Strength Training and the Menstrual Cycle

Effects of menstrual cycle based-training on muscle strength, muscle volume and muscle cell parameters in women with and without oral contraception

Dissertation

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ABSTRACT

PURPOSE: The menstrual cycle shows fluctuation of various endogenous hormones between the follicular phase and the luteal phase in women, who do not take oral contraceptives (OC). With the intake of OC biosynthesis and secretion of the endogenous hormones estrogen and progesterone are suppressed and other sex steroids are altered in different ways. Variations of these hormonal milieus might influence trainability of strength performance differently either between the menstrual cycle phases or between women with oral contraceptives and women without oral contraceptives.

Therefore, this thesis aimed to investigate hormone profiles during the menstrual cycle and the effects of the menstrual phase-based strength training physiologic and microscopic measures of strength capacity in eumenorrheic women, who do not take any oral contraceptive (non-OC users) and in women, who take the a combined monophasic oral contraceptive (OC users).

Study 1: investigated follicular phase-based (FT) vs. luteal phase-based (LT) strength training in non-OC users.

Study 2: investigated quasi-follicular phase-based (qFT) vs. quasi-luteal phase-based (qLT) strength training in monophasic OC-users.

Study 3: compared hormonal profiles and parameters of strength performance from both studies between non-OC users and OC users.

METHOD: Twenty non-OC users and seventeen OC users completed one-leg strength training on leg press for three menstrual cycles. One leg was trained mainly in the first half of the menstrual cycle (follicular phase training (FT) and quasi-follicular phase training (qFT), respectively) and the other leg mainly in the second half of the cycle (luteal phase training (LT) and quasi-luteal phase training (qLT), respectively). Venous blood samples were taken on day 11 of the menstrual cycle in the follicular phase (FP) / quasi-follicular phase (qFP) and on day 25 of the menstrual cycle in the luteal phase (LP) / quasi-luteal phase (qLP) to analyze values of 17-beta estradiol (E2), progesterone (P4), total testosterone (T),

free testosterone (free T) and DHEA-s. Maximum isometric muscle strength (F_{max}) and muscle diameter (Mdm) were analyzed before and after training intervention as well as muscle fiber composition (number of type I and type II fibers), fiber diameter (Fdm) and cell nuclei to fiber ratio (N/F) in subgroups of nine and six subjects, respectively.

RESULTS: Study 1: Concentrations of T and free T were higher in FP compared to LP ($P<0.05$). The increase in F_{max} after FT was higher than after LT (267 N vs. 188 N, $P < 0.025$). FT also showed a higher increase in Mdm than LT (0.57 cm vs. 0.39 cm, $P < 0.025$). Moreover, we found significant increases in Fdm of fiber type II and in N/F only after FT; however, there was no significant difference from LT. With regard to change in fiber composition, no differences were observed between FT and LT.

Study 2: Prior to training E2, P4, DHEA-s and T were not significantly different between the two phases, while free T was lower in qLP compared to qFP. After three months of strength training, P4, DHEA-s and T became higher in qFP compared to qLP ($P < 0.05$), while the difference in free T was no longer detectable. F_{max} and Mdm increased significantly after qFT and qLT without any differences between the two types of training periodization. OC pills with or without androgenicity did not have any influence on the development of F_{max} and Mdm. Number of fiber type II tended to increase after qFT, however remained the same after qLT, while the other muscle cell parameters were unaffected by any training periodization.

Study 3: Concentrations of E2, DHEA-s, T and free T were significantly ($p < 0.05$) higher in non-OC users as compared to OC users. Absolute increase of F_{max} after training intervention was the lowest ($P < 0.05$) after LT in non-OC users (Δ188.3N) as compared to FT (Δ 268.6N) of non-OC users, qFT (Δ 266.4N) and qLT (Δ 282.2N) of OC users.

CONCLUSIONS: In non-OC users, FT showed a significantly pronounced effect on muscle strength, on muscle diameter and on Fdm of fiber type II compared to LT. this might be due to the specific hormonal milieu during

each phase of the cycle. In OC users, however, no differences were found between the two training interventions qFT and qLT. This is presumably due to the constant doses of estrogen and progestin in monophasic OC. As a result, OC users had a more stable hormonal milieu for training adaptation processes at least during the consumption phase of 21 days, resulting in comparable trainability of strength performance throughout the cycle. Further studies with longer lasting training periods are needed in order to analyze if the late response in strength training adaptation becomes more pronounced after more than three months of menstrual cycle-based training in non-OC users. As no menstrual cycle-specific training responses have been observed, we recommend that untrained and moderately trained OC users perform their strength training independently from their pill cycle. Further studies are necessary in order to understand possible effects of androgenicity of OC pills on development of strength performance. Furthermore, more subjects have to be included in muscle biopsy analysis in order to understand possible underlying mechanisms of cycle-dependent strength training adaptations.

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Bochum im Mai 2012 Eunsook Sung

감사의 글

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제가 학업을 마칠 때까지 편찮으신데 없이 건강히 계셔주신 외할머니.
올해 **팔순** 생신 축하드립니다. 할머니의 기도가 없었으면 무사히 학업을 마칠수 있었을까요. 할머니 건강히 오래오래 사세요 사랑합니다.

