

**EFFECTS OF HORMONAL CONTRACEPTIVES ON
PHYSICAL PERFORMANCE AND BODY COMPOSITION**

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Master's Thesis
Exercise Physiology
Spring 2016
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ABSTRACT

Myllyaho, Moona. 2016. Effects of hormonal contraceptives on physical performance and body composition. Master's thesis in Exercise Physiology, Department of Biology of Physical Activity, University of Jyväskylä. 79 pp.

The purpose of the present study was to examine the effects of hormonal contraceptive use on neuromuscular, cardiorespiratory and body composition adaptations following combined strength and endurance training period.

Methods. Nineteen recreationally active women; 11 who had at least one year of hormonal contraceptive use (age 29 ± 4 yr; BMI 21 ± 2 kg/m²; VO_{2max} 44 ± 4 ml/kg/min) and 8 who had never used hormonal contraceptives (age 31 ± 5 yr; BMI 22 ± 2 kg/m²; VO_{2max} 41 ± 4 ml/kg/min) participated in the present study of combined strength and endurance training for 10 weeks. Training consisted of two maximal and explosive strength training sessions, and two high-intensity interval training sessions per week. Serum basal hormone levels, maximal bilateral isometric strength, maximal unilateral isometric knee extension and flexion, maximal bilateral dynamic leg press (1RM), counter movement jump (CMJ), upper body isometric strength, dynamic muscle endurance, a 3000 m running test and an incremental maximal running test were measured prior to the intervention and after the 10 weeks training period.

Results. There were no statistically significant differences between groups' improvements in any measured strength, endurance or body composition variables. The hormonal contraception users (HC) significantly improved their performance in maximal isometric leg press ($14\pm 15\%$, $p<0.05$), isometric knee flexion ($11\pm 14\%$, $p<0.05$), isometric bench press ($9\pm 7\%$, $p<0.001$), dynamic leg press ($10\pm 5\%$, $p<0.001$), countermovement jump ($6\pm 7\%$, $p<0.01$), push-ups ($14\pm 16\%$, $p<0.05$) and sit-ups ($22\pm 25\%$, $p<0.05$). The non-users (NHC) had significant improvements in isometric leg press ($6\pm 6\%$, $p<0.05$), isometric knee extension ($9\pm 11\%$, $p<0.05$), dynamic leg press ($7\pm 4\%$, $p<0.01$), countermovement jump ($11\pm 6\%$, $p<0.001$), push-ups ($20\pm 17\%$, $p<0.05$) and sit-ups ($13\pm 11\%$, $p<0.05$). Maximal oxygen consumption (VO_{2max}) remained unchanged in both groups. In the 3000 m time trial the HC group decreased their running time by $2\pm 4\%$ ($p=0.162$) and the NHC group by $3\pm 2\%$ ($p<0.05$). The improvement in time trial was significant only within the NHC group, however, there was not a significant difference between groups' improvements ($p=0.559$). There were no statistically significant differences between groups' improvements in body composition, however, a trend for greater increase in fat free mass was observed in NHC than in HC women ($p=0.065$). The HC group increased fat free mass by $1\pm 1\%$ ($p<0.05$) and the NHC group by $2\pm 1\%$ ($p<0.001$). Total fat was reduced in the HC group by $5\pm 10\%$ ($p=0.158$) and in the NHC group by $5\pm 6\%$ ($p<0.05$).

Summary and conclusions. High-intensity combined strength and endurance training resulted in significant improvements in neuromuscular performance and fat free mass in both groups. In addition, there were significant improvements in 3000 m running performance and decreases in total body fat within the NHC group. These findings suggest that combined strength and endurance training is a useful method for improving physical fitness and health in recreationally active women, and hormonal contraception use does not appear to impair the physical performance or body composition.

Keywords: hormonal contraceptives, combined strength and endurance training, physical performance, body composition, hormonal adaptations

ABBREVIATIONS

1RM	one repetition maximum
FSH	follicle-stimulating hormone
GnRH	gonadotropin-releasing hormone
HC	hormonal contraceptive group
HIIT	high-intensity interval training
LH	luteinizing hormone
MVC	maximum voluntary contraction
NHC	non-hormonal contraceptive group
OC	oral contraceptive
PMS	premenstrual syndrome
RFD	rate of force development
SHBG	sex hormone binding globulin
VO _{2max}	maximal oxygen consumption

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1 INTRODUCTION

Sports performance is a result of many cardiorespiratory, musculoskeletal, biomechanical, cellular, and enzymatic adaptations (Lebrun 2000, 37). Combined strength and endurance training has been of a great interest to researchers in the past years. Many studies have found that combined training of strength and endurance has a positive effect on health and sports performance in women (Taipale et al. 2010, Taipale et al. 2014, Sillanpää et al. 2009, Sillanpää et al. 2010).

Previous research examining the hormonal adaptations to combined strength and endurance training has been conducted most often with male subjects (Consitt et al. 2002, Costello et al 2014). The female body is more complicated than the male body with changing patterns of endogenous and exogenous hormones that may have consequences for the cardiorespiratory, neuromuscular, and metabolic systems (Lebrun 2000, 37). Studies with careful monitoring of the menstrual cycle are rare (Casazza et al. 2004).

Research investigating large fluctuations of estrogen and progesterone during pregnancy, menopause, and hormone replacement therapy has shown that the female sex hormones, estrogen and progesterone, have many physiological effects on the body that influence, for example, substrate metabolism, respiratory and neuromuscular systems. This can have an indirect influence on sports performance. (Janse de Jonge 2003.)

Investigators, however, are not in agreement about the effects of the menstrual cycle or the use of hormonal contraceptives on sports performance. There is concern among female athletes that exogenous hormones in the hormonal contraceptives affect athletic performance by blunting physiological adaptations to training. Despite the widespread use of hormonal contraceptives, studies examining the effects of synthetic sex steroids on the metabolic adaptations to physical exercise are limited. (Casazza et al. 2004.) For recreational and elite female athletes, it is very important to get reliable results about the

effect of the use of hormonal contraceptives in order to optimize athletic performance and maintain their health.

The aim of this study was to examine how hormonal contraceptive use may affect neuromuscular, cardiorespiratory and body composition adaptations to 10 weeks of high-intensity combined strength and endurance training performed on separate days in recreationally active women.

2 WOMEN AND HORMONES

Hormones are signaling molecules that regulate physiological and metabolic functions, such as growth, metabolism, and reproduction. Hormones are produced by secretory cells all over the body and transported in the blood stream. By acting on receptors of target cells, hormones induce specific responses. (Borer 2003, 1.) Hormones work both synergistically and independently to stimulate many metabolic functions in the body. For example, growth hormone and insulin like growth factor-1 both have a net anabolic effect enhancing whole body protein synthesis (Mauras et al. 2005).

The female body is complicated with changing patterns of hormones. Women must deal with fluctuations of endogenous hormones during development, the menstrual cycle, pregnancy, parturition, and menopause. (Lebrun 2000, 37.) The endogenous female hormones can be divided into gonadotropic hormones, and sex hormones, which are secreted from the hypothalamus, anterior pituitary gland, ovaries, and adrenal glands (Figure 1) (Guyton & Hall 2006, 906).

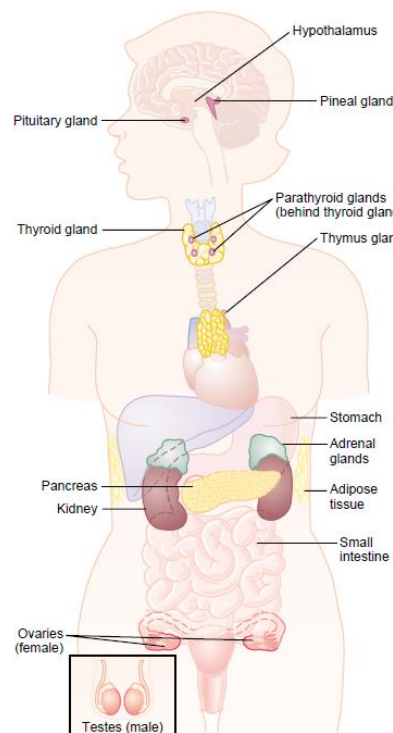


FIGURE 1. The principal endocrine glands and tissues of the body (Guyton & Hall 2006, 906)

2.1 Gonadotropic hormones

Gonadotropin-releasing hormone (GnRH) is released from the hypothalamus in short pulses once every 90 minutes and the concentration remains quite stable throughout the day. GnRH initiates secretion of the two anterior pituitary sex hormones: *follicle-stimulating hormone* (FSH) and *luteinizing hormone* (LH). In the blood FSH and LH are bound to sex hormone binding globulin (SHBG) or to albumin. FSH and LH act synergistically; they stimulate their ovarian target cells and activate the growth and proliferation of these cells. Concentrations of FSH and LH are increased close to ovulation. (Guyton & Hall 2006, 1012–1014.)

2.2 Sex hormones

Sex hormones can be divided into ovarian hormones and androgens. These steroid hormones are synthesized from cholesterol and acetyl coenzyme A. There are two types of ovarian hormones, the estrogens and the progestins. Both estrogens and progestins are secreted by corpus luteum in the ovaries in response to FSH and LH. In the blood estrogens and progestins are transported bound with plasma albumin and with specific estrogen- and progestin-binding globulins. (Guyton & Hall 2006, 1016.)

2.2.1 Estrogens

The most important estrogen is estradiol (β -estradiol) but also estrone and estriol are present in small amounts in the plasma of the human female (Guyton & Hall 2006, 1016). In women estrogens are synthesized and secreted primarily by the ovaries and to a lesser extent by adrenals (Kraemer & Ratamess 2005). In addition, estrogen can be synthesized locally in different tissues. Human skeletal muscle is able to synthesize estrogen by aromatization of androgens. (Pöllänen et al. 2011, Pöllänen et al. 2015.)

Exposure to estrogens during different life phases has long-term effects on women's health and wellbeing (Sipilä et al. 2015). Estrogens have many functions in the human body including the cardiovascular, musculoskeletal, immune, and central nervous

systems. The primary function of estrogens is the development and maintenance of normal sexual and reproductive system function including the initiation of the growth of the breasts. Estrogens promote the proliferation and growth of sex organs and specific cells that are related to reproduction. (Enns & Tiidus 2010.)

Estrogens have a growth-promoting effect not only in sex organs but also in the bones and fat tissues. One important function of estrogen is to stimulate bone growth by inhibiting osteoclastic activity in the bones. Estrogens also increase the amount of fat accumulation in the subcutaneous tissues. Therefore, the percentage of body fat is generally greater in females compared to males. (Guyton & Hall 2006, 1018.)

The secretion of estrogen is increased significantly at puberty and is further increased during the first few years of reproductive life. The secretion is progressively decreased toward the end of reproductive life, and beyond menopause there is almost no estrogen or progesterone secretion (Figure 2) (Guyton & Hall 2006, 1022). In recent years, hormone replacement therapy for postmenopausal women has been proposed as potentially therapeutic agent (Enns & Tiidus 2010).

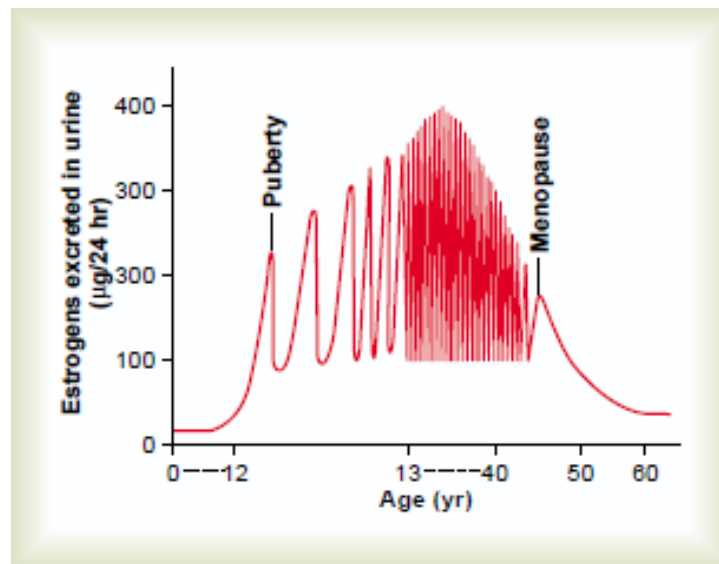


FIGURE 2. The changing levels of estrogen during life and during the monthly sexual cycle. (Guyton & Hall 2006, 1022)

2.2.2 Progestins

Progesterone is the most important progestin. However, small amounts of another progestin, 17- α -hydroxyprogesterone, are secreted as well. For practical purposes, progesterone is usually considered as the only important progestin. The primary function of progestins is to prepare the uterus for pregnancy by promoting secretory changes in the uterine endometrium during the luteal phase of the monthly menstrual cycle. They also prepare the breasts for lactation by causing the breasts to swell and proliferation of the alveolar cells into secretory cells. (Guyton & Hall 2006, 1018.)

Progestins have some 'anti-estrogenic' effects, for example, progesterone may play a secondary role in substrate metabolism as it has been reported to oppose the lipolytic effects of estrogen (Ashley et al. 2000). In addition, progesterone seems to have a central thermogenic action, since high levels of progesterone have been associated with increased core temperature (Hessemer & Bruck 1985). It has also been speculated whether progesterone has negative (Sarwar et al. 1996) or positive effects on muscle force production (Greeves et al. 1999).

2.2.3 Androgens

Androgens are generally considered as male sex hormones because they have masculinizing effects but, however, females have also smaller concentrations of androgens. In fact, androgens are necessary for the development of reproductive function and hormonal homeostasis in females. In addition, androgens affect bone density, muscle mass and strength, adipose tissue, mood and sexual desire. Testosterone is the most abundant androgen and it promotes muscle hypertrophy by enhancing protein synthesis. In females, testosterone is produced in the ovaries and in the outer layer of the adrenal glands. (Bachmann et al. 2002.)

2.3 Growth hormone, insulin like growth factor-1 and cortisol

Growth hormone (GH) is an anabolic hormone that stimulates cellular growth, proliferation, repair, regeneration, and protein synthesis. It acts synergistically and independently with insulin like growth factor-1 (IGF-1) to stimulate many anabolic functions in the body. (Mauras et al. 2005.) Circulating IGF-I has been shown to be positively associated with measures of aerobic fitness, muscular endurance, and body composition (Nindl et al. 2011).

In contrast, cortisol secreted by the adrenal cortex is a stress hormone that has catabolic functions. Cortisol opposes the effects of anabolic hormones since it stimulates lipolysis in adipose cells, increases protein degradation and decreases protein synthesis in muscle cells. (Izquierdo et al. 2004, Kraemer & Ratamess 2005.)

2.4 Female monthly menstrual cycle

The monthly rhythmical pattern of the secretion of the female hormones and the physical changes in the sexual organs is called the female monthly menstrual cycle. The average menstrual cycle is 28 days, but can range from 20 to 35 days (Guyton & Hall 2006, 1012.) Eumenorrhea refers to regular menstruation, oligomenorrhea to infrequent menstruation, and amenorrhea to the absence of menstruation (Matzkin et al. 2015).

The whole menstrual cycle is highly controlled by the hypothalamic-pituitary-ovarian axis and influenced by many physiological factors (Lebrun 2000, 39). Significant fluctuations in the concentrations of female sex hormones take place during the menstrual cycle. Two distinct hormone phases can be separated by ovulation; pre-ovulation follicular phase and post-ovulation luteal phase. (Redman et al. 2003.)

At the beginning of follicular phase, levels of both estrogen and progesterone are low. Estrogen secretion starts to increase during the middle of the follicular phase. Just prior to ovulation, estrogen levels reach a peak. Because of the negative feedback to the hypothalamus, estrogen levels fall. After ovulation and at the beginning of the luteal

phase, the secretion of both estrogen and progesterone starts to increase. The levels stay quite stable for a few days and then start to fall. If conception or implantation does not occur, the endometrium is detected as useless and shed as menstrual blood flow. The levels of both hormones are low and the entire process begins anew. (Lebrun 2000, 37-39, Guyton & Hall 2006, 1015.) The changing levels of FSH, LH, estradiol and progesterone during the normal female menstrual cycle are presented in figure 3.

