of the menstrual cycle

and we also showed that women with OC can use the same periodisation scheme.

To ensure that training effects (paper I) likely reflected strength gains and that potential effects due to neurogenic facilitation were avoided, the participants had to have an experience of strength training in the specific machines for leg-press and leg-curl for a minimum of 2 months, 3 times·w<sup>-1</sup>, prior the start of the study. Further, the women in the intervention study (papers I-II) were encouraged to continue their ordinary training and carefully record all training in the log book due to be able to especially study the impact of the changed leg training.

To ensure that the pre- and post-tests in the intervention study (papers I-II) were performed as planned in the early phase of the menstrual/OC cycle, day  $7 \pm 2$ , oestrogen levels were checked and were low as expected in this phase. In the observational study (papers III-IV) in order to ensure that various days of the menstrual/OC cycle were covered, the blood samples were collected once a month for each subject (with a maximal one-week difference) at different days of the menstrual/OC cycle.

At inclusion in these studies (I-IV) type of OC was not used for training group selection. Though most of the OC were combined OC types with hormones in monophasic or triphasic patterns with synthetic female sex hormones, corresponding to the endogenous sex hormones in the menstrual cycle (oestrogen and progesterone). The triphasic OC, have similarly distributed hormones as in the menstrual cycle. In the monophasic OC the hormone levels are high throughout the entire OC cycle. In the intervention study (papers I-II) the OC users were randomly distributed in the three groups by the assignation to training group in consecutive order. In group 1; 10 triphasic and 1 with gestagen, in group 2; 3 triphasic and 7 monophasic and in the control group; 7 triphasic and 4 monophasic, resulted in comparable hormones to the menstrual cycle during training. In the observational study (papers III-IV) the majority of OC users, (13 out of 15 women) used combined oestrogen-gestagen, one used combined anti-androgen and one used OC with only gestagen, hence assumed not to have influenced these results. Though, it could not be excluded that different type of OC might have different effects on the variables studied.

To ensure assertive blood sampling procedures during these studies (papers I-IV), methodological and standardisation requests were considered. Further, in OT and CBG analysis a rapid treatment of blood samples were performed to minimise a decomposition process after the sample collection. Hence, these analyses are considered to be confident.



We have used the “golden standard” methods for the measurement of body composition (BMD, LBM and fat mass), i.e. DEXA. Physical performance tests used for measurement of isokinetic muscle torque, jump height and aerobic capacity were performed by well-established standard methods, previously tested for reliability and described in detail (for references, see Material and methods). Though a possible learning effect should always be considered as a limitation when performing physical tests. This possibility was partially controlled in these studies by the subjects performing familiarisation with the techniques before the final tests were recorded.

The POMS questionnaire was used in the observational study (paper III-IV) with the standard instructions of the questionnaire (McNair, Lorr, and Dropplemann 1971) and filled in with the same procedure in a calm environment at pre- and post-test and once a month during the observational study. Although research generally support the use of psychological assessments for identifying individuals at risk of developing OTS, some problems exist that can limit accuracy. The subjects can falsely complete the questionnaire in order to present themselves in a more positive or negative light (Meeusen et al. 2013). In this study this was limited by an appropriate administration of the test, and by explaining the use of this test and emphasising that there are no right or wrong way to respond to the questionnaire. Moreover, the subjects were guaranteed that their data will remain confidential (Meeusen et al. 2013). Another concern regarding the POMS test is that the sensitivity of the mood subscales to training load is not uniform. For example, confusion score barely change following increase in training compared to the tension score (Raglin, Morgan, and O`Connor 1991). Though, since it is believed that OTS is a function of the total sum of stressors an athlete is exposed to, this questionnaire which capture broad moods, feeling or perceptions are most appropriate. Main et al (2016) suggest that a combination of self-report measures and physiological performance tests may provide the clearest picture of an athlete's current training state, the prediction of athletic performance changes and readiness for competition. However, it remains a need for additional test protocols that effectively integrate psychological information with biological assessments to enhance their efficacy (Meeusen et al. 2013).