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LIST OF ABBREVIATIONS

Δ	absolute difference
ATPase	adenosinetriphosphatase
DHEA-s	dehydroepiandrosterone-sulfate
E	estrogen
E2	estradiol
E/P	estrogen to progesterone ratio
Fdm	muscle fiber diameter
F_{max}	maximum isometric muscle strength
FP	follicular phase
FSH	follicle stimulating hormone
free T	free testrosterone
FT	follicular phase-based strength training
GnRH	gonadotropin releasing hormone
HE	hematoxylin and eosin
M.	muscle
N	number
LH	luteinzing hormone
LP	luteal phase
LT	luteal phase-based strength training
Mdm	muscle diameter
N/F	muscle cell nuclei to fiber ratio
NO	Muscle fiber type distribution
OC	monophasic oral contraceptive
P4	progesterone
qFP	quasi follicular phase
qFT	quasi follicular-phase based strength training
qLP	quasi luteal phase
qLT	quasi luteal-phase based strength training
T	total testosterone
yr	year

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INTRODUCTION

In past decades, it has repeatedly been verified that serum concentrations of luteinizing hormone (LH), follicle-stimulation hormone (FSH), estradiol (E_2) and progesterone (Prg) fluctuate during the menstrual cycle and that the level of androstenedione and testosterone reaches its peak prior to, or at the time of ovulation (Longcope, 1986; Van Look & Baird, 1980). The fluctuation of hormones during the menstrual cycle might influence muscle strength and training ability of strength (Friden, Hirschberg & Saartok, 2003; Marsh & Jenkins, 2002).

Recently, the use of oral contraceptives (OC) is increasing. The number of female athletes using OC is also increasing for reasons like birth control, management of premenstrual symptoms, dysmenorrhea, less menstrual blood loss, lower risk of musculoskeletal injury and time-shifting of the menstrual cycle, which could provide benefits for the female athletes (Bennell, White & Crossley, 1999; Constantini, Dubnov & Lebrun, 2005; Wojtys, Huston, Boynton, Spindler & Lindenfeld, 2002). Due to the intake of fixed doses of synthetic E2 and P4 in OC, endogenous E2 and P4 are suppressed in women using OC.

Since E2, P4 and other sex steroids are discussed to be important factors for strength capacity, there might be yet unknown different influences on strength training adaptation in both non-OC users and OC users. To the authors' knowledge, just a single study investigating trainability of strength during the menstrual cycle was performed by Reis et. al (1995). They report that strength training in the follicular phase is more effective on muscle strength than regular training. Our pilot study (Han & Sung, 1996) investigating muscle strength and microscopic parameters with muscle biopsy samples also showed pronounced effects after follicular phase based training as compared to leteal phase based training. It seems that more strength adaptation on skeletal muscle in the follicular phase.

The aim of this thesis, therefore, was to investigate the hormone profile in the follicular phase and the luteal phase of the menstrual cycle and effects of two different menstrual phase-based strength trainings – follicular phase-based strength training (FT) versus luteal phase-based strength training (LT) – on physiologic and microscopic measures of strength capacity.

This thesis on ‘Strength Training and the Menstrual Cycle’ is one part of series of studies on ‘Trainability and the Menstrual Cycle’. The other part was on ‚Endurance Training and the Menstrual Cycle’ and was carried out by Ms Ahreum Han.

Study 1 examined twenty eumenorrheic women who did not take any oral contraceptives (non-OC users).
Study 2 examined seventeen women who took combined monophasic oral contraceptives (OC users).
Study 3 compares the results of both studies between non-OC users and OC users.

1. STUDY 1: EFFECTS OF FOLLICULAR VERSUS LUTEAL PHASE-BASED STRENGTH TRAINING IN UNTRAINED WOMEN

ABSTRACT

PURPOSE: Hormonal variations during the menstrual cycle may influence trainability of strength. For this reason, we investigated the effects of follicular phase-based (FT) strength training on muscle strength, muscle volume and microscopic parameters, comparing it to luteal phase-based (LT) strength training.

METHODS: Eumenorrheic women without oral contraception (N = 20) completed strength training on a leg press for three menstrual cycles. They trained one leg mainly in the follicular phase (FP) and the other leg mainly in the luteal phase (LP). Concentrations of 17-beta estradiol (E2), progesterone (P4), total testosterone (T), free testosterone (free T), and DHEA-s were analyzed in blood samples taken during FP and LP. Maximum isometric force (F_{max}), muscle diameter (Mdm), muscle fiber composition (No), fiber diameter (Fdm) and cell nuclei-to-fiber ratio (N/F) were analyzed before and after training.

RESULTS: Concentrations of T and free T were higher in FP compared to LP ($P<0.05$). The increase in F_{max} after FT was higher than after LT (267 N vs. 188 N, $P < 0.025$). FT also showed a higher increase in Mdm than LT (0.57 cm vs. 0.39 cm, $P < 0.025$). Moreover, we found significant increases in Fdm of fiber type II and in N/F only after FT; however, there was no significant difference from LT. With regard to change in fiber composition, no differences were observed between FT and LT.

CONCLUSIONS: FT showed a higher effect on muscle strength, muscle diameter and Fdm of fiber type II than LT. As a result, we recommend that eumenorrheic female athletes without oral contraception base the periodization of their strength training on their menstrual cycle.

1.1. INTRODUCTION

Women between the ages of approximately 13 and 50 experience a circamensal rhythm referred to as the menstrual cycle, in which the ovarian hormones fluctuate predictably over, 23–38 days on average (Oosthuyse & Bosch, 2010; Reilly 2000). 17-beta estradiol (E2) peaks prior to ovulation and during the luteal phase (LP), while progesterone (P4) reaches its highest values during LP after ovulation (Van Look & Baird, 1980). In both sexes, androgens are produced by the reproductive organs and the adrenals. The most important androgen secreted is testosterone; the adrenal glands and the ovaries produce very little testosterone but secrete weaker androgens. In particular, dehydroepiandrosterone (DHEA; and its sulfoconjugate) secreted by the adrenals, and androstenedione secreted by the adrenals and the ovaries are of physiological importance in women (Enea, Boisseau, Fargeas-Gluck, Diaz & Dugue, 2011). In addition to E2 and P4, androgens also fluctuate over the menstrual cycle. The levels of androstenedione and testosterone, for instance, reach their peaks prior to, or at the time of ovulation (Longcope, 1986).