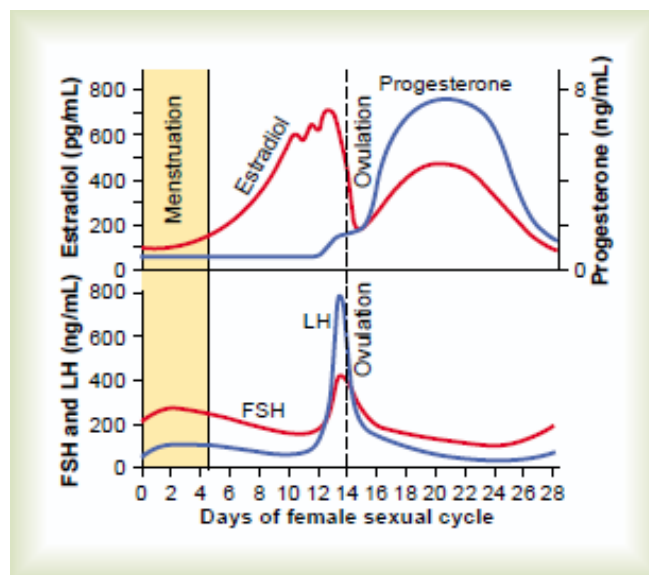


FIGURE 3. Approximate plasma concentrations of the follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol and progesterone during the normal female menstrual cycle. (Guyton & Hall 2006,1013)

Inadequate energy intake and strenuous physical training can lead to abnormal menstrual cycles. For example elite athletes may have a shortened luteal phase, anovulatory cycles or amenorrhea. (Lebrun 2000, 39.) Menstrual dysfunction with chronically low ovarian hormones may increase the risk for osteoporosis (Hergenroeder et al. 1997, Rickenlund et al. 2004). The female athlete triad refers to the interrelationships among energy availability, menstrual function, and bone mineral density (Figure 4) (Matzkin et al. 2015).

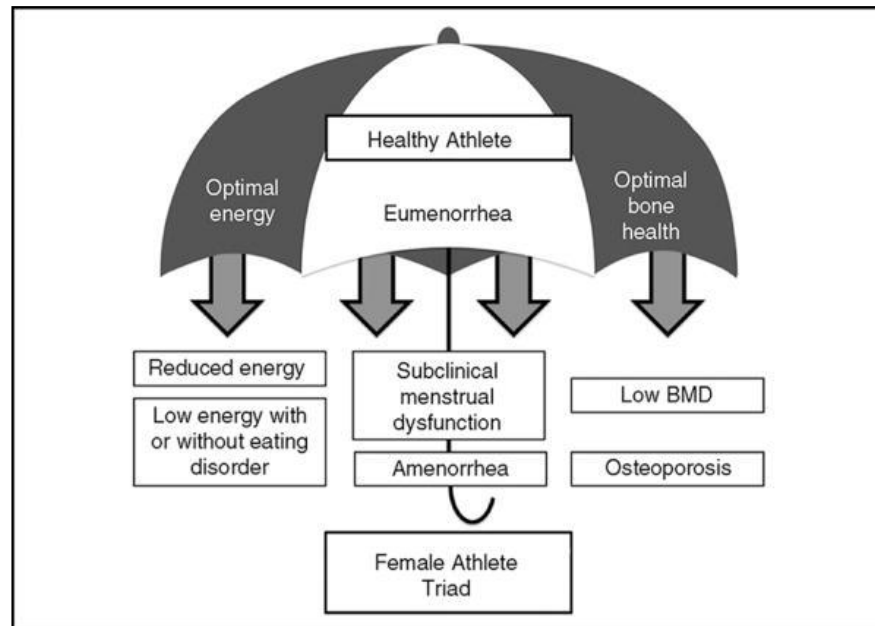


FIGURE 4. The female athlete triad umbrella. BMD = bone mineral density. (Matzkin et al. 2015)

2.5 Exogenous hormones and hormonal contraceptives

There are different types of synthetic sex steroids, also known as exogenous hormones. Synthetic androgens are usually referred to as anabolic steroids but combinations of synthetic female sex hormones are frequently used in hormonal contraceptives. These synthetic hormones reduce cyclical variability and provide a consistent menstrual cycle while also inhibiting ovulation and preventing pregnancy. A significant number of women are prescribed hormonal contraception for purposes such as contraception and cycle regulation, as well as the treatment of painful menstruation. Hormonal contraceptives are even used to protect bone density in amenorrheic athletes. (Davis & Westhoff 2001, Hergenroeder et al. 1997, Rickenlund et al. 2004.)

Mechanism of hormonal contraception. The main mechanisms of hormonal contraceptives are ovulation inhibition and changes in the cervical mucus that inhibit sperm penetration (Rivera 1999). Hormonal contraception pills systematically control the concentrations of endogenous female sex hormones by providing synthetic ovarian hormones for 21 out of 28 days. Actions on the hypothalamus and anterior pituitary gland

via negative feedback lead to the suppression of GnRH, FSH and LH (Figure 5). Therefore, the natural production of endogenous estrogens and progestins are reduced to levels indicative of menopause, and, depending on the type of hormonal contraception administered, 3-5 times more exogenous estrogen and 1-3 times more exogenous progesterone than endogenous levels can be provided. (Burrows & Peters 2007, Elliott et al. 2005, Lebrun 2000, 37–39.) Hormonal contraceptives even reduce the levels of total and free testosterone, by inhibiting ovarian and adrenal androgen synthesis and by increasing levels of sex hormone-binding globulin (Bachmann et al. 2002, Rickenlund et al. 2004, Wiegratz et al. 2003, Zimmermann et al. 2014).

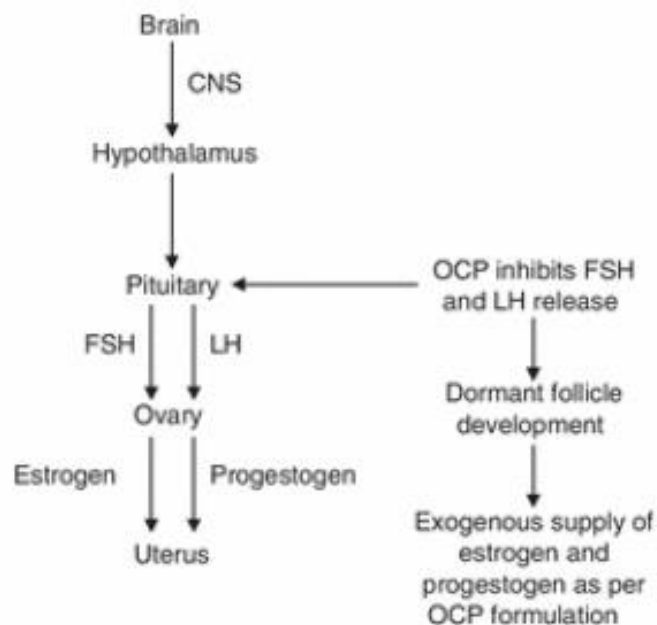


FIGURE 5. The oral contraceptive pill (OCP) and control of endogenous sex hormones. (Burrows & Peters 2007)

Types and formulations. There is a variety of different types and formulations of hormonal contraceptives in which the doses of synthetic estrogens and progestins vary. Synthetic estrogens are found as ethinylestradiol and synthetic progestins as levonorgestrel, norethindrone acetate, desogestrel, norgestimate, norgestrel or etynodiol. (Burrows & Peters 2007.) Combination pills contain both estrogen and progesterone. The exogenous hormone dose is much lower in the newer forms, and the estrogenic and progestogenic content of pills between different brands can range from 0.02 to 0.5 mg

and 0.1 to 1.0 mg, respectively. (Elliott et al. 2005.) In contrast, the mini pills contain only progesterone, and it tends to have milder side effects, but more intermenstrual bleeding, than combination pills. Progesterone in hormonal contraceptive pills opposes the actions of estrogen, depending on its potency and androgenicity. Potency refers to the power of the progesterone to produce its desired effects, while androgenicity refers to the ability to produce masculine characteristics. (Burrows & Peters 2007.)

Hormonal contraceptives can also be divided into monophasic, biphasic and triphasic groups. In monophasic hormonal contraceptives the amounts of estrogens and progestins remain constant over 21 days, followed by 7 days of a placebo. Biphasic hormonal contraceptives contain a fixed amount of estrogens and two different doses of progestins during the 21 days of consumption, followed by 7 days of placebo. Triphasic hormonal contraceptives contain three different doses of estrogen and/or progestin during the pill cycle. (Burrows & Peters 2007.) Triphasic hormonal contraceptives can mimic more closely the ovarian hormone variation that occurs during the normal menstrual cycle (Rechichi et al. 2009).

Side-effects. Hormonal contraceptives are generally well tolerated, but some women experience side effects, such as headache, nausea, breast tenderness, and weight gain (Rickenlund et al. 2004). However, in past decades the development of hormonal contraceptives has attenuated adverse side-effects such as fluid retention and increases in blood pressure (Lebrun, 2000, 52). Healthy non-smoking women using hormonal contraception do not appear to have an increased risk of myocardial infarction, embolic stroke, or venous thrombosis (Cedars 2002). Nevertheless, the clinical consequences of the suppressed testosterone levels due to hormonal contraceptive use have so far gained little attention (Zimmermann et al. 2014).

Beneficial side-effects of hormonal contraceptives often include a reduction in dysmenorrhea, premenstrual syndrome (PMS) and iron-deficiency anemia because of excessive monthly blood loss (Bennell et al. 1999, Davis & Westhoff 2001, Lebrun et al. 2003). Hormonal contraceptives may also help to maintain a predictable hormonal milieu (Cedars 2002). In women with a low bone mineral density and menstrual disturbances,

hormonal contraception treatment may help increase bone mineral density and prevent osteoporosis (Rickenlund et al. 2004).

Contraceptive options. In prevention of pregnancy, there are many contraceptive options. The condom, diaphragm, cervical cap, and spermicides demand responsibility and consistency of use, but are popular due to the lack of side-effects and possible alterations in sports performance. (Lebrun 2000, 51.) Intrauterine contraception is a highly effective long-acting reversal contraceptive that prevents fertilization by reducing the opportunity of sperm to fertilize an ovum. It is available as either a copper intrauterine device or the levonorgestrel intrauterine system. (Diedrich et al. 2015.) The levonorgestrel intrauterine system is a T-shaped plastic frame containing levonorgestrel, which is released at a rate of approximately 12 –20 µg per day (Gupta et al. 2015).

3 SPORTS PERFORMANCE AND SEX HORMONES

Women and men respond to training with many of the same physiological adaptations. Therefore, training methods seem to be similar. (Lebrun 2000, 88.) However, the effect of the female sex hormones and menstrual cycle on sports performance is a very confused topic. Investigations about the effects of the menstrual cycle as well as the use of hormonal contraceptives on the physiological adaptations to training are not in complete agreement. Research in this area is complicated by multiple factors related to subjects such as age, fitness status, menstrual cycle history and cycle length, as well as the high variability in hormonal contraceptive formulations. Therefore, the interpretation of previous studies is difficult. (Bennell et al. 1999, Joyce et al. 2013, Rechichi et al. 2009.)

Reported results vary when different types of exercise, hormonal contraceptives, and menstrual cycle phase have been studied. The most important factor is the method of menstrual cycle phase verification via hormone measurements so that in the long-term research the same phase is compared multiple times (Kraemer & Ratamess 2005). Usually two different phases of the menstrual cycle, the follicular phase and the luteal phase are compared. (Smekal et al. 2007, Vaiksaar et al. 2011.)

3.1 Strength training

Muscular strength is defined as the maximum force or tension generated by a muscle (Mittleman & Zacher 2000, 29). The development of strength is determined by the complex integration of a number of physiological factors, such as neurological adaptations, muscle hypertrophy, alterations in muscle fibre composition, and hormonal responses (Kraemer et al. 1998). Strength training can be divided into maximal, explosive, and endurance strength (Kraemer & Ratamess 2005). Women respond to resistance training with similar patterns of relative strength gains and changes in muscle hypertrophy as men do (Lebrun, 2000, 85). Women at all ages can have marked improvements in muscle strength and hypertrophy after moderate to high intensity strength training (Sillanpää et al. 2009, Sillanpää et al. 2010). It is well known that strength training has acute and chronic effects on circulating hormone levels such as

testosterone, growth-hormone, and cortisol (Kraemer & Ratamess 2005). However, there may be a potential gender difference in the hormone adaptation to strength training (Kraemer et al. 1998). In addition, there is little research examining the chronic adaptations of female sex hormone synthesis and secretion in response to long-term strength training (Consitt et al. 2002).

In some strength training studies there have not been statistically significant changes in resting levels of sex hormones including estradiol, progesterone, testosterone, FSH, LH or SHBG, over the study period (Häkkinen et al. 1990, Häkkinen et al. 1992, West & Phillips 2012). However, Kraemer et al. (1998) and Marx et al. (2001) demonstrated increases in testosterone levels after 8–24 weeks of resistance training in untrained women (Figure 6).

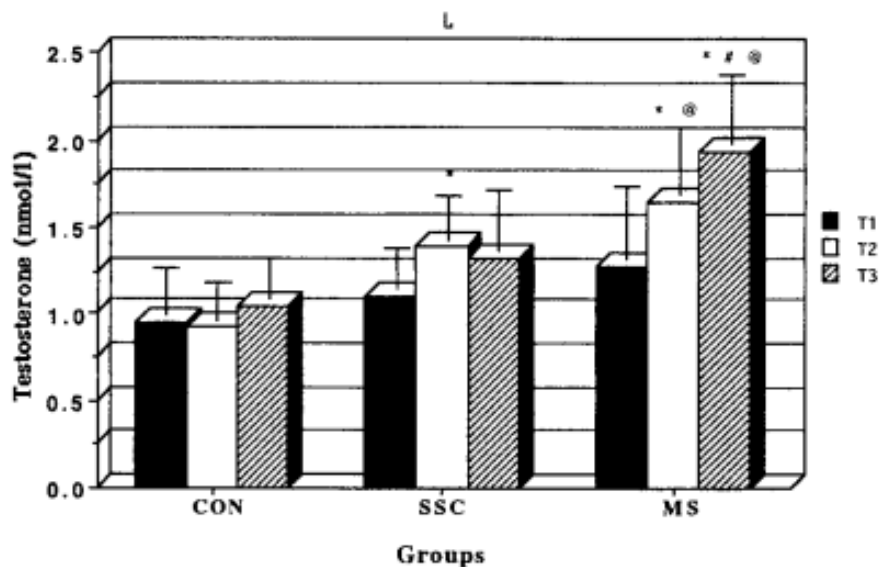


FIGURE 6. The effects of resistance training on resting serum testosterone concentrations in control group (CON), low-volume, single-set circuit group (SSC), and periodized high-volume multiple-set group (MS) during pre-training (T1), after 12 weeks (T2), and after 24 weeks of training in healthy, untrained women. * $P < 0.05$. (Marx et al. 2001)

Studies about postmenopausal women have revealed that at the time of menopause, estrogen secretion decreases and maximum voluntary force (MVF) relative to muscle cross-sectional area (CSA) is also decreasing. Hormone replacement therapy (HRT) in

postmenopausal women has shown to diminish age-associated muscle loss, loss in muscle power, and accumulation of fat in skeletal muscle. Further HRT raises the protein synthesis rate in skeletal muscle after resistance training, and has an anabolic effect upon connective tissue in both skeletal muscle and tendon. (Phillips et al. 1993, Sipilä et al. 2013, Skelton et al. 1999.) These facts suggest that estrogen may have a muscle-strengthening action. In addition, the protective role of estrogen on cardiac, smooth and skeletal muscle has been speculated over the past years. Estrogen has been reported to play an important role in inflammation process and stimulation of muscle repair and regenerative processes. (Enns & Tiidus 2010.)

In addition to ovarian production, estrogen can be synthesized locally, for example in human skeletal muscle. Interestingly, intramuscular sex steroids have been strongly associated with muscle strength and power regulation in female muscle. The systemic estrogen levels, however, do not seem to be related to intramuscular hormone levels. This suggests that tissue-specific steroid hormones and their local synthesis may have functions which are independent from ovarian hormone secretion. (Pöllänen et al. 2011, Pöllänen et al. 2015.)