## General conclusions

The results from this thesis further enhance that periodisation of resistance training should be based on the women's individual menstrual cycle, and preferable in the first two weeks, when concentration of oestrogen is dominant and progesterone is low. The hormones oestrogen and progesterone have mostly opposing influences, for example oestrogen suppresses protein catabolism and progesterone promotes protein catabolism, and the responses may be dependent on the concentrations of the respective ovarian hormone. A different trainability between the follicular phase and the luteal phase was demonstrated with no evident differences in the training effects between OC users and non-users. After four consecutive cycles with resistance training, firstly, we detected a significantly higher effect on strength, power and lean body mass when the training was periodised to the first two weeks of the menstrual/OC cycle. Secondly, we were not able to detect evident differences in the outcome variables between OC users and non-users. Thirdly, we did not find any exercise related negative health consequences due the periodised training regimes, and moreover the periodised training performed the first two weeks was experienced as more positive compared to periodised training the last two weeks of the cycles and to regular performed training. Based on these results, we recommend trained women to perform periodised resistance training in the first two weeks of their menstrual cycle. Since there are different training effects between the two menstrual phases, female athletes and coaches should take this into consideration in training programs, and also investigators in sports research in women.

It is important to optimise performance in sports, though it is critical to balance between training stress and recovery throughout a whole training and competition period. That is why sufficient monitoring instruments are important to assess the athlete's mood, need for recovery, and current life circumstances. Cortisol, associated with exercise-related disorders in female athletes, could be used as a possible hormone marker. Another hormone, besides cortisol, to identify physiological and psychosocial stress is OT, due to its relation to different forms of positive social interactions and stress management. However, we found an impact of season on cortisol and OT, with different pattern in OC users compared to non-users. Hence, these hormones could not be suggested as optimal, diagnostic, biological markers alone or in combination with other markers to detect OR and OTS in physically active women.

## **The major conclusions based on the results of the studies described in this thesis:**

- High frequency periodised leg resistance training the first two weeks of the menstrual/oral contraceptive cycle resulted in, a larger gain in isokinetic peak torque in right and left knee flexor, improved squat jump and countermovement jump, and increased lean body mass of the legs, compared to training during the last two weeks of the menstrual/oral contraceptive cycle. No evident differences in training effects between oral contraceptive users and non-user were found.
- No exercise-related negative consequences on, sex hormones, growth hormone, cortisol, total body fat mass and bone mineral density in the spine, was associated with the high frequency periodised leg resistance training irrespectively of if the training was performed the first two weeks or the last two weeks of the menstrual/oral contraceptive cycle. More women experienced the periodised leg resistance training the first two weeks of the menstrual/oral contraceptive cycle as positive.
- An impact of season on oxytocin and cortisol levels was found, with different pattern in oral contraceptive users and non-users respectively, hence these hormones could not be suggested as optimal, diagnostic markers alone or in combination with others to detect overreaching and overtraining syndrome in physically active women.

## **Future prospects**

This thesis has raised some questions with regard to strategies to improve effects of training without causing negative exercise-related health consequences in female athletes. The high frequency periodised resistance training during the first two weeks of the menstrual/OC cycle resulted in gains in power, strength and lean body mass. Though, it remains to clarify the precise mechanism for these menstrual/OC cycle related training effects in future studies. Moreover, future research should elucidate whether menstrual/OC cycle related periodised endurance training could improve training effects without exercise-related negative health consequences. Multiple factors must then be investigated in the prevention of menstrual disorders, including duration and intensity of training, prevention of negative caloric balance that could lead to a negative impact on components in the female athletic triad and/or to OR and OTS. Athletes and professional in sports would benefit greatly if a specific, sensitive simple diagnostic test existed for early detection of factors to diagnose OR/OTS and to distinguish OR from OTS. Hence, to identify reliable diagnostic markers in female athletes, OC users and non-users are warranted, due to that our result showed that OT and cortisol are inapplicable.

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