The fluctuation of hormones during the menstrual cycle may influence exercise performance and the trainability of muscle strength (Constantini, Dubnov & Lebrun, 2005; Janse de Jonge, 2003; Lebrun, 1994). During perimenopausal and postmenopausal periods, a striking decline in muscle strength occurs that can be reversed by hormone replacement therapy (HRT), especially by estrogens, suggesting that estrogens and gestagens are important modulators of muscle physiology (Barros & Gustafsson, 2011). Indeed, a meta-analysis of data from female patients receiving HRT confirmed the beneficial effects of estrogens on muscle strength (Greising, Baltgalvis, Lowe & Warren, 2009). Most animal studies have demonstrated that female estrogen-supplemented rodents exhibit less skeletal muscle myofiber injury and inflammation following exercise-induced muscle injury.

In addition, estrogen may also influence post-damage repair processes through activation and proliferation of satellite cells (Enns & Tiidus, 2010). Although the role of androgens in female physiology has not been well established, several recent clinical trials have indicated that testosterone supplementation at physiological doses in androgen-deficient women induces improvements in lean body mass. These physiological effects may be critical for athletic performance. However, the effect of testosterone supplementation in women with serum androgen concentrations within the health-related reference interval has not been studied (Enea, Boisseau, Fargeas-Bluck, Diaz & Dugue, 2011).

The potential mechanism(s) underlying estrogenic action remain elusive. Among others, the discovery of three types of estrogen receptors (ERs) has led to the discovery that estrogen may govern the regulation of a number of downstream genes and molecular targets (Enns & Tiidus, 2010; Lowe, Baltgalvis & Greising, 2010). One recent study comparing postmenopausal females with or without HRT use reported that those women using HRT had significantly greater up-regulation of pro-anabolic gene expression both at rest and following eccentric exercise (Dieli-Conwright, Spektor, Rice, Sattler & Schroeder, 2009). Furthermore, it has recently been postulated that the beneficial effect of estrogens on muscle strength is accomplished by improving the intrinsic quality of skeletal muscle, whereby fibers are enabled to generate force, i.e., myosin strongly binds to actin during contraction (Lowe, Baltgalvis & Greising, 2010).

The biological actions of androgens are mainly mediated by the androgen receptor (AR). AR complexes interact with various factors (e.g. co-activators or corepressors) to modulate transcription of androgen target genes via binding to specific DNA sequences. Androgens may also regulate cellular activity via a more rapid nongenomic mechanism involving membrane receptors and/or cytosolic receptors. These steroid receptors are able to activate intracellular signaling molecules, such as the mitogen-activated protein kinase 1 (MAPK1), by transcription-independent mechanisms (Enea, Boisseau, Fargeas-Bluck, Diaz & Dugue, 2011).

Only very few data exist on the physiological effects of P4 on the female skeletal muscle cell. Recent studies have consistently found amino acid oxidation and protein degradation to be greater in LP compared with the follicular phase (FP) at rest and during exercise. It appears that P4 is responsible for the consistent finding of increased protein catabolism in LP, while estrogen may reduce protein catabolism (Oosthuyse & Bosch, 2010).

Overall, the existing data indicate a more anabolic state in FP and the peri-ovulatory phase of the menstrual cycle as compared to a more catabolic state in LP. The only available strength training intervention study using the different hormonal milieu of FP and LP as modulators of training adaptability analyzed the possible divergent effects of training stimuli in either FP or LP on the amount of strength gain in healthy women (Reis, Frick & Schmidtbleicher, 1995). The authors described a slightly higher trainability of isokinetic strength of one-leg knee extensor muscles in seven healthy young women when the respective leg was mainly trained for four weeks in FP (every other day in FP and once a week during the rest of the cycle) as compared to a training periodization without any regard for the phase of the cycle (every third day throughout the whole cycle). As the number of subjects was very small, and one of the subjects additionally had a luteal phase insufficiency, the training period of the specific training on one leg was short (4 weeks), and no muscle biopsy samples were taken, these results are very preliminary. Despite the wide inter-individual variability, however, all subjects of this study showed higher strength adaptations during the follicular-phase based training.

1.2. AIMS

The aim of this study was to further investigate the effects of a longer-lasting follicular phase-based (FT) strength training on macroscopic and microscopic parameters of skeletal muscle adaptations compared to luteal phase-based (LT) strength training in an in vivo controlled training intervention study in healthy young females.

1.3. METHODS

1.3.1. Subjects

Twenty healthy eumenorrheic women, with a mean (± SD) age of 25.9 ± 4.5 yr, height of 164.2 ± 5.5 cm and weight of 60.6 ± 7.8 kg volunteered to participate in this study. Subjects were untrained or moderately trained and they were currently not performing resistance training. Moreover they had not been taking oral contraceptives or any other hormonal treatments during the year prior to participation in this study and had no history of any endocrine disorders. Only women who reported a regular menstrual cycle were recruited.

Prior to the study, participants were informed about the purpose, procedures and risks of the study and written informed consent was obtained from each participant. Approval for the experimental protocol was obtained from the Ethics Committee of the Ruhr-University Bochum, Germany.

1.3.2. Experimental design

Participants performed a strength training program of the left and right knee extension muscle groups, separately for each leg, on a leg press machine over a period of three menstrual cycles each. Subjects were randomly divided to two groups according to single-leg muscle strength in order to reduce effects of leg preference: one group (N = 10) mainly

trained the left leg during the follicular phase (FT), while the right leg was mainly trained during the luteal phase (LT). The other group (N = 10) mainly trained the right leg during FP (FT), while the left leg was trained during LP (LT). For further analysis, both follicular phase-trained legs and both luteal phase-trained legs were taken together in the FT- or LT-trained leg group, respectively.

1.3.3. Study schedule

The duration of the study for each participant was based on the individual length of the menstrual cycle. The entire study took five menstrual cycles (2 control cycles followed by 3 training cycles). During the overall study period, individual cycle integrity was analyzed by daily measurements of basal body temperature.