3.1.1 Effect of menstrual cycle on strength

The presence of changes in muscle strength during the menstrual cycle has been a subject of controversy. Studies have investigated muscle strength, endurance, and power performance in women during various phases of the menstrual cycle such as during menstruation, follicular, ovulation or luteal phase (Elliott et al. 2005, Friden et al. 2003, Sarwar et al. 1996). Many hormonal changes take place around ovulation when the highest peak in estrogen concentration is observed. In addition, there is an increase in concentrations of testosterone, LH and FSH. Levels of estrogen and progesterone are higher in the luteal phase compared with the follicular phase. (Sarwar et al. 1996.)

Different components of the neuromuscular system such as muscle, tendon, and nervous system, may be influenced by estrogens (Sipilä et al. 2015). Some studies suggest that the menstrual phase does not affect muscle contractile characteristics and strength performance (Elliott et al. 2005, Friden et al. 2003), while some studies have suggested

that strength performance is enhanced during menstruation when estrogen and progesterone levels are low (Davies et al. 1991), or during the follicular phase (Phillips et al. 1996), or at the time of ovulation (Sarwar et al. 1996). For example, Sarwar et al. (1996) found significant enhancement in maximum quadriceps and handgrip strength during ovulation. This can, however, be explained by other mechanisms than estrogen and progesterone. Testosterone receptors are also present in the muscle and have the potential to play a role in this variation because testosterone levels also increase during ovulation. (Lebrun 2000, 48.)

3.1.2 Effect of hormonal contraceptives on strength

The literature about the physiological effects of hormonal contraceptive use and exogenous female sex steroids on strength performance is limited. Some studies with postmenopausal women have demonstrated that synthetic estrogen in hormone replacement therapy may have a muscle strengthening action (Phillips et al. 1993, Skelton et al. 1999). Therefore, high levels of exogenous estrogen caused by hormonal contraceptive use could also have a positive effect on strength in premenopausal women. In addition, androgenic components of hormonal contraceptives could have some positive effects on strength (Burrows & Peters 2007). On the other hand, hormonal contraception use may increase levels of sex hormone binding globulin and decrease levels of total and free testosterone (Bachmann et al. 2002, Zimmermann et al. 2014). Most studies, however, suggest that hormonal contraceptive use does not affect maximum force production (Elliott et al. 2005, Sarwar et al. 1996) or isokinetic strength (Lebrun et al. 2003).

According to Sarwar et al. (1996), maximal voluntary force of the quadriceps or hand muscles did not change in young sedentary women using hormonal contraception (Sarwar et al. 1996). Elliott et al. (2005) showed similar results when examining the effects of hormonal contraception use on maximal dynamic and isometric leg strength in young sedentary women. The results suggest that hormonal contraceptive use would not affect the maximum force generating capacity. (Elliott et al. 2005.)

3.2 Endurance training

Endurance means the ability to perform repeated, continuous skeletal muscle contractions for prolonged periods at a given submaximal power or speed (Hawley 2009). The determining factors of endurance performance consist of maximal oxygen uptake, mitochondrial density, performance efficiency and body composition (Shangold & Mirkin 1988, 65). Maximal oxygen uptake (VO_{2max}) and peak oxygen uptake (VO_{2peak}) have been considered as the 'gold standard' for assessing aerobic exercise capacity and cardiorespiratory performance (Taylor et al. 1955, Blomqvist & Saltin 1983). The relative changes that result in endurance training seem to be same in men and women (Shangold & Mirkin 1988, 66).

3.2.1 Effect of menstrual cycle on endurance

It has been suggested that estrogen and progesterone fluctuation during the menstrual cycle have the potential to affect endurance performance through secondary effects on fuel availability, the oxygen transport system and ventilation (Janse de Jonge 2003). For example, there can be alterations in blood pressure, blood volume, and body temperature. In addition, estrogen may alter secretion rates of growth hormone, insulin and glucagon, which play a central role in substrate metabolism. (Ashley et al. 2000, Lebrun 2000, 39.)

Some studies have demonstrated that estrogen may enhance fatty acid oxidation since lipids are the preferred fuel in the luteal phase. This is supported by findings of a lower respiratory exchange ratio (RER) during the luteal phase when there is usually an increase in estrogen levels (Ashley et al. 2000, Redman et al. 2003, Vaiksaar et al. 2011). For example, Redman et al. (2003) demonstrated a lower RER in the LP at VO_{2peak} (Figure 7). These results suggest that high estrogen levels may have a glycogen sparing effect and be advantageous for prolonged exercise.

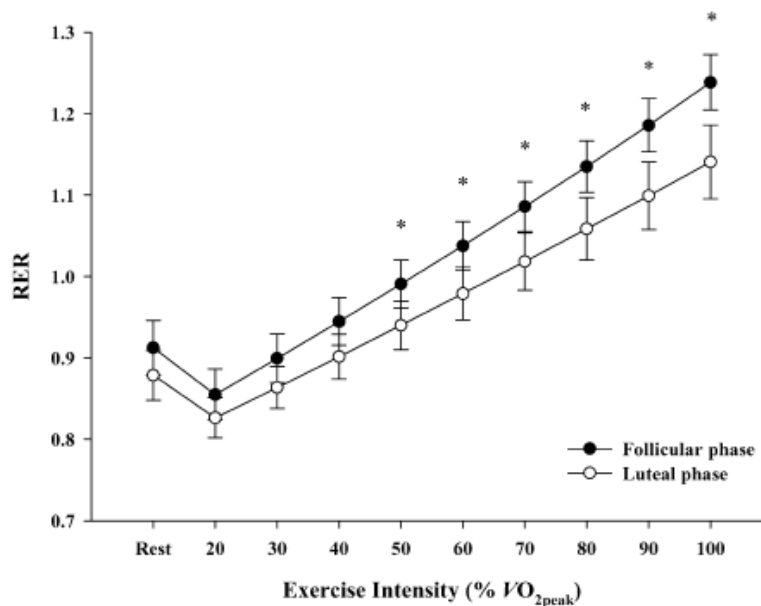


FIGURE 7. Effect of menstrual cycle phase on respiratory exchange ratio (RER) during incremental exercise to exhaustion in sedentary women. (Black circles represent follicular phase and white circles luteal phase). Values are mean (SE). * Significantly different from follicular phase, $P < 0.05$. (Redman et al. 2003)

In general, the cyclic variations of endogenous estrogen and progesterone levels across the normal menstrual cycle do not appear to affect aerobic performance (Casazza et al. 2002, Redman et al. 2003, Smekal et al. 2007, Vaiksaar et al. 2011) or maximal anaerobic performance (Giacomoni et al. 2000). The $\dot{V}O_{2max}$, a critical determinant of aerobic exercise capacity, appears to be independent of menstrual cycle phase. In addition, sport-specific endurance performance, maximal power output, or anaerobic threshold do not appear to vary during pre- and post-ovulatory phases in sedentary (Redman et al. 2003), active (Giacomoni et al. 2000, Smekal et al. 2007) or highly trained women (Vaiksaar et al. 2011).

Hormonal contraception users have not typically been included in the majority of menstrual cycle research (Rechichi et al. 2009). Many studies that have examined the hormonal contraceptive cycle phase, however, are in agreement that sport-specific endurance performance is not influenced by the cycle phase (Giacomoni et al. 2000, Vaiksaar et al. 2011, Rechichi & Dawson 2012).

For example, Rechichi and Dawson (2012) observed no differences in a 200 m time trial at 3 time points of a single hormonal contraceptive cycle, during the consumption phase, early, and late in the withdrawal phase. Similarly, Giacomoni et al. (2000) demonstrated that menstrual cycle phase does not influence maximal cycling and jumping anaerobic performances in females regardless of whether they were using hormonal contraceptives or not. However, the premenstrual and menstrual symptoms may have a negative influence on performance in women regardless of whether they use hormonal contraceptives or not (Giacomoni et al. 2000).

3.2.2 Effect of hormonal contraceptives on endurance

There is some concern that the exogenous ovarian hormones in hormonal contraceptives may have cardiovascular, hemodynamic, and metabolic effects, such as changes in blood lipids, substrate metabolism, decreased running economy or VO_{2max} (Burrows & Peters 2007, Casazza et al. 2004, Lebrun et al. 2003, Suh et al. 2003). However, the effect of longitudinal hormonal contraceptive use on endurance performance has not been thoroughly examined (Joyce et al. 2013).

Some studies have demonstrated a reduction (4% to 15%) in VO_{2peak} when measured before and during triphasic hormonal contraceptive use in moderately active and highly trained women (Casazza et al. 2002, Lebrun et al. 2003, Suh et al. 2003). Lebrun et al. (2003) conducted a double blind, placebo controlled study with 14 trained athletes. The VO_{2max} was decreased by 4.7% in the triphasic hormonal contraceptive group compared with 1.5% improvement in the placebo group. Increases in blood pressure and decreases in O_2 pulse with hormonal contraception use could explain part of the decreases in VO_{2max} . On the other hand, high levels of synthetic estrogen caused by hormonal contraception could enhance fatty acid oxidation, spare glycogen and be advantageous for prolonged exercise. (Ashley et al. 2000, Burrows & Peters 2007, Casazza et al. 2004, Lebrun et al. 2003, Redman et al. 2003, Suh et al. 2003, Vaiksaar et al. 2011.)

It seems that the effects of hormonal contraceptives on aerobic capacity are more pronounced in triphasic formulations than in monophasic formulations (Burrows & Peters 2007). Many studies that involve low dose, monophasic, combination pills suggest that

hormonal contraceptive use does not affect endurance capacity (Rickenlund et al. 2004, Joyce et al. 2013). In addition, effects of hormonal contraception on physiological tests do not always translate into effects on performance in the field setting. For example, reduced peak oxygen uptake (VO_{2peak}) does not necessarily translate to impaired performance in the field (Bennell et al. 1999, Joyce et al. 2013).

Joyce et al. (2013) investigated the effect of long-term monophasic hormonal contraceptive use on cycling performance in recreationally active women. The study demonstrated that long-term hormonal contraceptive users had a lower VO_{2peak} at the anaerobic threshold compared with normally menstruating women. Despite a reduction in VO_{2peak} , long-term hormonal contraceptive use did not appear to alter endurance exercise performance as indicated by exercise economy and time to exhaustion. However, the researchers noted that the effect of hormonal contraception use on exercise economy may be dependent on the mode of exercise and the duration of hormonal contraception use. (Joyce et al. 2013.) It is also possible that cardiorespiratory adaptations to hormonal contraceptive use occurs (Bennell et al. 1999).

3.3 Combined strength and endurance training

Researchers have examined combined strength and endurance training over the past years. The pioneering work was completed by Hickson (1980) who concluded that training strength and endurance simultaneously leads to a compromised adaptation (Figure 8). Strength and endurance training adaptations are distinct due to genetic and molecular mechanisms and, therefore, concurrent training can be described as a compromise, compared with training for either exercise mode alone. This is called 'the interference effect'. (Hickson 1980.) Anabolic and catabolic hormones may play an important role in the adaptation processes of simultaneous strength and endurance training (Izquierdo et al. 2004).

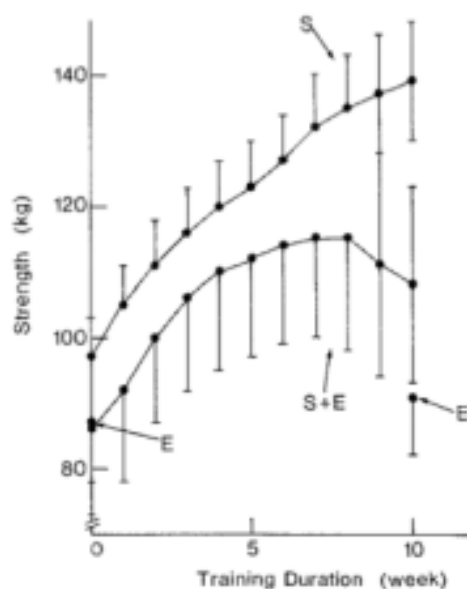


FIGURE 8. The development of strength in response to strength (S), endurance (E) or combined (S+E) training over a 10 week period (Hickson, 1980)

There is some evidence that endurance training may inhibit maximal strength or muscle mass development during high volume and high intensity training for longer training periods (Hickson 1980, Coffey & Hawley 2007, Hawley 2009, Wilson et al. 2012). In contrast, the influence of combined endurance and strength training on endurance performance has shown equivocal results. Authors have reported no effect on endurance performance (Hickson 1980, Bell et al. 2000) and improvements in endurance performance (Mikkola et al. 2011). In addition, the duration, intensity, and type of exercise, particularly endurance training modality, influence interference. For example, in a meta-analysis by Wilson et al. (2012) it is concluded that resistance training concurrently with running, but not cycling, is associated with significant decrements in both hypertrophy and strength. The overall effect sizes for strength, endurance and combined training are presented in figure 9.

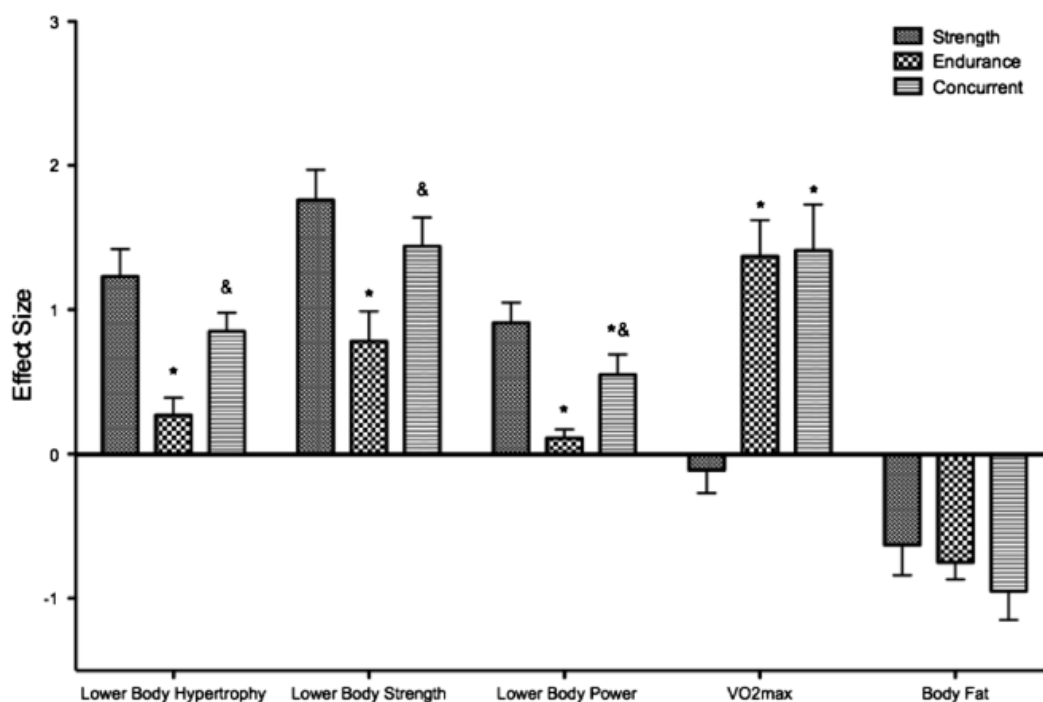


FIGURE 9. Overall effect sizes for strength, endurance, and concurrent training: the mean overall ES (mean \pm 6 SE) for lower-body strength, lower-body hypertrophy, power, VO₂max, and body fat. * Significant difference from strength training ($p < 0.05$). & Significant difference from endurance training ($p < 0.05$). (Wilson et al. 2012)

Many studies have found that combined training of strength and endurance has a positive effect on health and sports performance, and it can be more effective in improving physical fitness, body composition, and metabolic health than either method alone (Sillanpää et al. 2009, Sillanpää et al. 2010). For example, Sillanpää et al. (2010) investigated concurrent strength and endurance training in healthy middle-aged women. The results revealed that combined training for 21 weeks led to marked improvements in cardiorespiratory fitness, body composition, and metabolic risk factors. Total body lean mass, maximal cycling power, and knee extension strength were increased significantly.

It should be noticed that most of the studies investigating combined training have been performed in men. Gender differences in basal hormone levels and hormone metabolism should be considered in endocrine research on the female population. (Asikainen et al. 2004, Consitt et al. 2002, Sillanpää et al. 2009.) The table 1 represents the summary of hormone adaptations to training in women. However, little is known how metabolic

health and fitness is influenced by prolonged combined endurance and strength training in women. (Asikainen et al. 2004, Sillanpää et al. 2009.) In recent years, exercise endocrinological research on women has started to accumulate (Consitt et al. 2002) but research about effects of hormonal contraceptive use on combined strength and endurance training is non-existent.

TABLE 1. Summary of research on anabolic and catabolic hormone adaptations to endurance and strength training in women. ↑ indicates increases; ↓ indicates decreases; ↔ indicates no changes or equivocal results, number of arrows indicates the strength of research. (Modified from Consitt et al. 2002)

	Endurance training	Strength training
Testosterone	↓↔	↑↔
Estradiol	↓	↔
Growth hormone	↑↔	↔
IGF-1	↑↔	↑↔
Cortisol	↔↔↔	↓↔

4 BODY COMPOSITION AND SEX HORMONES

Sex steroids have been shown to be associated with metabolic function and mechanisms of regulation (Rickenlund et al. 2004). Because of the regional distribution of receptors for sex steroid hormones, there is a gender difference in fat accumulation. In premenopausal women, for example, estrogens increase the amount of fat accumulation in the subcutaneous tissues. (Borer 2003, 119.) In addition, progesterone may have effects on bodyweight because of water regulation and fluid retention via aldosterone (Burrows & Peters 2007). In athletes, even small changes in body composition may have a huge impact on performance (Rickenlund et al. 2004).

4.1 Effect of the menstrual cycle on bodyweight

There are potential changes in the distribution of body fluids throughout the menstrual cycle since many women report changes in bodyweight and a bloated feeling (Janse de Jonge 2003). However, most studies with hormone verification have not found significant changes in bodyweight over the normal menstrual cycle (Casazza et al. 2002, Lebrun et al 1995, Redman et al. 2003). Thus, it is suggested that estrogen and progesterone changes during the menstrual cycle do not significantly affect fluid regulation and body weight (Janse de Jonge 2003).

4.2 Effect of hormonal contraceptives on body composition

Despite worldwide use of hormonal contraceptives, the effects on body composition are not clear. Individual responses to hormonal contraceptive use may involve some weight gain as a result of either fluid retention or appetite stimulation (Rickenlund et al. 2004, Rosenberg et al 1998). In addition, significant increases (1% to 5%) in total body fat percentage have been reported with triphasic hormonal contraceptive use (Casazza et al. 2002, Lebrun et al. 2003, Suh et al. 2003) and monophasic hormonal contraceptive use (Rickenlund et al. 2004). For example, Casazza et al. (2002) and Suh et al. (2003) demonstrated that 4 months of a low-dose, triphasic hormonal contraceptive use significantly increased body weight and fat mass in moderately physically active young

women. Similarly, Lebrun et al. (2003) reported in a double blind, placebo controlled trial a significant increase in the sum of skinfolds in women taking triphasic hormonal contraceptive compared with those taking placebo (Figure 10).

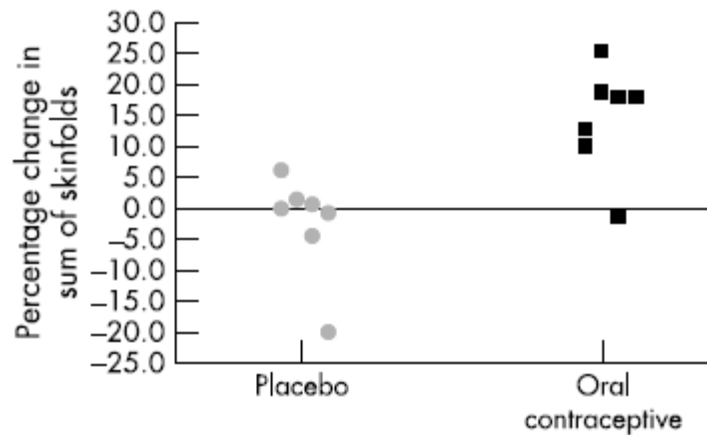


FIGURE 10. Percentage change in the sum of skinfolds for women in the placebo and oral contraceptive groups (Lebrun et al. 2003)

Rickenlund et al. (2004) investigated if hormonal contraceptive use affects body composition and physical performance in female endurance athletes with and without menstrual disturbances and in sedentary controls. There were significant increases in weight and fat mass only in the athlete group with menstrual disturbances and not in athletes or controls with regular menstruation. In addition, hormonal contraceptive treatment significantly increased bone mineral density (BMD) in those with low BMD at baseline. They concluded that hormonal contraceptive treatment in female athletes has primarily beneficial effects on body composition without adverse effects on physical performance. (Rickenlund et al. 2004.)

However, some studies have not found an overall effect of hormonal contraceptive use on body composition or weight (Lloyd et al. 2002, Rosenberg et al 1998). For example, Lloyd et al. (2002) demonstrated that the use of hormonal contraceptive in young women was associated with less favorable blood lipid patterns, but not with weight gain or increased body fat.

Overall, it seems that the increases in body mass and body fat percentage occur within the first few months of hormonal contraceptive use (Lebrun et al. 2003, Suh et al. 2002). In addition, the effect of hormonal contraceptives on body composition depends on the potency and androgenicity of the progesterone within the hormonal contraception pill. Triphasic formulations with higher progestogenic and androgenic activity may have more pronounced effects on body composition in the short term compared with formulations with lower potency and androgenicity. (Burrows & Peters 2007, Casazza et al. 2002, Suh et al. 2003.)

5 PURPOSE OF THE STUDY

This study was designed to investigate effects of 10 weeks high-intensity combined strength and endurance training on serum hormone levels and physical performance in recreationally active women. The primary focus of this study was to examine effects of hormonal contraceptives on hormonal, neuromuscular and cardiorespiratory adaptations when training with high intensity. The secondary focus of this study was to examine the influence of hormonal contraceptives on changes in body composition.

The research questions are as follows:

- 1) How does the neuromuscular and cardiorespiratory performance change after 10 weeks of high-intensity combined strength and endurance training?
- 2) Will combined strength and endurance training cause changes in the basal serum hormone levels?
- 3) Are there differences in the changes of serum hormone levels or physical performance between hormonal contraception users and non-users?
- 4) Are there differences in the changes of body composition between hormonal contraception users and non-users?

6 METHODS

6.1 Subjects

Twenty-four healthy women aged 18–40 from the Jyväskylä region were recruited to participate in the study. Requirements for participation included the subjects to be recreationally active, BMI of less than 30 kg/m² to avoid pronounced overweight, and a Cooper running test result of minimum of 2300m. In addition, subjects were required to be free of any chronic illnesses, musculoskeletal and cardiac problems that would prohibit resistance and endurance training and testing. Training background of the subjects consisted of different endurance activities such as jogging, orienteering, and cross-country skiing, but no elite athletes were included in the study.

All subject candidates completed a health questionnaire and a screening of resting ECG before inclusion in the study. Subjects agreed to participate after receiving detailed information about the upcoming measurements and procedures. They were informed about their possibility to drop out of the study at any time. All subjects signed an informed consent document prior to participation in the study. The methodology of the present study was approved by the Ethical Committee at the University of Jyväskylä, and by the Ethical Committee of the Central Finland Health Care District.

Five of the chosen subjects had to drop out during the control period, because of personal reasons. After the control period 19 subjects started the intervention, and all of them were able to finish the entire 10 week intervention. The study group of 19 subjects consisted of 11 women who had at least one year of hormonal contraceptive use (HC), and 8 women who had never used hormonal contraceptives (NHC). The anthropometric data of the subjects is presented in table 2.

Hormonal contraception products used by the subjects in the study consisted of combined pills and progesterone-only pills, as well as two different intrauterine systems. All the hormonal contraception products used by the subjects are presented in table 3.

TABLE 2. Anthropometric data of hormonal contraception group (HC) and non-hormonal contraception group (NHC) measured at PRE-tests. (mean \pm SE)

Group	n	Age (years)	Height (cm)	Weight (kg)	BMI(kg/m²)	Body fat (%)
HC	11	29.2 \pm 4.2	167.4 \pm 5.0	59.6 \pm 5.1	21.3 \pm 1.9	23.3 \pm 6.4
NHC	8	30.5 \pm 5.1	167.6 \pm 4.9	60.4 \pm 6.2	21.5 \pm 1.8	23.6 \pm 7.2

TABLE 3. Hormonal contraception used in this study

Name of the product	Type	Active ingredients
Mercilon	Combined pill Monophasic	Ethinylestradiol, Desogestrel
Femoden	Combined pill Monophasic	Ethinylestradiol, Gestodene
Yasminelle	Combined pill Monophasic	Ethinylestradiol, Drospirenone
YAZ	Combined pill Monophasic	Ethinylestradiol, Drospirenone
Meliane	Combined pill Monophasic	Ethinylestradiol, Gestodene
Cypretyl	Combined pill Monophasic	Ethinylestradiol, Cyproterone
Mini-Pill (Pfizer)	Progesterone-only pill	Norethisterone
Cerazette	Progesterone-only pill	Desogestrel
Jaydess	Intrauterine system	Levonorgestrel (13.5 mg)
Mirena	Intrauterine system	Levonorgestrel (52 mg)

6.2 Study design

This study was a part of a larger research project (VoKe project) which was conducted between June and December 2014. Subjects acted as their own controls over a 2-3 month period prior to the 10 week high-intensity combined strength and endurance training intervention. All subjects were initially familiarized with the training- and measurement protocols of the current study before proceeding to basal measurements of maximal strength and endurance. The measurements were done prior to the control period (CTRL), as well as at the beginning (PRE), in the middle (MID), and at the end (POST) of 10 week intervention. Results used in this thesis are only the results of the PRE- and POST-measurements of the 10 week intervention period. The study design is presented in figure 11.

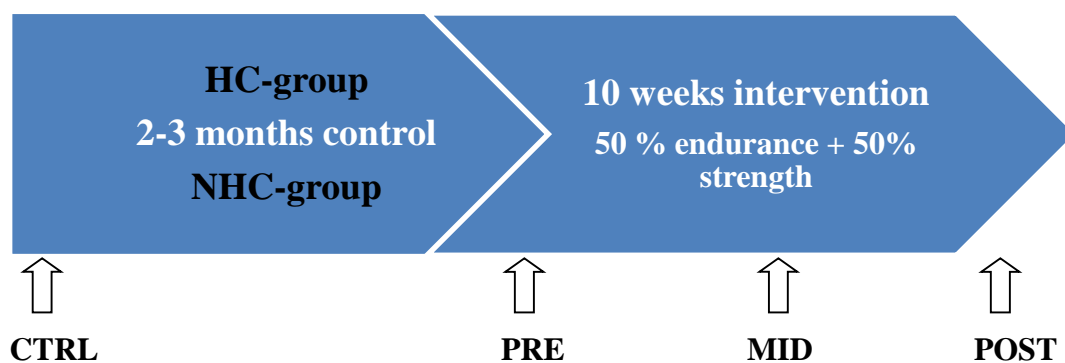


FIGURE 11. Study design for hormonal contraception group (HC) and non-hormonal contraception group (NHC).

6.2.1 Physical training

High-intensity combined strength and endurance training over a 10 week training period was planned to improve body composition and metabolic function. A total of four high intensity training sessions per week was performed by each subject and consisted of two strength training sessions and two running interval training sessions. In addition, subjects were recommended to complete one low-intensity aerobic exercise as a recovering exercise every week. Other physical activity was allowed, as long as it did not inhibit the

good quality of training sessions in the intervention. This physical activity was recorded in training diaries (not reported in this thesis).

Subjects were instructed to do two days of training in a row followed by a rest day, but not to do two same training modalities in subsequent days. In order to control the circadian variations in hormones and muscle strength, the training sessions were completed at the same time of day.

During the 10-week intervention subjects executed 99.1% of the training sessions. Each training session took 45–90 minutes. The intervention was progressive, as the training intensity of the two first training weeks was lower, and the training intensity in strength and endurance training was increased throughout the training period. However, due to MID- and POST-measurements on training weeks five and ten, these weeks were reduced on volume with only one strength workout and one endurance workout.

6.2.2 Strength training

In order to increase muscle strength and induce positive changes in body composition, the strength training sessions twice a week consisted of combined maximal and explosive strength training. The strength training was targeted for the lower extremities, and every training session consisted of several multi-joint movements with high loads, and continued with a biomechanically similar power movement. For example, heavy squats were followed by countermovement jumps, and calf raises were followed by calf jumps. For the main exercises there was no exercise order. Loads in the main exercises increased progressively during the training period from 50 to 85% 1 RM, depending on the exercise. The overview of every strength training session is presented in table 4.

Strength training sessions were performed in a gym that was built for research purposes and supervised by members of research staff. All performed sets, reps and loads were recorded in individual training diaries.

TABLE 4. Overview of strength training sessions. The dispersion in the load means that at the beginning of study the intensity was lower and was increased throughout the study.

Main exercises (twice a week)				
	Sets	Reps	Load (%1 RM)	Rest (min)
Squats (~100° knee angle)	1–2 warm up	8–10	50–70	2
	1 maximal	5–6	70–85	2
Countermovement jumps (~100° knee angle)	1 explosive	10	bodyweight	2
Leg press	1–2 warm up	8–10	50–70	2
	1 maximal	5–6	70–85	2
	1 explosive	10	30	2
Knee flexion	1–2 warm up	8–10	50–60	2
	1 maximal	8–10	70–85	2
Calf raise	1 warm up	8–10	50–70	2
	1 maximal	5–6	70–85	2
Calf jump	1 explosive	6	bodyweight	2

After the main exercises listed in table 4, subjects performed plyometric exercises that are presented in table 5. Exercises from list A (drop jumps and plyometric strides) were performed in the first strength training session of the week, and exercises from list B (step-ups and hurdle jumps) were performed in the second strength training session.

After the plyometric exercises, subjects performed core exercises, such as plank, back extension, and torso rotation. Core exercises are presented in table 6.

TABLE 5. Plyometric exercises

A (once a week)				
	Sets	Reps	Load	Rest (min)
Drop jumps	2 explosive	6	bodyweight	2
Plyometric strides	2 explosive	5+5	bodyweight	2
B (once a week)				
Step-ups (using a bench)	2 explosive	10	bodyweight	2
Hurdle jumps	2 explosive	5	bodyweight	2

TABLE 6. Upper body and core exercises

In every training session				
	Sets	Reps	Load	Rest (min)
Bench press	2–4	8–10	70–80% 1RM	1–2
Plank	2	60 sec	bodyweight	2
Back extension	2	10–15	0–10 kg	2
Torso rotation	2	10–15	0–10 kg	2

6.2.3 Endurance training

Endurance training sessions included high-intensity interval training and sprint training. High-intensity interval training included 4x4 min running intervals at 70–90% of heart rate max with 4 min rests between. This training method is based on the article by Helgerud et al. (2007). In order to improve subjects' running velocity endurance training included also sprint training with 3x3x100 m sprints, with 2 min rest after every sprint and 5 min rest between sets. Overview of the endurance training sessions is presented in table 7.

For both endurance training sessions, the warm-up consisted of 10 min and cool-down of 15 min at 60–70% heart rate max. In addition, subjects performed some dynamic stretching in warm-up and cool-down.

Most of the endurance training sessions were supervised by the members of research staff, but subjects were also allowed to perform endurance training on their own. Heart rate values were recorded from every interval and used in the determination of the training intensity.

TABLE 7. Overview of the endurance training sessions. The dispersion in intensity means that the intensity was increased throughout the study.

	Sets	Reps	Intensity (%HRmax)	Rest (min)
Running	4	4 min	75–90%	4 (60–70% HRmax)
Running	3x3	100 m	80–100%	2 (60–70% HRmax) (5 min between sets)

6.3 Data collection and analysis

PRE- and POST-measurements consisted of anthropometric measurements, strength and neuromuscular fitness measurements, cardiorespiratory fitness measurements, field tests, and collection of blood samples. The number of measurements was reduced in the MID-measurements with only collection of blood samples and a lower number of strength and neuromuscular fitness measurements. The CTRL-, PRE-, MID- and POST-measurements were done at the same time of day for each subject to control for circadian variations. Results used in this thesis are only the results from PRE- and POST-measurements.