In the first control cycle, the individual menstrual cycle integrity was analyzed by measurements of basal body temperature for non-OC users.
In the second control cycle, blood samples for hormone analysis were taken from a cubital vein on day 11 (late FP) and on day 25 (late LP) of the menstrual cycle. Additionally, maximum isometric strength of the knee extension muscles (F_{max}) was determined on the same days. Furthermore, the diameter (Mdm) of three single muscles of the quadriceps muscle was measured on day 25, and muscle biopsies were taken from the vastus lateralis muscle on day 27 (late LP) of the second control cycle.
During the three training cycles, F_{max} was repeatedly measured during each cycle on day 25 in LP. During the third training cycle, venous blood samples were taken again on days 11 and 25, Mdm was determined on day 25, and muscle biopsies were taken on day 27.

1.3.3.1. Monitoring of menstrual cycle integrity

The fluctuation of basal body temperature was used to identify the phases of the menstrual cycle including ovulation in order to individually determine the exact training and testing schedule. Subjects were instructed to

measure their basal body temperature orally with a digital thermometer for one minute every morning throughout the entire study period at the same time before getting out of bed. The occurrence of ovulation was defined when an increase in basal body temperature of at least 0.3 ºC was measured (Kelly, 2006; Owen, 1975). A subject was excluded from the study if no significant increase in basal body temperature, i.e. no ovulation, was detected during any of the five menstrual cycles.

1.3.3.2. Strength training program

The subjects completed three cycles of a one-leg strength training program with different training quantities of the right and left leg in FP and LP, respectively, while the total number of single-leg training sessions in one menstrual cycle remained the same in FP and LP. In principle, the training was performed four times a week: three times a week (typically on Monday, Wednesday and Friday) under supervision on a leg press machine and once a week (typically on Saturday) at home with the subject's own body weight (one-leg squats). On the days on which both legs had to be trained separately, subjects performed exercises for both legs one after the other in randomized order. On the leg press, subjects performed a submaximum strength training (about 80% of maximum strength of the respective leg) with three sets of 8–12 repetitions until exhaustion and with 3–5 min recovery between sets. The respective weight on the leg press machine was increased by 10 kg in the following training session if the subject was able to perform more than 12 repetitions during the last of the three sets. Training load of each individual one-leg training session on the leg press machine was documented. At home, subjects performed three sets of 15–20 one-leg squats with 3–5 min recovery between sets.

One leg was mainly trained in FP (FT) and the other leg mainly in LP (LT). In FT, subjects trained eight times in FP and around ovulation (typically between day 1 and day 14) and just twice in LP for FT during a "typical" menstrual cycle with a total length of 28 days. In LT, they trained eight

times in LP (typically between day 15 and day 28) and just twice in FP. When the individual cycle lasted less than 28 days, the number of training sessions was adapted accordingly so that the total number of sessions was the same for both legs. When the cycle lasted longer than 28 days and the number of single-leg training sessions in LT reached the number in FT (e.g. typically N = 10), subjects continued their single-leg strength training with both legs for another one or two sessions to avoid differences in the total number of training sessions between FT and LT in a single menstrual cycle.

1.3.3.3. Hormone analysis

Venous blood was centrifuged after blood clotting, and the serum was kept frozen at -80° C until analysis. Each sample was analyzed for E2, P4, total testosterone (T) and free T, and dehydrotestosterone-sulfate (DHEA-s). E2, P4, T, and DHEA-s were assayed by immunochemistry (Elecsys® 1010 System, Roche Diagnostics GmbH), and free T was assayed by radioimmunoassay (Multi-Crystal LB 2111 gamma counter, Berthold Technologies GmbH & Co. KG).

1.3.3.4. Measurement of isometric muscle strength

Maximum isometric knee extension muscle strength (F_{max}) of the right and left leg was measured separately once in late FP (day 11) and once in the late LP (day 25) in the second control cycle and in each training cycle. F_{max} was determined on a leg press machine (Medizinische Sequenzgeräte, Compass, Germany) using a combined force and load cell (GSV-2ASD, ME-Messsysteme GmbH, Hennigsdorf, Germany). The intraclass correlation coefficient of repeated measurements (ICC) was 0.998, indicating a high internal consistency (reliability) of the system. Prior to testing the subjects underwent a 10-min warm-up period of aerobic, low-resistance ergometer cycling and were then familiarized with the test procedure and the testing position (knee angle: 90°, ankle angle: 90°) on the leg press. Each measurement was repeated three times with

30 s rest between the tests. The best result was selected for data analysis. Due to time schedule issues, for subjects were not able to perform the maximum isometric strength tests during the training cycles, but were able to continue their strength training program without any reduction in training load. For the determination of the increase in F_{max} over time, strength values of the different tests during the training cycles were compared with the mean of both measurements in the control cycle.

1.3.3.5. Determination of muscle diameter

Mdm of rectus femoris, vastus intermedius and vastus lateralis muscle of the right and left leg was measured by real-time ultrasound imaging prior to and after training at day 25 in LP of the second control cycle and the third training cycle analyzing the distances between the outer and inner muscle fasciae. Previous studies showed that muscle cross-sectional area might reliably be measured using real-time ultrasound imaging (Martinson & Stokes, 1991). We used a Vivid I CE 0344 ultrasound device (GE Medical System, Solingen, Germany) with a parallel scanner (8L-RS, 4.0–13.3 MHz), which provides 10 cm penetration depth of the sound wave and enables high quality analysis of deeper lying muscles. Subjects prevented long-lasting static muscular tension for at least 30 minutes prior to the measurement in order to avoid alterations in Mdm (Reimer, 2004). All subjects lay supine with outstretched legs on an examination table without any pad, cushion or pillow underneath. Ultrasound images were obtained exactly half-way between the spina iliaca anterior superior and the upper margin of the patella. The transducer was placed gently on the skin to avoid compression and distortion of the underlying tissue (Reimer, 2004). The transducer was held at angles of 90° towards the skin and towards the longitudinal direction of the muscles to ensure a clear cross-sectional image. The images were frozen on the screen to measure muscle diameter. The position of the transducer was recorded for each muscle to reproduce the exact position after training intervention. The mean of three measurements of each of the three analyzed muscles was taken for both legs and the sum of the 3 Mdm was calculated for both

sides of the body. Reliability analysis was performed for Mdm determination. The obtained ICC was 0.997, indicating a high reliability of the ultrasound imaging of Mdm used in this study.