6.3.1 Anthropometrics and body composition

Anthropometric measurements were completed in a fasted (12h) state and in underwear. The height of each subject was measured using standard methods. In order to give

immediate feedback to subjects, muscle and fat tissue estimates were measured with bioimpedance (Inbody 3.0, Biospace Co). Estimates of muscle and fat tissues as well as bone mineral density for the whole body, trunk, android, and both arms and legs, were measured using dual-energy x-ray absorptiometry (DXA) (Lunar Prodigy Advance, GE Medical Systems – Lunar, Madison WI USA). Subjects were scanned using the default scan mode for total body scanning automatically selected by the Prodigy software (enCORE 2005, version 9.30 and Advance 12.30).

6.3.2 Neuromuscular performance

Prior to strength and neuromuscular measurements, subjects warmed up on a bicycle ergometer for 5 min at the intensity of the subjects' choice. Strength was measured using both isometric and dynamic actions. PRE- and POST-measurements were similar, and consisted of all the following measurements. In MID-measurements the number of tests was reduced, and it consisted of countermovement jump, isometric leg press, isometric knee extension and isometric knee flexion only.

Maximal bilateral isometric strength and rate of force development (RFD). Isometric strength of the leg extensor muscles was measured using an electromechanical isometric leg extension device. The horizontal leg press is presented in figure 12. (Designed and manufactured by the Department of Biology of Physical Activity, University of Jyväskylä, Finland). In this test, the subjects were in a sitting position so that the knee angle was 107°, measured using the greater trochanter, lateral tibiofemoral joint space and lateral malleolus as reference points. The subjects were instructed to exert their maximal force as fast as possible during a period of approximately 3 s. A minimum of three trials was completed for each subject. If the maximum force during the last trial was greater than 5% compared to the previous one, an additional trial was performed. However, no more than five maximal trials were performed. The best performance in terms of maximal force measured in Newtons (N) was used for statistical analysis. Force data was collected at a sampling frequency of 2000 Hz, and then filtered (20 Hz low pass filter). Force development was assessed over 20 ms (± 10 s from maximal rate of force development). Force data was analyzed using customized scripts (Signal 4.04, CED, UK).



FIGURE 12. The isometric leg press used in evaluation of isometric strength of leg extensor muscles.

Maximal unilateral isometric knee extension and flexion. A David 200 dynamometer modified for strength testing (Figure 13) was used to measure the unilateral isometric knee extension and flexion force. Subjects' hips were stabilized with seat belt, and in isometric knee extension the subjects' ankle was secured to the cushion in order to inhibit any possible kicking effect. Knee angle was adjusted to be 107 degrees, using the same reference points as used for maximal bilateral isometric leg press. The dynamometer was re-adjusted for measurement of knee flexion. In addition, the measurement protocol, force collection and computer program were the same as with isometric leg press. On verbal command, the subject performed a maximum isometric knee flexion and extension.



FIGURE 13. The modified David 200 used for isometric knee extension and isometric knee flexion.

Maximal bilateral dynamic leg press. Maximal bilateral dynamic concentric strength was estimated with one repetition maximum (1RM) of leg press (Figure 14, David Sports Ltd., Helsinki, Finland). At first subjects performed sub-maximal warm-up sets with increasing weight and decreasing repetitions: 5 x ~70% 1RM, 2 x ~80-85% 1RM and 1 x ~90-95% of estimated 1RM, with one minute of rest between sets. Following this warm-up, the greatest weight that the subject could successfully lift was determined as 1RM. The result was recorded with the accuracy of 1.25 kg. Verbal encouragement was given to promote maximal effort. The action started from a knee angle of approximately 60°, and legs were extended to a full range of motion (180°). Subjects were, however, instructed not to lock their knee joints at full extension, and to keep constant contact with the seat and backrest during leg extension.



FIGURE 14. The maximal bilateral dynamic leg press used in the estimation of one repetition maximum (1RM).

Countermovement jump (CMJ). Dynamic explosive force characteristics of the leg muscles were measured on a force platform by using countermovement jump (CMJ). Subjects were instructed to jump as high as possible with an explosive countermovement action before the concentric phase of the movement. Subjects were instructed to do as deep and quick a countermovement as they can. The hands were kept on the hips during the jump. When ground contact was achieved subjects were allowed to bend their knees to ease off the landing. Subjects performed three jumps with one minute rests between. The jump height was calculated from the flight time (Signal 4.10, CED, UK).

Upper body isometric strength. Upper body strength was measured using isometric bench press (Modified David 200). A modified David 200 dynamometer was applied for the

recording of the bilateral isometric force of the bench press action, including the triceps brachii, anterior deltoid, and pectoralis major muscles. Subjects sat on the dynamometer and pushed with their arms against a horizontal bar with their elbows at 90°.

Dynamic muscle endurance. Dynamic muscle endurance tests consisted of sit-ups, push-ups and standing long jump according to the guidelines of the Finnish Defense Forces (Pihlainen et al. 2011, 41–43). Sit-ups and push-ups were performed as many repetitions as possible in 1 min with good technique. Standing long jump was performed into a sand box.

6.3.3 Cardiorespiratory performance

Cardiorespiratory performance was measured in a similar way in PRE- and POST-measurements. Both field and laboratory tests were used.

Field test. A 3000 m time trial was used as a field test. It was performed in indoor athletics track on a 200 m track. After a 10 min warm-up subjects ran 3000 m as fast as they could. Split times for each kilometer were recorded.

Laboratory test. An incremental exercise test to exhaustion on a treadmill was used as a laboratory test. In the treadmill test the incline remained a constant 1-degree throughout the test, but the running velocity began at 7–9 km·h⁻¹ and was increased by 1 km·h⁻¹ every third minute until volitional exhaustion. Heart rate was monitored using a heart rate monitor (Suunto t6, Vantaa, Finland), and mean heart rate values from the last minute of each stage were used for analysis. Expired gases were continuously sampled and analyzed breath-by-breath throughout the test using a portable gas analyzer (Oxycon Mobile®, Jaeger, Hoechberg, Germany).

Maximal oxygen uptake (VO_{2max}) was determined to the highest average of oxygen consumption (VO₂) value of 1 min. Other factors such as a heart rate, and respiratory exchange ratio were monitored for determination of maximal effort. Fingertip blood samples were taken in the end of each work stage to measure blood lactate concentrations. For blood sampling, the treadmill was stopped for approximately 15–20 seconds. Blood

lactates were analyzed using a Biosen S_line Lab+ lactate analyzer (EKF Diagnostic, Magdeburg, Germany). Lactate threshold (LT) and respiratory compensation threshold (RCT) were determined using blood lactate, ventilation, oxygen consumption (VO_2) and production of carbon dioxide (VCO_2).

6.3.4 Blood samples

Resting venous blood samples were collected from participants in a fasted state (12 h). Blood samples were collected using sterile needles into serum tubes (Venosafe, Terumo Medical Co., Leuven, Belgium) by a qualified lab technician. Whole blood was spun for 10 minutes at 2500 rpm (Megafuge 1.0R, Heraeus, Germany). Serum samples were frozen at -80°C until analysis.

Blood samples were collected for the determination of total testosterone (TES), follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), progesterone (P), sex hormone binding globulin (SHBG), cortisol (C), insulin-like growth factor I (IGF-I) and its binding proteins. Analyses were performed using chemical luminescence techniques (Immulite 2000 XPi, Siemens, Llanberis, UK) and hormone specific immunoassay kits (Siemens, New York, NY, USA). All samples of the test subject were analyzed in the same assay for each hormone.

The sensitivity for serum hormones were 0.5 nmol/l (TES), 0.1 mIU/ml (FSH), 0.1 mIU/l (LH), 55 pmol/l (E2), 0.1 ng/ml (P), 0.2 nmol/l (SHBG), 5.5 nmol/l (C), and 0.26 nmol/l (IGF-I). The inter assay coefficients of variation were 9.4% (TES), 6.0% (FSH), 7.8% (LH), 12.0% (E2), 8.5% (P), 5.4% (SHBG), 6.6% (C), and 6.4% (IGF-1).

6.3.5 Menstrual cycle

The menstrual cycle phases were estimated from the first menstrual bleed. The subjects were instructed to schedule a measurement at the beginning of the cycle, between days 1–7.

6.4 Statistical Analysis

All data was analyzed and graphed using Microsoft Excel 2010 and IBM SPSS Statistics computer software. Independent-samples T-test was used for analyzing between group differences. Since the number of subjects was low, even non-parametric Wilcoxon-Mann-Whitney test was used. Repeated levels ANOVA with two levels (PRE, POST) was used for analyzing within group differences at different time points. The significance for all tests was set at * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. In addition, # $p < 0.075$ presents a statistical trend.

7 RESULTS

7.1 Changes in neuromuscular performance

Overall, the study group improved both power and strength characteristics, such as isometric leg press by $11\pm 12\%$ ($p<0.001$), isometric knee extension by $6\pm 10\%$ ($p<0.05$), isometric knee flexion by $11\pm 15\%$ ($p<0.01$), isometric bench press by $8\pm 9\%$ ($p<0.001$), dynamic leg press by $8\pm 5\%$ ($p<0.001$), countermovement jump by $8\pm 7\%$ ($p<0.001$), 1 min push-ups by $16\pm 16\%$ ($p<0.001$), and 1 min sit-ups by $18\pm 20\%$ ($p<0.01$). When comparing the groups, there were no significant differences neither in PRE- nor POST-measurements. In other words, neuromuscular adaptations were similar in both groups.

The hormonal contraception users (HC) improved isometric leg press by $14\pm 15\%$ ($p<0.05$), isometric knee flexion by $11\pm 14\%$ ($p<0.05$), isometric bench press by $9\pm 7\%$ ($p<0.001$), dynamic leg press by $10\pm 5\%$ ($p<0.001$), countermovement jump by $6\pm 7\%$ ($p<0.01$), push-ups by $14\pm 16\%$ ($p<0.05$) and sit-ups by $22\pm 25\%$ ($p<0.05$). While, the non-users (NHC) improved isometric leg press by $6\pm 6\%$ ($p<0.05$), isometric knee extension by $9\pm 11\%$ ($p<0.05$), dynamic leg press by $7\pm 4\%$ ($p<0.01$), countermovement jump by $11\pm 6\%$ ($p<0.001$), push-ups by $20\pm 17\%$ ($p<0.05$) and sit-ups by $13\pm 11\%$ ($p<0.05$).

The strength results are presented in table 8. In addition, bilateral dynamic leg press and countermovement jump results are illustrated in figures 15 and 16.

TABLE 8. Strength characteristics in PRE- and POST-measurements for the whole study group (ALL), hormonal contraception (HC) and non-hormonal contraception groups (NHC). (mean \pm SE). * Presents significant within-group difference from PRE-measurements (* p <0.05, ** p <0.01 and *** p <0.001). (ALL: n =19, HC: n =11, NHC: n =8)

	ALL		HC		NHC	
	PRE	POST	PRE	POST	PRE	POST
Isometric leg press (N)	2744 \pm 509	3033 \pm 636***	2812 \pm 377	3206 \pm 621*	2651 \pm 669	2796 \pm 615*
Isometric knee extension (N)	742 \pm 103	792 \pm 160*	733 \pm 105	762 \pm 138	755 \pm 106	832 \pm 188*
Isometric knee flexion (N)	310 \pm 59	342 \pm 66**	304 \pm 69	336 \pm 83 *	319 \pm 47	350 \pm 35
Isometric bench press (N)	545 \pm 99	586.4 \pm 98 ***	533 \pm 88	580 \pm 93***	561 \pm 118	595 \pm 114
Dynamic leg press (kg)	116 \pm 16	126 \pm 18***	112 \pm 15	123 \pm 17***	123 \pm 16	131 \pm 19**
CMJ (cm)	25.8 \pm 4.1	27.9 \pm 4.3***	25.0 \pm 3.7	26.5 \pm 4.0**	26.9 \pm 4.6	29.7 \pm 4.1***
1 min push-ups	35 \pm 11	41 \pm 14***	33 \pm 13	37 \pm 5*	37 \pm 10	45 \pm 16*
1 min sit-ups	35 \pm 9	40 \pm 7**	32 \pm 8	38 \pm 5*	38 \pm 9	42 \pm 9*
Standing long jump (cm)	206 \pm 16	209 \pm 14	197 \pm 13	202 \pm 9	218 \pm 11	219 \pm 14

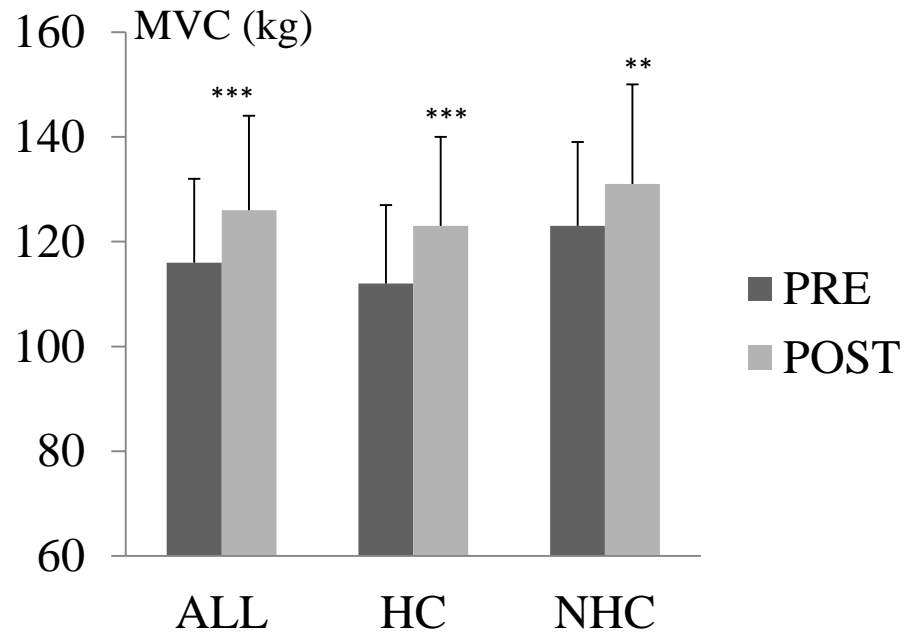


FIGURE 15. Bilateral dynamic leg press results presented in absolute values (kg) in PRE- and POST-measurements for the whole study group (ALL), hormonal contraception (HC) and non-hormonal contraception groups (NHC). * Presents significant within-group difference from PRE-measurements (** $p < 0.01$, *** $p < 0.001$).

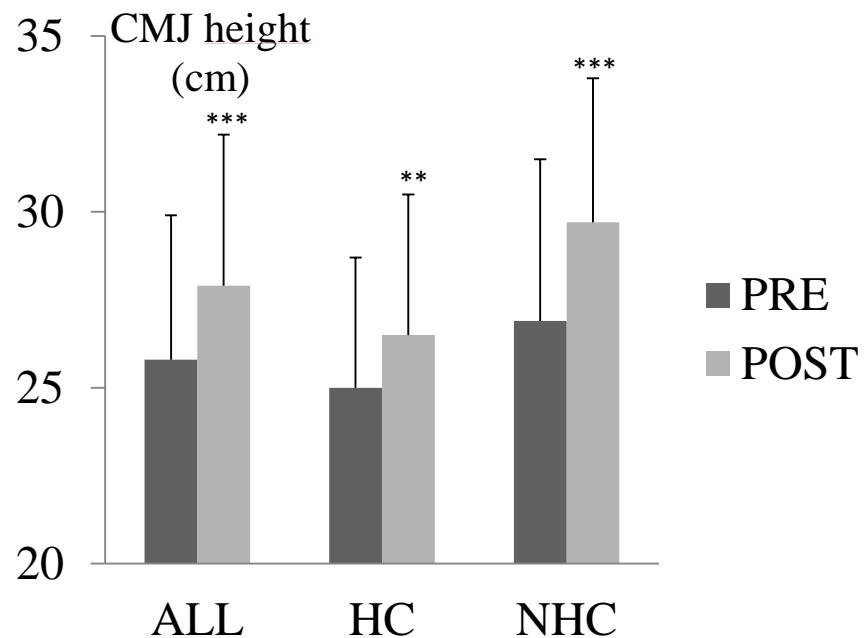


FIGURE 16. Countermovement jump heights in absolute values (cm) in PRE- and POST-measurements for the whole study group (ALL), hormonal contraception (HC) and non-hormonal contraception groups (NHC). * Presents significant within-group difference from PRE-measurements (** $p < 0.01$, *** $p < 0.001$).