Mdm of rectus femoris, vastus intermedius and vastus lateralis muscle of the right and left leg was measured by real-time ultrasound imaging prior to and after training at day 25 in LP of the 2nd control cycle and the 3rd training cycle analysing the distances between the outer and inner muscle fasciae. Previous studies showed that muscle cross sectional area might reliably be measured using real-time ultrasound imaging (Martinson et al. 1991). We used a Vivid I CE 0344 ultrasound device (GE Medical System, Solingen, Germany) with a parallel scanner (8L-RS, 4.0 – 13.3 MHz), which provides 10 cm penetration depth of the sound wave and enables high quality analysis of deeper lying muscles. Subjects prevented long-lasting static muscular tension for at least 30 minutes prior to the measurement in order to avoid alterations in Mdm (Reimer, 2004). All subjects lay supine on the back with stretched legs on an examination couch without any pad, cushion or pillow underneath. Ultrasound images were obtained exactly half-way between the spina iliaca anterior superior and the upper margin of the patella. The transducer was placed gently on the skin to avoid compression and distortion of the underlying tissue (Reimer, 2004). The transducer was held at angles of 90° towards the skin and towards the longitudinal direction of the muscles to ensure a clear cross-sectional image. The images were frozen on the screen to measure muscle diameter. The position of the transducer was recorded for each muscle to reproduce the exact position after training intervention. The mean of 3 measurements of each of the 3 analysed muscles was taken at both legs and the sum of the 3 Mdm was calculated for both sides of the body. Reliability analysis was performed for Mdm determination. The obtained ICC was 0.997, indicating a high reliability of the ultrasound imaging of Mdm used in this study.

1.3.3.6. Histochemical analysis of muscle samples

Nine subjects volunteered to participate in muscle needle biopsies taken on day 27 of the second control cycle and of the third training cycle. After local anesthesia with 1% lidocaine and incision of the skin and fascia, percutaneous muscle biopsy samples (70–300 mg) were obtained from the vastus lateralis muscle of both the right and left leg by a standard needle biopsy technique (Bergström, 1962). Directly after sampling, the tissue was removed from the needle, mounted cross-sectionally in a Tissue-TEK® embedding medium, frozen in isopentane, put into an aluminum container, cooled further with liquid nitrogen, and stored at -80°C for subsequent analysis.

Thin sections (10 µm) of the frozen tissue were cut in a cryostat at -20°C and mounted on cover glasses for further staining. Histochemical analysis for the determination of muscle fiber types (types I and II) was performed with adenosine-triphosphatase (ATPase) staining procedures using an alkaline pre-incubation at pH 4.3 and 9.6 (Brooke et al. 1970). Moreover, muscle cell nuclei were stained with hematoxylin and eosin for nuclei-to-fiber ratio analysis (Yan, 2000). Fiber type counting and measurements were performed on photographs by two investigators to standardize the procedure. All fibers of one sample were counted and measured twice and the average of the two counts was taken for statistical analysis. If the variation between the two counts or measurements was greater than 1%, fibers were counted a third time and the average of the two counts with the smaller variation was used for analysis. For muscle fiber type classification, an average of 288 fibers from each sample was counted, the fiber type (Type I or Type II) identified, and the percentage of each type was calculated. For the determination of muscle fiber diameters (Fdm), an average of 62 fibers (range 20–119) from each fiber type was selected. Cellular diameters were determined using cell life science documentation software (Olympus Life and Material Science Europe GmbH, Germany).

1.3.4. Statistical Analysis

Data are presented as mean values with SD. Normality of distributions was proved by the Kolmogorov-Smirnov test. A one-Tailed paired *t*-test was used to evaluate differences in training workload, F_{max}, Mdm, fiber composition, fiber diameter and muscle nuclei-to-fiber-ratio between values before (pre) and after the training intervention (post) (see below: a, b) and between FT and LT (see below: c), respectively. In all cases, P values < 0.025 were taken to indicate statistical significance. Statistics were tested with a hierarchical procedure: a) FT_{post} better than FT_{pre}; b) LT_{post} better than LT_{pre}; c) if a) significant: ΔFT better than ΔLT; if b) significant: ΔLT better than ΔFT (ΔFT: absolute difference between FT_{pre} and FT_{post}, ΔLT: absolute difference between LT_{pre} and LT_{post}). A two-Tailed paired *t*-test was used to compare hormone concentration between FP and LP and between prior to and after training and to compare training units between FT und LT for three training cycles. Significance was defined as $P < 0.05$. The intraclass correlation coefficient of repeated measurements (ICC) (McGraw et al. 1996) was determined to evaluate reliability of the determination of F_{max} and Mdm.

1.4. RESULTS

1.4.1. Menstrual cycle integrity

Basal body temperature showed a significant increase during LP compared to FP in all three training cycles of the 20 subjects included in the study.

1.4.2. Number of training sessions

The total number of single-leg training sessions was approx. 28 sessions per leg and did not different between FT and LT (FT: N = 28.6 ± 1.7; LT: N = 28.1 ± 1.9; P > 0.05).

1.4.3. Training load

Mean training load did not differ between FT and LT at the beginning of the training period (FT: 69.4 ± 12.4 kg; LT: 68.1 ± 10.5 kg, P > 0.05). Training load was elevated continuously according to the increase in muscle strength from the beginning of the training period to the last training session in FT and LT. Due to a higher increase in muscle strength, the increase in training load was slightly higher at the end of FT compared to LT (FT: 102.5 ± 11.8 kg; LT: 97.5 ± 13.4 kg, P < 0.05), and mean training load was also higher slightly higher during FT compared to LT (FT: 88.1 ± 9.8 kg; LT: 84.7 ± 10.2 kg, P < 0.05).

1.4.4. Hormonal concentrations

TABLE 1-1: Serum concentrations of E2, P4, DHEA-s, T and free T in the follicular phase (FP, day 11) and the luteal phase (LP, day 25) before and after strength training (N = 20)

	Pre-Training		Post-Training	
	FP	LP	FP	LP
E2 (pg/ml)	124 ± 104	114 ± 71	92 ± 70	142 ± 41 †
P4 (ng/ml)	0.82 ± 0.53	5.66 ± 3.93 †	0.78 ± 0.50	8.36 ± 3.33 †
DHEA-s (ug/ml)	2.65 ± 1.13	2.52 ± 0.83	2.55 ± 0.73	2.58 ± 0.73
T (ng/ml)	0.44 ± 0.20	0.35 ± 0.18 †	0.37 ± 0.14 *	0.37 ± 0.15
Free T (pg/ml)	2.57 ± 0.86	1.94 ± 0.62 †	2.14 ± 0.62 *	2.06 ± 0.60

E2: estradiol, P4: progesterone, T: testosterone, pre/post-training: before/after three months of strength training, FP: follicular phase, LP luteal phase, *: $P < 0.05$ post training vs. pre training, †: $P < 0.05$ LP vs. FP

We did not find any significant differences in the serum concentrations of E2 and DHEA-s between day 11 and day 25 of the menstrual cycle prior to training, while P4 was significantly higher, and T and free T were significantly lower on day 25 as compared to day 11 (Table 1-1). After the strength training period, E2 and P4 were significantly higher on day 25 compared to day 11, while the differences in T and free T between both days were no longer detectable, and DHEA-s remained the same on both days. Three months of strength training induced a significant decline in the serum concentrations of T and free T on day 11 without any effect on day 25 or on the other hormones. The kind of training (FT vs. LT) did not have any different effect on any of the hormones (data not shown).

1.4.5. Maximum isometric muscle strength

F_{max} of knee extension muscles increased significantly ($P < 0.025$) after both types of training periodization as compared to the pretraining level (Figure 1-1). Absolute increase in F_{max} was significantly smaller after LT (ΔLT: 188 ± 98 N) compared to FT (ΔFT: 267 ± 101 N) ($P < 0.025$).

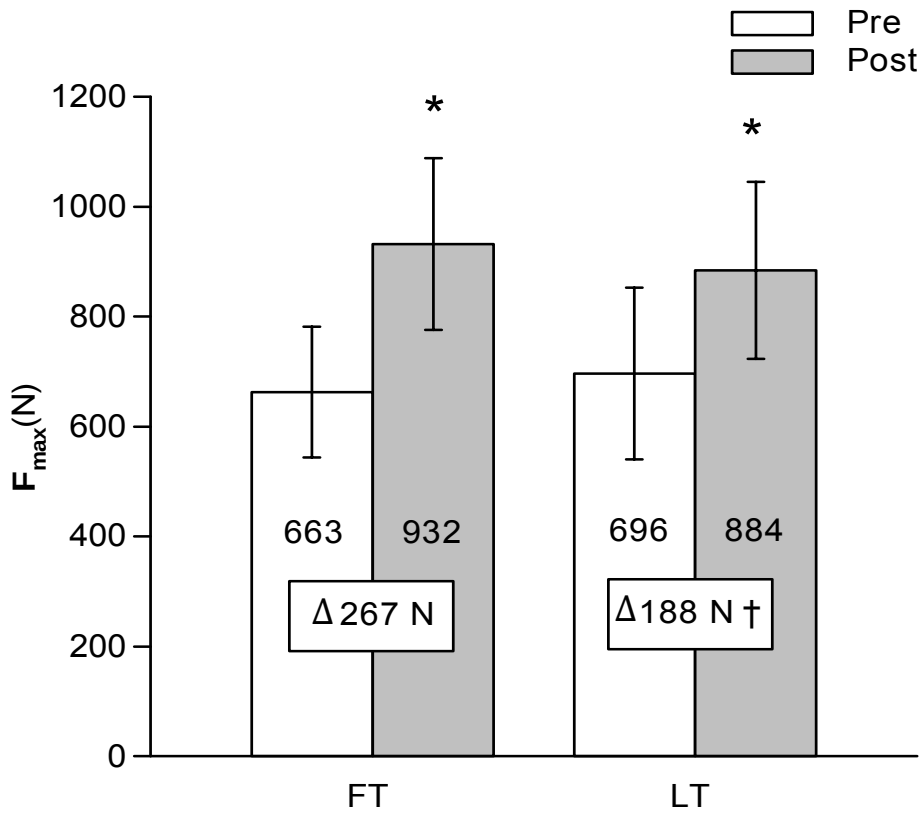

FIGURE 1-1: F_{max} before and after three months of follicular phase-based (FT) or luteal phase-based (LT) strength training (N = 20); Pre: before training, Post: after training, *: $P < 0.025$ post training vs. pre training, †: $P < 0.025$ FT vs. LT

F_{max} increased progressively during FT and LT as compared to the mean of both measurements in the control cycle, apart from the first strength test in LT, in which the slight increase in F_{max} did not reach the level of significance (Figure 1-2). From the first measurement in the first training cycle, increase in FT was significantly higher compared to LT and remained elevated to the same extent throughout the remaining training period.