7.2 Changes in cardiorespiratory performance

The overall changes in maximal oxygen consumption (VO_{2max}) were negligible when analyzing the incremental exercise tests results. There were no significant differences between groups in VO_{2max} neither in PRE- nor POST-measurements. Neither of the groups had statistically significant changes in VO_{2max} . The results are presented in table 9.

The overall improvement in 3000 m time trial was $2\pm 3\%$ ($p < 0.05$) when analyzing all subjects as a one group. When comparing the groups, there were no significant differences between groups in the 3000 m time trial neither in PRE- nor POST-measurements. The group using hormonal contraception (HC) decreased their running time by an average of $18\pm 32s$ ($2\pm 4\%$, $p = 0.162$) and the non-hormonal contraception group (NHC) decreased their running time by an average of $20\pm 16s$ ($3\pm 2\%$, $p = 0.018$). The improvement of the 3000 m time trial was significant only for the non-hormonal contraception group (NHC) ($p < 0.05$), but there was not a significant difference between groups' improvements ($p = 0.559$). The 3000 m time-trial results are presented in table 9.

TABLE 9. Maximal oxygen consumption (VO_{2max}) results presented in $ml \cdot kg^{-1} \cdot min^{-1}$ and 3000 m time-trial (TT) results presented in seconds for the whole study group (ALL), hormonal contraception (HC) and non-hormonal contraception groups (NHC). (mean \pm SE). * Presents significant within-group difference from PRE-measurements ($*p < 0.05$). (ALL: n=19, HC: n=11, NHC: n=8)

	ALL		HC		NHC	
	PRE	POST	PRE	POST	PRE	POST
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	42.8 \pm 4.1	42.7 \pm 3.8	43.7 \pm 4.3	43.3 \pm 3.1	41.3 \pm 3.6	41.7 \pm 4.9
3000 m TT (s)	813.0 \pm 86.8	794.6 \pm 73.2*	815.0 \pm 83.2	797.3 \pm 66.0	810.0 \pm 100.0	790.5 \pm 89.4*

7.3 Changes in body composition

Overall, body mass was unaltered, fat free mass was increased by $1\pm 1\%$ ($p<0.001$) and total fat was reduced by $5\pm 8\%$ ($p<0.05$). When comparing the groups, there were no significant differences in the body composition neither in PRE- nor POST-measurements. However, a trend for greater increase in fat free mass was observed in NHC than in HC women ($p=0.065$). In the HC group fat free mass was increased by $1\pm 1\%$ ($p=0.027$) and in the NHC group by $2\pm 1\%$ ($p=0.000$). In addition, the decrease in total body fat was significant only within the NHC group ($p<0.05$), but there was not a significant difference between groups' improvements ($p=0.906$). Body fat was decreased by $5\pm 10\%$ ($p=0.158$) in the HC group and by $5\pm 6\%$ ($p=0.030$) in the NHC group. The body composition results are presented in table 10.

TABLE 10. Body composition results of PRE- and POST-measurements for all subjects (ALL), hormonal contraception (HC) and non-hormonal contraception groups (NHC). (mean \pm SE). * Presents significant within-group difference from PRE-measurements (* $p<0.05$, *** $p<0.01$). (ALL: $n=19$, HC: $n=11$, NHC: $n=8$)

	ALL		HC		NHC	
	PRE	POST	PRE	POST	PRE	POST
Body mass (kg)	59.9 \pm 5.3	59.8 \pm 5.0	59.6 \pm 4.9	59.5 \pm 5.0	60.4 \pm 6.2	60.4 \pm 5.5
Fat free mass (kg)	46.2 \pm 3.3	46.9 \pm 3.5***	46.2 \pm 3.8	46.6 \pm 3.9*	46.4 \pm 2.8	47.4 \pm 2.9***
Total fat (%)	23.4 \pm 3.3	22.3 \pm 6.3*	23.3 \pm 6.4	22.4 \pm 6.5	23.6 \pm 7.2	22.3 \pm 6.4*

7.4 Hormonal adaptations

Overall, there were no changes in serum hormone levels from PRE- to POST-measurements. When comparing the groups there were no significant between-group differences in PRE- or POST-measurements. However, a statistical trend was observed as HC group had lower levels of luteinizing hormone in PRE-measurement ($p=0.073$) and lower levels of estradiol in POST-measurements ($p=0.063$) compared to the NHC group.

HC group did not experience significant changes in hormonal levels between PRE- and POST-measurements. In NHC group progesterone was increased significantly ($p<0.05$) from PRE- to POST-measurements. In addition, the decrease of luteinizing hormone from PRE- to POST-measurements showed a statistical trend ($p=0.074$).

Blood sample of POST-measurement was missing for one person in the NHC group, so sample size for hormonal data is 7 for the NHC group. Hormonal levels of both groups are presented in table 11.

TABLE 11. Serum hormonal levels of PRE- and POST-measurements for the whole study group (ALL), hormonal contraception (HC) and non-hormonal contraception groups (NHC). (mean \pm SE). * Presents significant within-group difference from PRE-measurements (* p <0.05). # Presents a statistical trend compared to NHC group (p <0.075). (ALL: n =18, HC: n =11, NHC: n =7)

	ALL		HC		NHC	
	PRE	POST	PRE	POST	PRE	POST
Testosterone (nmol/l)	0.6 \pm 0.4	0.5 \pm 0.2	0.7 \pm 0.4	0.5 \pm 0.2	0.4 \pm 0.3	0.5 \pm 0.2
Cortisol (nmol/l)	520.0 \pm 155.0	536.0 \pm 189.0	539.0 \pm 174.0	541.0 \pm 194.5	488.0 \pm 128.0	527.0 \pm 194.0
SHBG (nmol/l)	78.5 \pm 50.1	85.2 \pm 61.5	93.2 \pm 64.3	100.5 \pm 81.5	61.7 \pm 24.3	67.8 \pm 21.0
IGF1 (nmol/l)	29.6 \pm 7.4	28.9 \pm 8.2	30.3 \pm 7.1	29.5 \pm 8.8	28.6 \pm 8.5	28.1 \pm 7.7
Progesterone (ng/ml)	0.5 \pm 0.8	0.8 \pm 2.2	0.7 \pm 0.9	1.3 \pm 2.8	0.3 \pm 0.2	0.4 \pm 0.2*
LH (mIU/l)	5.0 \pm 3.4	4.3 \pm 2.3	3.8 \pm 3.8#	3.7 \pm 2.5	6.7 \pm 1.5	5.2 \pm 1.9
FSH (mIU/l)	7.5 \pm 4.2	6.3 \pm 3.8	6.3 \pm 4.5	6.1 \pm 4.2	8.8 \pm 3.2	6.5 \pm 2.7
Estradiol (pmol/l)	216.0 \pm 281.3	237.0 \pm 203.0	226.6 \pm 344.3	147.8 \pm 73.1#	198.3 \pm 161.2	377.0 \pm 264.7

7.5. Associations between changes in physical performance and serum hormones

Associations between changes in physical performance and serum hormones are presented by the Pearson's correlation. The correlations were examined comparing the change in physical performance characteristics and the PRE-values of hormones, as well as the change in hormones between PRE- and POST-measurements ($\Delta\%$). All significant correlations between physical performance and PRE-levels of serum hormones are presented in table 12, and changes in hormones in table 13. The corresponding correlations for all groups, even non-significant, are presented in the tables for the sake of comparison.

Baseline testosterone levels correlated positively with the change in isometric knee extension ($r=0.61$; $p<0.05$) and flexion ($r=0.70$; $p<0.05$) in the HC group, but negatively with the change in isometric knee flexion ($r=-0.78$; $p<0.05$) in the NHC group. Baseline cortisol levels correlated positively with the change in isometric knee flexion ($r=0.61$; $p<0.05$) and with the change in isometric bench press ($r=0.62$; $p<0.05$) in the HC group. Baseline SHBG levels were associated with the change in isometric leg press ($r=0.83$; $p<0.05$) in the NHC group, and with the change in isometric knee extension ($r=0.74$; $p<0.05$) in the HC group. In addition, a high negative correlation was observed between SHBG PRE and the change in 1 min push-ups ($r=-0.93$; $p<0.01$) in the NHC group. Baseline progesterone levels correlated positively with the change in 3000 m time trial ($r=0.61$; $p<0.05$) and with the change in standing long jump ($r=0.57$; $p<0.05$) in the whole study group (ALL). In addition, there was a high negative correlation between progesterone PRE and the change in VO_{2max} in the whole study group ($r=-0.75$; $p<0.01$) and in the HC group ($r=-0.79$; $p<0.01$).

Correlation coefficients between changes in physical performance and hormone PRE-values are illustrated in table 12. Figures 17–19 represent correlations for the whole study group (ALL), HC group, and NHC group, respectively.

TABLE 12. Correlation coefficients between changes in physical performance ($\Delta\%$) and hormone PRE-values. (ALL: n= 18, HC: n=11, NHC: n=7)

	ALL		HC		NHC	
	r	p	r	p	r	p
Isometric leg press ($\Delta\%$)						
FSH PRE	0.411	0.09	0.691*	0.018	-0.062	0.895
SHBG PRE	0.162	0.548	0.01	0.979	0.833*	0.020
Isometric knee extension ($\Delta\%$)						
Testosterone PRE	0.239	0.339	0.611*	0.046	0.148	0.751
SHBG PRE	0.307	0.247	0.736*	0.024	0.091	0.846
Progesterone PRE	-0.444	0.065	-0.627*	0.039	0.177	0.704
Isometric knee flexion ($\Delta\%$)						
Testosterone PRE	0.169	0.504	0.697*	0.017	-0.781*	0.038
Cortisol PRE	0.172	0.495	0.614*	0.045	-0.585	0.168
Isometric bench press ($\Delta\%$)						
Cortisol PRE	0.284	0.253	0.619*	0.042	-0.213	0.647
VO_{2max} ($\Delta\%$)						
Progesterone PRE	-0.752**	0.000	-0.785**	0.004	-0.565	0.186
FSH PRE	0.486*	0.041	0.513	0.107	0.305	0.506
Time in 3000m time-trial ($\Delta\%$)						
Progesterone PRE	0.611*	0.02	0.652	0.057	-0.354	0.559
1 min sit-ups ($\Delta\%$)						
SHBG PRE	0.349	0.243	0.441	0.322	-0.932**	0.007
Standing long jump ($\Delta\%$)						
Progesterone PRE	0.574*	0.025	0.773*	0.014	-0.380	0.457

r = Pearson's correlation coefficient, p-value = significance (2-tailed)

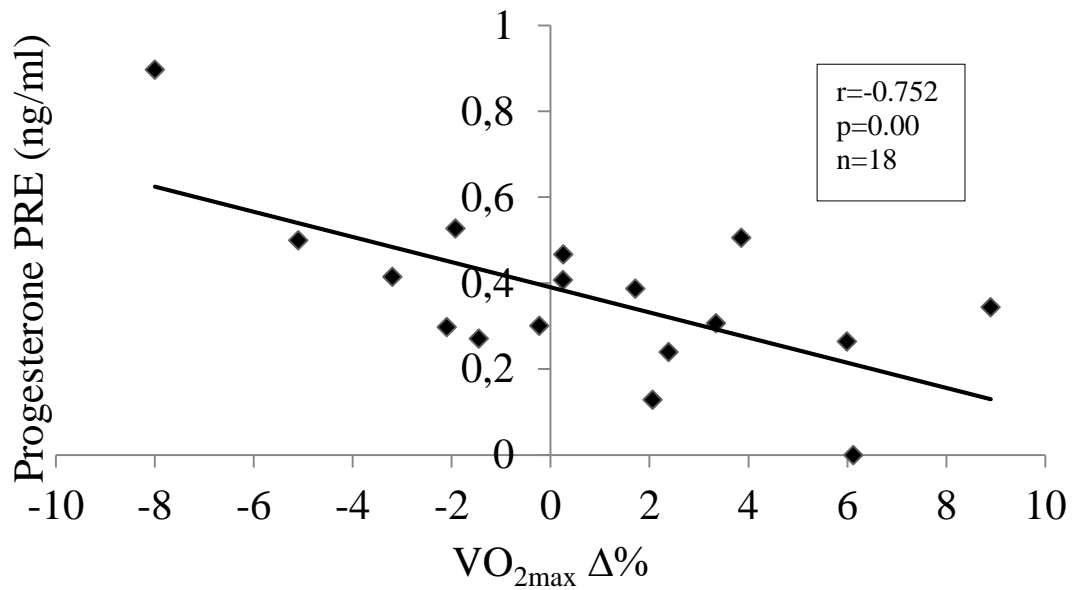


FIGURE 17. The correlation of change in maximal oxygen uptake (VO_{2max}) and the progesterone at PRE-measurements for the whole study group (ALL).

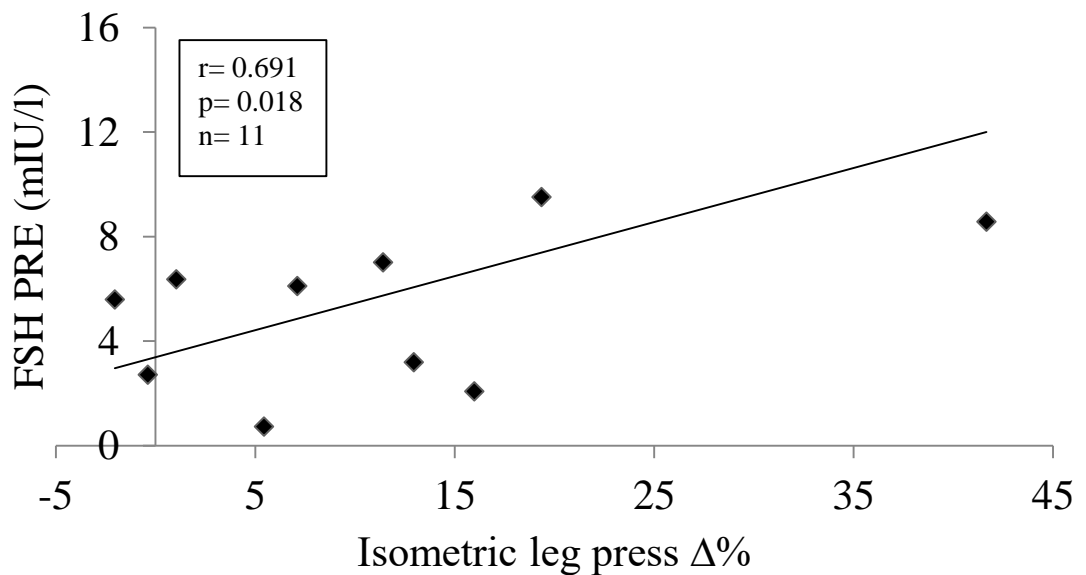


FIGURE 18. The correlation of change in isometric leg press and the follicle stimulating hormone at PRE-measurements for the HC group.

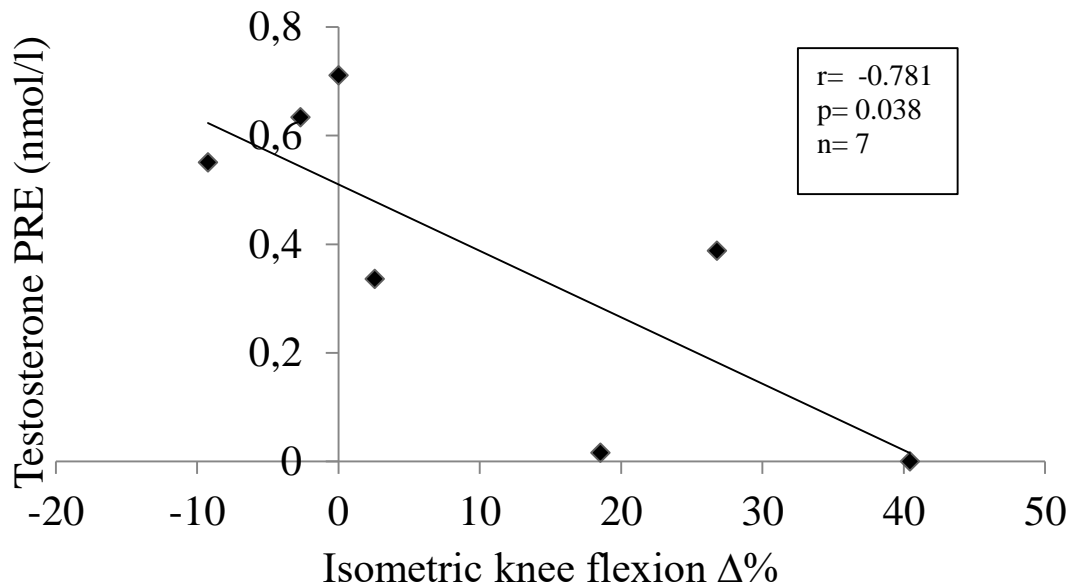


FIGURE 19. The correlation of change in isometric knee flexion and testosterone at PRE-measurements for the NHC group.