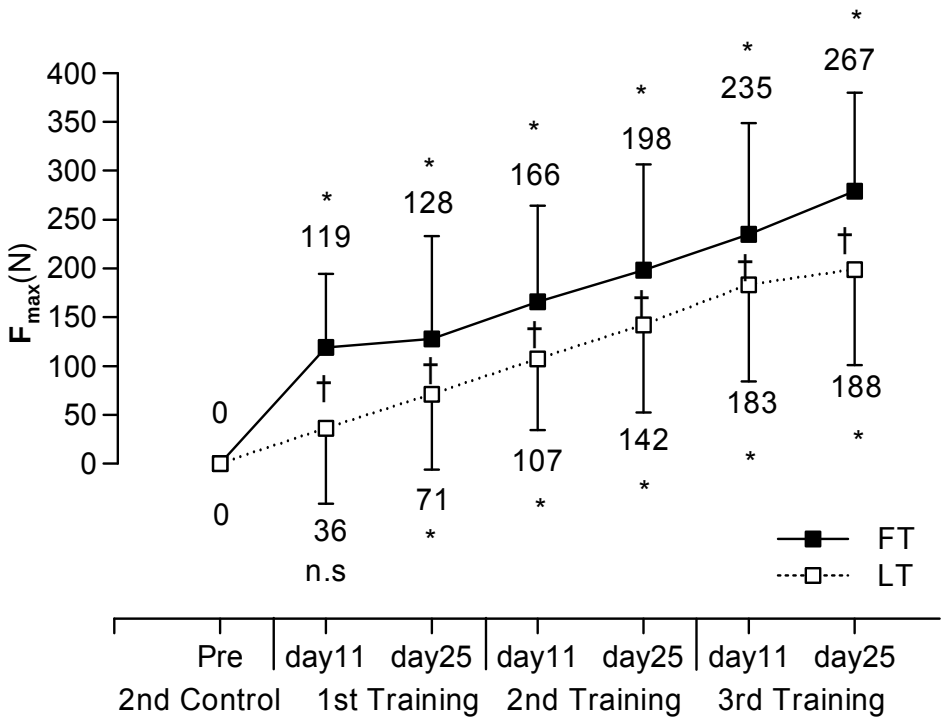

FIGURE 1-2: Increase in F_{max} compared to the pre-training value during follicular phase-based (FT) or luteal phase-based (LT) strength training (N = 18)
Pre: before training, Control: control cycle, Training: training cycle,
n.s.: not significant; *: $P < 0.025$ compared to pre training, †: $P < 0.025$ FT vs. LT

1.4.6. Muscle diameter

The sum of Mdm of the three muscles increased significantly ($P < 0.025$) after both types of training periodization compared to the pretraining level (Figure 1-3). Absolute increase in Mdm was significantly smaller after LT (ΔLT: 0.39 ± 0.38 cm) compared to FT (ΔFT: 0.57 ± 0.54 cm) ($P < 0.025$, Figure 1-3).

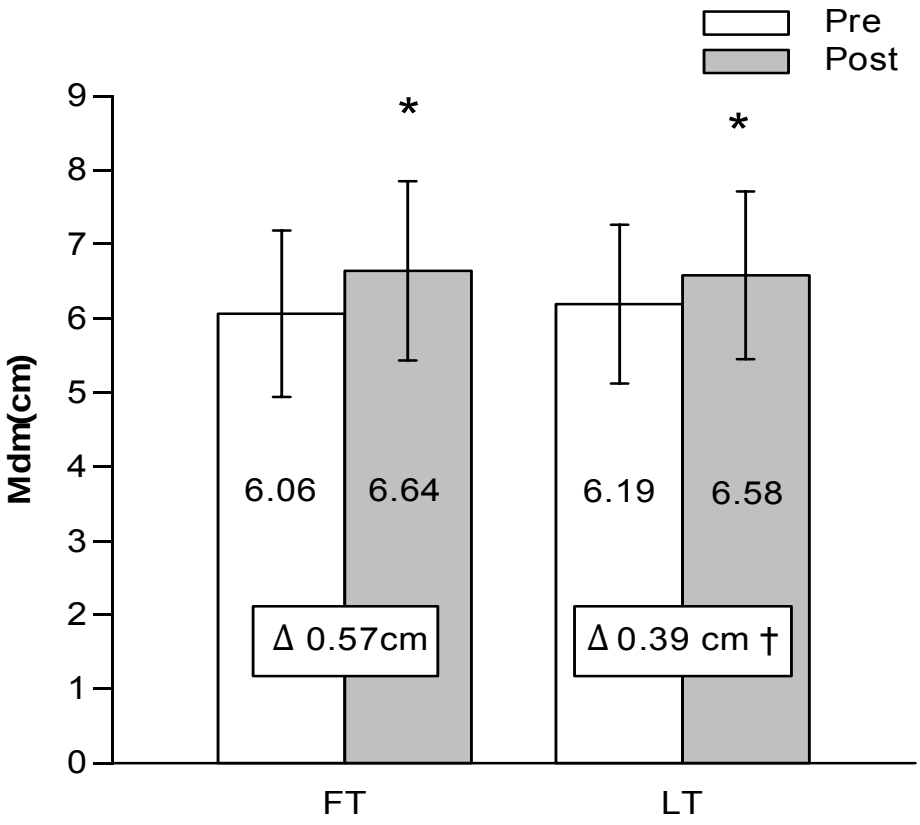

FIGURE 1-3: Sum of the diameters of rectus femoris, vastus intermedius and vastus lateralis muscle before and after three months of follicular phase-based (FT) or luteal phase-based (LT) strength training (N = 20); Pre: before training, Post: after training, *: $P < 0.025$ post training vs. pre training, †: $P < 0.025$ FT vs. LT

1.4.7. Muscle fiber characteristics

Freeze damage, created by freeze-thawing during preparation, is a major artifact that affects morphological analysis in this type of study. Although the least-damaged fibers in well-preserved regions were selected, there was still evidence of minor damage. The volume of artifacts varied between individuals, but pre- and post-training sample quality was similar so the results were not affected. Fiber type distribution remained nearly the same after both kinds of strength training periodization with about 40% type I fibers and 60% type II fibers (Table 1-2). Fdm increased significantly after FT in type II fibers ($P < 0.025$) and tended to increase after LT in type II fibers ($P = 0.045$), but remained the same in type I fibers after FT and LT. The nuclei-to-fiber ratio increased significantly after FT ($P < 0.025$) and remained unchanged after LT.