The change in LH correlated with strength, power and endurance variables. For example, the change in LH correlated positively with the change in isometric knee extension in the NHC group ($r=0.76$; $p<0.05$), but negatively with the change in countermovement jump in the whole study group (ALL) ($r=-0.54$; $p<0.05$) and in the HC group ($r=-0.66$; $p<0.05$). In addition, the change in LH correlated with change in 3000 m time trial time in the NHC group ($r=0.90$; $p<0.05$).

The change in cortisol correlated with the change in VO_{2max} in the whole study group (ALL) ($r=0.67$; $p<0.01$) and in the HC group ($r=0.74$; $p<0.01$), and with the change in 1 min push-ups in the NHC group ($r=0.92$; $p<0.01$). Correlation coefficients between changes in physical performance and changes in hormones are presented in table 13. Figure 20 represents correlation for the NHC group.

TABLE 13. Correlation coefficients between changes in physical performance ($\Delta\%$) and changes in hormones ($\Delta\%$). (ALL: n= 18, HC: n=11, NHC: n=7)

	ALL		HC		NHC	
	r	p	r	p	r	p
Isometric leg press ($\Delta\%$)						
SHBG $\Delta\%$	-0.184	0.496	0.146	0.708	-0.810*	0.027
Isometric knee extension ($\Delta\%$)						
Testosterone $\Delta\%$	-0.037	0.889	-0.645*	0.032	-0.129	0.808
Isometric knee flexion ($\Delta\%$)						
LH $\Delta\%$	-0.033	0.897	-0.184	0.589	0.761*	0.047
Dynamic leg press $\Delta\%$						
Cortisol $\Delta\%$	0.276	0.267	0.158	0.642	0.769*	0.043
Isometric bench press ($\Delta\%$)						
Testosterone $\Delta\%$	0.400	0.112	-0.626*	0.039	0.701	0.121
CMJ ($\Delta\%$)						
LH $\Delta\%$	-0.535*	0.022	-0.661*	0.027	0.309	0.500
FSH $\Delta\%$	-0.586*	0.011	-0.651*	0.030	-0.356	0.433
VO_{2max} ($\Delta\%$)						
Cortisol $\Delta\%$	0.667**	0.003	0.738**	0.01	0.495	0.258
Time in 3000m time-trial ($\Delta\%$)						
Progesterone $\Delta\%$	0.452	0.121	0.433	0.244	0.988*	0.012
LH $\Delta\%$	-0.200	0.494	-0.289	0.450	0.895*	0.040
1 min push-ups $\Delta\%$						
Cortisol $\Delta\%$	0.734**	0.002	0.622	0.073	0.919**	0.010
1 min sit-ups ($\Delta\%$)						
SHBG $\Delta\%$	0.306	0.310	0.266	0.565	0.902*	0.014

r = Pearson's correlation coefficient, p-value = significance (2-tailed)

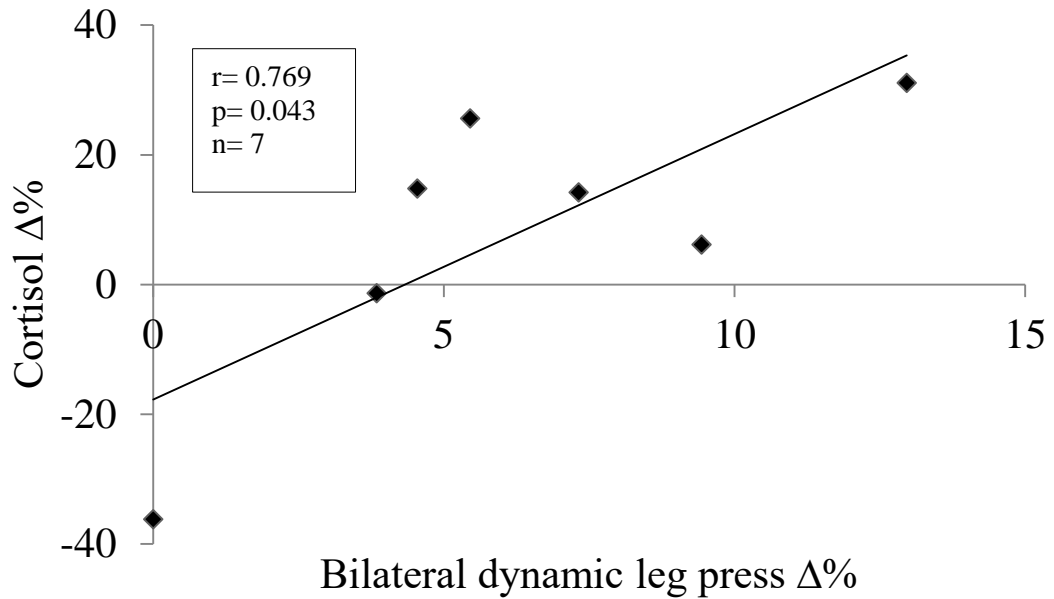


FIGURE 20. The correlation of change in bilateral dynamic leg press and change in cortisol for the NHC group.

7.6. Associations between changes in body composition and hormones

Associations between changes in body composition and hormones are presented by Pearson's correlation. The correlations were examined comparing the change in body composition and the PRE-values of hormones (Table 14), as well as the change in hormones between PRE- and POST-measurements ($\Delta\%$) (Table 15). The corresponding correlations for all groups, even non-significant, are presented in the tables for the sake of comparison.

In the whole study group (ALL), and in the HC group there were positive correlations between the change in body mass and progesterone PRE ($r=0.58$; $p<0.05$, $r=0.70$; $p<0.05$, respectively). In addition, progesterone PRE correlated positively with the change in total fat in the whole study group ($r=0.50$; $p<0.05$). In the NHC group the change in fat free mass was correlated positively with testosterone PRE ($r=0.94$; $p<0.01$), and progesterone PRE ($r=0.82$; $p<0.05$).

Correlation coefficients of changes in body composition and hormone PRE-values are illustrated in table 14. Figures 21 and 22 represent correlations for the NHC group.

TABLE 14. Correlation coefficients of changes in body composition ($\Delta\%$) and hormone PRE-values. (ALL: n=18, HC: n=11, NHC: n=7)

	ALL		HC		NHC	
	r	p	r	p	r	p
Body mass ($\Delta\%$)						
Progesterone PRE	0.581*	0.012	0.699*	0.017	-0.418	0.350
Total fat ($\Delta\%$)						
Progesterone PRE	0.496*	0.036	0.555	0.077	-0.419	0.349
Fat free mass ($\Delta\%$)						
Testosterone PRE	-0.221	0.379	-0.318	0.341	0.940**	0.002
Progesterone PRE	-0.021	0.935	0.053	0.876	0.816*	0.025

r = Pearson's correlation coefficient, p-value = significance (2-tailed)

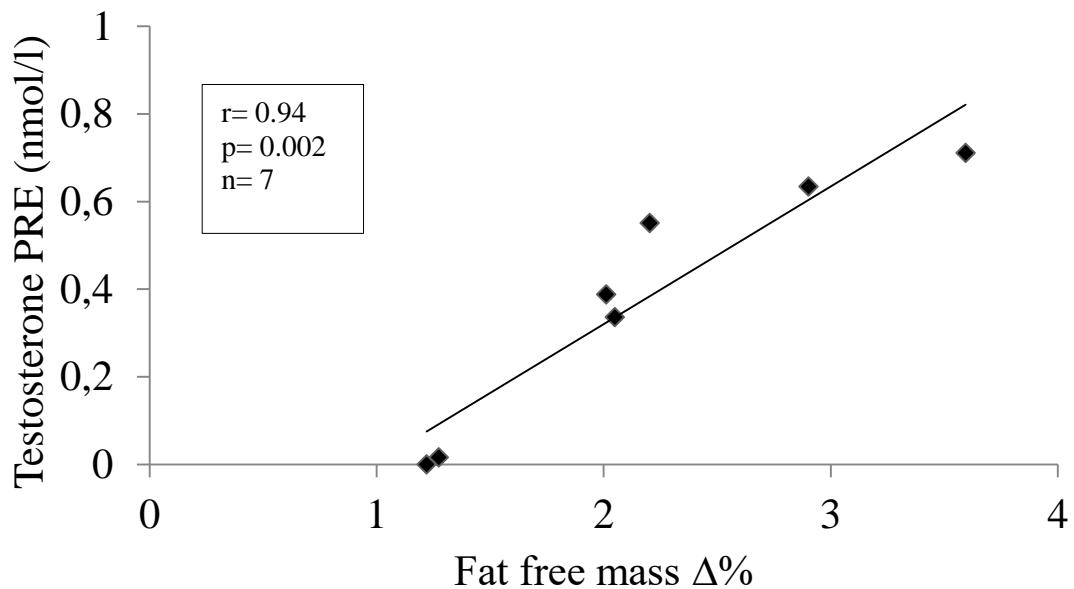


FIGURE 21. The correlation of change in fat free mass and testosterone at PRE-measurements for the NHC group.

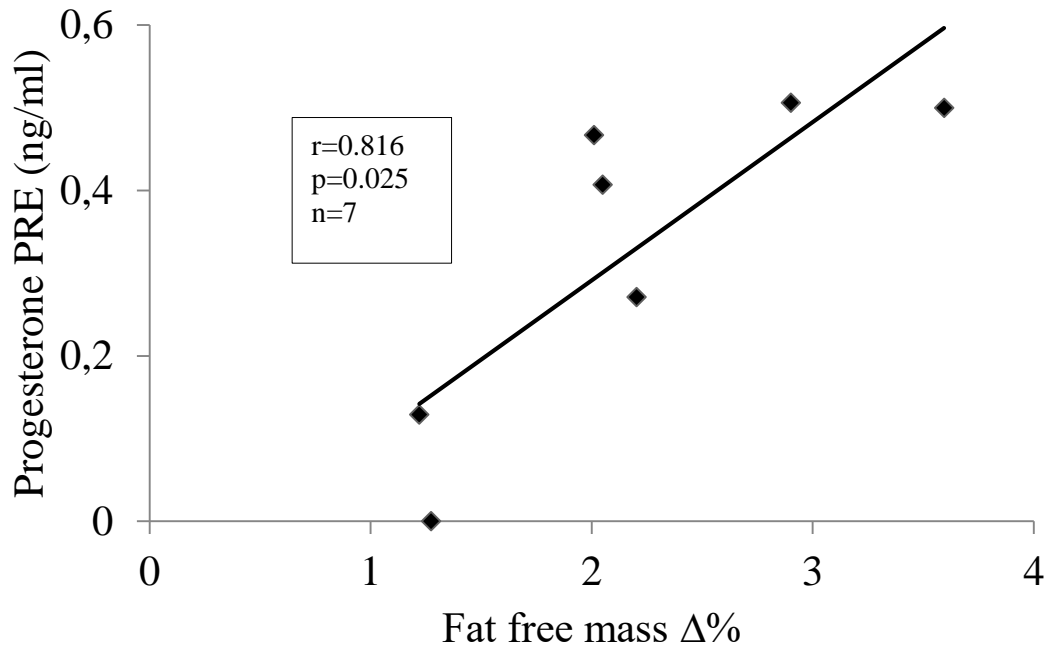


FIGURE 22. The correlation of change in fat free mass and progesterone at PRE-measurements for the NHC group.

In the HC group the change in body mass correlated positively with the change in testosterone ($r=0.75$; $p<0.05$), and with the change in progesterone ($r=0.64$; $p<0.05$). In addition, the change in fat free mass correlated negatively with the change in SHBG ($r=-0.70$; $p<0.05$). Correlation coefficients of changes in body composition and changes in hormones are illustrated in table 15.

TABLE 15. Correlation coefficients of changes in body composition ($\Delta\%$) and changes in hormones ($\Delta\%$). (ALL: $n=18$, HC: $n=11$, NHC: $n=7$)

	ALL		HC		NHC	
	r	p	r	p	r	p
Body mass ($\Delta\%$)						
Testosterone $\Delta\%$	0.191	0.462	0.748*	0.008	0.297	0.568
Progesterone $\Delta\%$	0.463	0.061	0.638*	0.035	0.463	0.061
Fat free mass ($\Delta\%$)						
SHBG $\Delta\%$	-0.316	0.234	-0.704*	0.034	-0.443	0.320

r = Pearson's correlation coefficient, p-value = significance (2-tailed)

8 DISCUSSION

The purpose of this study was to examine the effects of high-intensity combined strength and endurance training on physical performance, body composition and serum hormone levels in recreationally active women. The primary focus of this study was to compare responses among hormonal contraception users (HC) and non-users (NHC). The main findings of this study were:

- 1) Isometric leg press, isometric knee extension, isometric knee flexion, isometric bench press, dynamic leg press, countermovement jump, 1 min push-ups, 1 min sit-ups, and 3000 m time trial running performance improved significantly after 10 weeks of high-intensity combined strength and endurance training.
- 2) Combined strength and endurance training did not cause significant changes in the resting serum hormone levels.
- 3) There were no differences in the changes of serum hormone levels or neuromuscular performance between HC and NHC groups. The 3000 m time trial performance was improved only within the NHC group, however, there was not a significant difference between groups' improvements.
- 4) There were no differences in the changes of body composition between HC and NHC women. However, a trend for greater increase in fat free mass was observed in NHC than in HC women.

8.1 Neuromuscular performance

In this study the subjects were recreationally active women with mostly endurance training background, and none of the subjects had trained strength training regularly before the intervention. The present 10 weeks of combined maximal and explosive type strength and high-intensity endurance training resulted in marked improvements in neuromuscular performance. This is in line with some previous research of combined

training conducted on both men and women (Mikkola et al. 2007, Sillanpää et al. 2009, Taipale et al. 2014). For example, the observed improvement in isometric leg press of $11\pm 12\%$ ($p<0.01$) was in line with results of Mikkola et al. (2007), who reported an improvement of $8\pm 9\%$ ($p<0.05$) in isometric leg press in young distance runners following combined explosive strength and endurance training.

Maximal and explosive type strength training increased maximal isometric and dynamic strength, and this improvement was probably associated with training-induced neural adaptations as in other explosive type of strength training studies (Mikkola et al. 2007, Paavolainen et al. 1999), although electromyographic measurements of the muscles were not reported in this thesis to support this suggestion. In addition, without a control group, it is not possible to know if strength and muscle mass development was blunted or not by concurrently performing endurance activities, as observed in some other studies (Bell et al. 2000, Hickson 1980, Coffey & Hawley 2007, Hawley 2009, Wilson et al. 2012).

Both groups' neuromuscular adaptations were similar with significant increases in strength in both isometric and dynamic actions. Several published studies support the finding that hormonal contraceptive use does not seem to affect baseline maximal force production (Elliott et al. 2005, Lebrun et al. 2003, Sarwar et al. 1996), and this study's results suggest that they do not seem to affect strength development either.

8.2 Endurance performance

High-intensity interval training has shown to increase maximal oxygen consumption (VO_{2max}), muscle buffer capacity, time to fatigue, and therefore time trial performance (Edge et al. 2006, Helgerud et al. 2007). However, in the present study, a huge increase in VO_{2max} was not expected since the subjects' fitness level was already high at the PRE-measurements (42.8 ml/kg/min). The present combined strength and endurance training program did not induce changes in VO_{2max} but led to improvements in 3000 m time trial. The training method of 4x4 min of high-intensity interval running used in this study is based on the article by Helgerud et al. (2007) who reported an improvement of 7.2% in VO_{2max} after 8 weeks of interval training three times a week. In the present study, this

training method was executed only once a week, which was probably not enough to induce changes in VO_{2max} in recreationally active individuals.

Although VO_{2max} was not improved, it does not mean that this type of combined training would not be a useful method for improving endurance performance. Combining maximal and explosive strength and high-intensity endurance and sprint training resulted in an improvement of 2% in the 3000 m running performance in recreationally active women. Lactate threshold, running economy or hemodynamics were not reported in this study, however, it is likely that this improvement in 3000 m running performance was related to improved neuromuscular characteristics, as observed in the study of Paavolainen et al. (1999).

When comparing the groups, there were no differences in VO_{2max} . In the 3000 m time trial the within-group difference from PRE-measurements was significant only for the NHC group, but a significant difference between groups' improvements was not observed. Therefore, it cannot be concluded that hormonal contraceptives impaired endurance performance. Published studies have reported contradictory results of the effects of hormonal contraceptives on aerobic capacity depending on the type of hormonal contraceptives. Some studies examining triphasic hormonal contraceptives have demonstrated a reduction (4% to 15%) in VO_{2max} (Casazza et al. 2002, Lebrun et al. 2003, Suh et al. 2003), whereas some studies with monophasic pills suggest that hormonal contraceptive use does not affect endurance capacity at all (Rickenlund et al. 2004, Joyce et al. 2013). All of the combined pills used in the present study were monophasic, and the adaptation of VO_{2max} was similar in both groups. In addition, no significant differences in hormone levels were observed between HC and NHC groups. Thus, it can only be speculated if there was a difference in cardiorespiratory or neuromuscular adaptations between groups that led to improvements in the 3000 m time trial only for the NHC group.

8.3 Hormonal adaptations

Research examining sports performance in women with different hormonal status is rare. In this study, several hormones were examined in order to gain insights into the potential underlying mechanisms that may affect the physical performance adaptations. However,

high-intensity combined strength and endurance training did not induce changes in serum hormone levels in recreationally active women. This is in line with the findings of Häkkinen et al. (1990) who observed no statistically significant changes in sex hormones including estradiol, progesterone, testosterone, FSH, LH or SHBG during prolonged power type strength training in women. In contrast, Kraemer et al. (1998) and Marx et al. (2001) demonstrated changes in hormone levels, such as increases in resting testosterone, SHBG and IGF-1 concentrations, and decreases in resting cortisol concentrations after 8–24 weeks of resistance training in untrained women. It is possible that hormonal levels in recreationally active individuals, as in the present study, require longer periods of training for any changes to be evident. The hormonal data of the present study also suggests that the combined strength and endurance training was not too strenuous, because it did not create a catabolic environment as neither increase in cortisol levels nor decrease in anabolic hormones were observed, in other words the testosterone/cortisol ratio remained unaltered (Bell et al. 2000, Consitt et al. 2002, Mikkola et al. 2007).

According to many studies, exogenous ovarian hormones in hormonal contraceptives may increase levels of sex hormone-binding globulin, and reduce the levels of total and free testosterone (Bachmann et al. 2002, Burrows & Peters 2007, Casazza et al. 2004, Elliott et al. 2005, Rickenlund et al. 2004, Wiegratz et al. 2003, Zimmermann et al. 2014). In the present study, however, no significant between-group differences were observed. Contrary to expectations, hormonal contraceptives did not have a significant impact on resting hormone levels, such as SHBG or total testosterone.

It should also be taken into consideration that the blood samples were collected at the beginning of the menstrual cycle, between days 1–7, when levels of both estrogen and progesterone are low. It can be speculated that the ovarian hormone levels could have been significantly different between groups in another phase of menstrual cycle, as at ovulation or at the beginning of the luteal phase (Redman et al. 2003, Vaiksaar et al. 2011).

It should also be considered that inter-individual variation in hormone levels may be large (Lebrun 2000, 44), as observed in this study. The blood lipids were not measured in this study, but it would have been interesting to know if the use of hormonal contraceptives

had effects on the lipid profiles, as in the study of Lloyd et al. (2002) who demonstrated less favorable blood lipid patterns among hormonal contraception users. More research examining the associations of female sex hormones and combined strength and endurance training is needed.

8.4 Body composition

Body composition was measured with DXA which is considered to be a valid method for assessing small changes in fat and muscle tissue (Houtkooper et al. 2000). Combined strength and endurance training induced positive changes in body composition of the study group; fat free mass was increased by 1% and total body fat was decreased by 5%. This is in line with previous research of combined training in women (Sillanpää et al. 2009, Sillanpää et al. 2010). For example, Sillanpää et al. (2010) reported increased fat free mass by 2% ($p=0.001$) and decreased total body fat by 6% ($p<0.001$) following 21 weeks of combined training in middle-aged women.

When comparing the groups, there were no statistically significant differences in improvement of body composition. However, a trend for greater increase in fat free mass was observed in NHC than in HC women ($p=0.065$). In addition, a decrease in total fat was observed only within the NHC group. Plausible explanation for the non-significant decrease in total fat within the HC group, may be the larger standard deviation ($5\pm 10\%$, $p=0.158$) compared to the NHC group ($5\pm 6\%$, $p=0.03$).

In literature, there are no similar experimental study designs as in the present study, but some studies with triphasic hormonal contraceptives have reported increased fat mass in moderately active women (Casazza et al. 2004, Suh et al. 2003, Lebrun et al. 2003) and in female endurance athletes (Lebrun et al. 2003). It seems that the results depend on the use of different types and formulations of hormonal contraception, since the potency and androgenicity of the progesterone within the hormonal contraception pill may play a significant role (Burrows & Peters 2007, Casazza et al. 2002, Suh et al. 2003). In addition, the increases in body mass and body fat occur usually within the first months of hormonal contraception use (Burrows & Peters 2007, Casazza et al. 2004, Lebrun et al. 2003, Suh et al. 2003).

In the present study, all subjects had at least one year of hormonal contraceptive use thus any initial changes in body composition that might occur should be in the past. More importantly, there were three different types and ten different formulations of hormonal contraceptives used by the subjects. In addition, dietary conditions were not controlled. Therefore, it is challenging to draw conclusions, but the present study suggests that body composition adaptations following combined strength and endurance training does not differ between hormonal contraception users and non-users.

8.5 Associations between changes in serum hormones and physical performance and body composition

Testosterone, an anabolic hormone, seemed to correlate with changes in neuromuscular performance and changes in body composition. In the HC group testosterone PRE-values correlated positively with change in isometric knee extension ($r=0.61$; $p<0.05$) and change in isometric knee flexion ($r=0.70$; $p<0.05$). In the NHC group there was a positive correlation between testosterone PRE-values and change in fat free mass ($r=0.94$; $p<0.01$). These findings are in accordance with previous studies that have demonstrated that testosterone levels may be an important indicator of trainability in women (Häkkinen et al. 1990, Häkkinen et al. 1992). In addition, the changes in body composition, such as increase in weight and fat mass, can be associated with decline in androgen levels (Rickenlund et al. 2004). It was surprising, that in the NHC group, there was a negative correlation between testosterone PRE-values and change in isometric knee flexion ($r=-0.781$; $p<0.05$). However, it must be taken into account that the individual variations in testosterone levels in women are large, and increases in strength and muscle mass do not always coincide with increases in levels of testosterone (Consitt et al. 2002). It would have been interesting to see the testosterone levels in the middle of the training period.

Serum cortisol as a primary catabolic stress hormone plays several regulatory roles in metabolism, and negatively impacts protein metabolism. In the present study there was high correlations between the change in cortisol levels and the change in VO_{2max} (ALL: $r=0.67$; $p<0.01$, HC: $r=0.74$; $p<0.01$), as well as the change in repetitions of 1-min push-ups (ALL: $r=0.73$; $p<0.01$, NHC: $r=0.92$; $p<0.01$). In addition, the change in cortisol levels correlated positively with the change in bilateral dynamic leg press (NHC: $r=0.77$;

$p < 0.05$). The increases in cortisol concentrations reflect the overall training stress, and suggest that the training stimulus was adequate (Kraemer & Ratamess 2005). In addition, it suggests that a decrease in catabolic environment is not necessary to induce strength gains (Häkkinen et al. 1990). Usually, a reduction in resting cortisol concentration has been thought to contribute to the overall enhancement of strength, since an increase in resting testosterone or decrease in resting cortisol may improve tissue anabolism, and be more conducive to strength development (Kraemer et al. 1998, Marx et al. 2001). On the other hand, cortisol has also positive effects, and chronic changes in resting cortisol levels may be needed in tissue homeostasis involving protein metabolism (Kraemer & Ratamess 2005). In the HC group, the potential importance of cortisol was demonstrated with a positive relationship between cortisol levels at PRE-measurements and improvement in isometric knee flexion (HC: $r = 0.61$; $p < 0.05$) and in isometric bench press (HC: $r = 0.62$; $p < 0.05$). Casazza et al. (2004) pointed out the interrelationship between cortisol and ovarian hormones, and concluded that exogenous ovarian hormones in hormonal contraceptives appear to increase endogenous counterregulatory hormones related to lipolysis, such as cortisol at rest and during exercise.

Compared to testosterone and cortisol, female sex hormones did not demonstrate as clear correlations. It is interesting that estradiol levels were not associated with any of the variables. In contrast, progesterone showed both positive and negative correlations. For example, in the NHC group high baseline progesterone levels were associated with increase in fat free mass ($r = 0.82$; $p < 0.05$), but in the whole study group high baseline progesterone levels were associated with increase in total fat ($r = 0.50$; $p < 0.05$) and body mass ($r = 0.58$; $p < 0.05$). In addition, it seemed that low baseline progesterone levels were associated with more favorable changes in some physical performance variables compared to high levels. For example, low baseline progesterone levels were associated with increase in isometric knee extension (HC: $r = -0.63$; $p < 0.05$), increase in VO_{2max} (ALL: $r = -0.75$; $p < 0.01$, HC: $r = -0.79$; $p < 0.01$), and improvement in the 3000 m time-trial (ALL: $r = 0.61$; $p < 0.05$). This is an interesting finding that would suggest that lower level of progesterone is more conducive to physical performance, especially among hormonal contraception users. It may even give some support to the speculation that progesterone may modify body composition, thermoregulation, and hemodynamics (Lebrun 2000, 42), oppose the lipolytic effects of estrogen (Ashley et al. 2000), and impair the muscle force

production (Sarwar et al. 1996). However, in this study, there was a positive relationship between baseline progesterone levels and improvement in standing long jump (ALL: $r=0.57$; $p<0.05$, HC: $r=0.77$; $p<0.05$). The number of studies investigating the effects of progesterone on performance is limited but, for example, Greeves et al. (1999) found a positive relationship between relative force and progesterone. The associations between progesterone and physical performance and body composition found in this study indicate the demand for further investigation.

Other female sex hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were also correlated with physical performance variables. For example, high baseline levels of FSH were associated with increase in isometric leg press (HC: ($r=0.69$; $p<0.05$) and with increase in VO_{2max} (ALL: $r=0.49$; $p<0.05$). In addition, the change in countermovement jump correlated negatively with changes in FSH (ALL: $r=-0.54$; $p<0.05$, HC: $r=-0.66$; $p<0.05$) and LH (ALL: $r=-0.59$; $p<0.05$, HC: $r=-0.65$; $p<0.05$). It is not surprising that FSH and LH were correlated similarly since they act synergistically to stimulate estrogen and progesterone secretion from the ovaries during the female monthly menstrual cycle (Guyton & Hall 2006, 1012–1014). The effect of these sex hormones on physical performance and body composition, however, is unclear.

Sex hormone binding globulin (SHBG) seemed to correlate with many variables, too. High baseline levels of SHBG were associated with increases in isometric leg press in the NHC group ($r=0.83$; $p<0.05$), and increases in isometric knee extension in the HC group ($r=0.74$; $p<0.05$). However, low level of baseline SHBG was associated strongly with increases in 1 min push-ups in the NHC group ($r=-0.93$; $p<0.01$). In addition, the change in SHBG correlated negatively with the change in isometric leg press in the NHC group ($r=-0.81$; $p<0.05$) and the change in fat free mass in the HC group ($r=-0.70$; $p<0.05$). In other words, the more SHBG was reduced the more isometric strength of lower extremity or fat free mass was increased. This can be explained by the fact that SHBG binds to androgens and estrogens, and levels of SHBG are decreased by androgens (Guyton & Hall 2006, 1003). Beyond SHBG's role in the transport, it appears to be a potential anabolic compound that has an important function as a complex with testosterone in conditions with low testosterone levels. Therefore, the role of SHBG in the stimulation

of anabolic processes is more important in women compared to men. (Kraemer et al. 1998.)

8.6 Strengths and limitations of the present study

The present study seems to be the first to evaluate the effects of hormonal contraceptive use on high-intensity combined strength and endurance training. The strengths of the present study can be attributed to the well-designed training program that led to improvements in performance, as well as diverse and standardized measurements of power and strength, endurance, body composition and serum hormone levels.

Hormonal changes may have an important role in strength and endurance development (Kraemer et al. 1998, Kraemer & Ratamess 2005), and in this study several hormones were examined in order to gain insights into the potential underlying mechanisms that may affect the physical performance adaptations in women with different hormonal status. However, systemic hormone concentration measured in the serum cannot be directly taken as an estimate of the hormone content of a specific tissue, and the magnitude of the training induced hormonal changes is not always associated with other training adaptations (Pöllänen et al. 2011, Pöllänen et al. 2015, West & Phillips 2012). Since performance development can usually be explained mostly by local adaptations in neuromuscular system and heart, electromyographic measurements or muscle biopsies would have provided more information about the training adaptation.

The most notable limitation of the present study was the small sample size. The number of subjects at the end of the study was quite small in both groups (HC: n=11, NHC: n=8) since not all the subjects were able to complete the study. A small sample size with high inter-individual variation is prone to the inclusion of outliers. In addition, the MID-measurements could not be included in this thesis because all the subjects were not able to participate in the MID-measurements and, therefore, hormonal data of seven subjects was missing. The absence of blood samples from the middle of the training period may not provide the sufficient information needed for precise defining of the hormonal adaptation to training of an individual.

Most importantly, the hormonal contraception used in this study consisted of three different types of contraception; combined pills, progesterone-only pills and intrauterine systems, which all have different active ingredients and amount of exogenous hormones. Therefore, the impact on performance or training induced adaptation may vary, and it is not possible to generalize results about the exact effects of hormonal contraception.

In addition, without a randomized controlled trial, we can only speculate whether a possible discrepancy between hormonal contraception users and non-users is a true difference or a consequence of selectivity of women who end up using hormonal contraception. Moreover, when hormonal contraception is used as a medical treatment in randomized controlled clinical research, the subjects need to be aware of the use or non-use of hormonal contraceptives to follow ethical recommendations in preventing unintended pregnancy.

8.7 Conclusions and practical applications

High-intensity combined strength and endurance training 2 + 2 times in a week over a 10 week training period markedly enhanced neuromuscular performance and led to small improvements in endurance performance and body composition. These findings suggest that combined strength and endurance training is a useful tool for improving physical fitness in recreationally active women.

The significant inter-individual variability in hormone levels and a small sample size make it challenging to draw conclusions, but this study suggests that hormonal contraception use does not affect the neuromuscular or endurance performance in recreationally active women. Neither does hormonal contraception have negative effects on body composition.

It should be remembered, that changes in cardiovascular, neuromuscular, metabolic and hormonal factors due to hormonal contraception use probably have minimal impact on physical performance in recreational athletes, but may have more impact at the elite level. It should also be considered that hormonal contraceptives may have marked individual effects in some women (Lebrun 2000, 44).

In future studies, the type of hormonal contraception and different formulations should be defined more accurately when evaluating the effects of hormonal contraceptives. The hormonal status of each subject should also be monitored more thoroughly. It is possible that neuromuscular adaptations to the hormonal contraception use may occur (Bennell et al 1999). In addition, the deleterious effect of hormonal contraceptives on $\text{VO}_{2\text{max}}$ may be short term (<6 months) in nature (Joyce et al. 2013), and it appears that the increases in body mass and total body fat occur within the first few months of hormonal contraceptive use (Casazza et al. 2002, Lebrun 2003, Suh 2002). Therefore, well-designed randomized clinical trials are required to measure changes in performance with initial hormonal contraception use.

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