TABLE 1-2: Muscle fiber type distribution (No), fiber diameter (Fdm) and nuclei-to-fiber ratio (N/F) before and after three months of follicular phase-based or luteal phase-based strength training (N = 9)

	Pre-Training				Post-Training			
	FT		LT		FT		LT	
	Type I	Type II	Type I	Type II	Type I	Type II	Type I	Type II
No (%)	40.9 ± 9.1	59.1 ± 9.1	41.8 ± 13.6	58.2 ± 13.6	40.3 ± 11.1	59.7 ± 11.1	40.5 ± 13.0	59.5 ± 13.0
Fdm (µm)	54.5 ± 5.1	45.8 ± 5.8	54.0 ± 7.4	46.8 ± 7.9	56.7 ± 7.1	52.5 ± 7.0 *	57.0 ± 3.4	51.9 ± 7.3 #
N/F	2.9 ± 0.4		3.4 ± 0.8		3.8 ±1.1 *		3.4 ± 0.7	

FT: follicular phase-based training, LT: luteal phase-based training, *: $P < 0.025$ post-training versus pre-training, #: $P = 0.045$ post-training versus pre-training

1.5. DISCUSSION

The plasticity of skeletal muscle is reflected in its ability to adapt to altered metabolic and functional demands. Resistance training results in an increase in muscle strength accompanied by an increase in neural adaptation and muscle size. This increased muscular size is due to muscle fiber hypertrophy. Although hypertrophy occurs in all fiber types, that of type II fibers is most pronounced. Resistance training does not cause the conversion of type I slow to type II fast fiber. Women have the same physiological ability as males to tolerate and adapt to heavy resistance training (Wang, Hikida, Staron & Simoneau, 1993). However, only very few studies are concerned with the effects of the hormonal milieu throughout the menstrual cycle and the adaptation to strength training in women.

This study is the second one to address the planning of strength training with respect to hormonal fluctuations during the menstrual cycle, and the first to include the analysis of muscle cell parameters. In contrast to the first study by Reis et al. (Reis et al., 1995), we analyzed the effects of a longer-lasting training period (three menstrual cycles of one-leg FT vs. one-leg LT compared to two menstrual cycles of one-leg training with a change in the respective type of training for each leg after the first cycle). Furthermore, we clearly varied the strength training periodization between FP and LP, while Reis et al. (Reis et al., 1995) focused on a periodization between a follicular phase-based training versus a “regular training” with training loads every third day throughout the whole menstrual cycle.

The most important finding of our study is a slight but significantly higher increase in F_{max} after three months of FT compared to three months of LT (Figure 1-1). This is in line with the main finding of Reis et al. (Reis et al., 1995), who described a higher percent increase in F_{max} after the second training cycle in the follicular phase-trained leg compared to the regularly trained leg (33% increase vs. 13% increase in F_{max}). In contrast to Reis et al. (Reis et al., 1995), however, the difference between FT- and LT-induced increases in F_{max} in our study already occurred after the first

menstrual cycle and then remained nearly constant over the following two training cycles (Figure 1-2).

The second important finding of our study is a slight but significantly higher increase in the diameter of the rectus femoris, vastus intermedius and vastus lateralis muscles after FT compared to LT, which is in line with the higher increase in maximal isometric strength in FT. Reis et al. (Reis et al., 1995) measured muscle cross-sectional area (MSA) before and after strength training with two different regimes of menstrual cycle-triggered training periodization. While MSA increased slightly after both types of training, the authors did not include any data on significance in their study. The higher increase in muscle diameter after three months of follicular-phase based training in our study is associated with a higher ratio between protein synthesis and protein breakdown during or after each strength training session in the follicular phase compared to the luteal phase.

The more pronounced increase in muscle strength and muscle diameter in FT compared to LT could be explained at least in part by the higher concentrations of T and free T during FP compared to LP in the pre-training and probably at least also in the early training period in this study (Table 1-1). Since androgen secretion from the ovary is under luteinizing hormone (LH) control at least in part, it is not unexpected that ovarian androgen secretion varies through the cycle: the blood levels of T have been described as lowest in the early follicular phase and then rising to their highest levels just prior to, or at the time of, ovulation and then gradually fall during the luteal phase (Alexander, Sherwin, Bancroft & Davidson, 1990; Longcope, 1986). In females, serum T, however, may also originate from the adrenal gland or from peripheral conversion (Enea, Boisseau, Fargeas-Gluck, Diaz & Dugue, 2011). Early studies have shown that the production rate of T from the adrenals is about 50 µg/day, with the ovaries secreting an additional 50 µg/day and the major source of T is the peripheral conversion of androstenedione (around 100 µg/day) (Longcope, 1986). This mixture and production interrelationship may explain why some studies did not find any changes in serum T concentration

throughout the menstrual cycle (Jabbour, Kelly, Fraser & Critchley, 2006), and why T and free T were no longer different between FP and LP after three months of strength training in our study, or even declined over time in FP after strength training compared to FP prior to strength training. DHEA-s, the main metabolite of the adrenal glands but not of the ovaries, remained completely unaffected by the phase of the cycle and throughout the training intervention period in this investigation. In a very recent review of physical exercise-induced changes in the concentration of circulating androgens in women, the authors concluded that studies regarding the effect of resistance exercise on circulating androgens in women (Enea, Boisseau, Fargeas-Gluck, Diaz & Dugue, 2011) are still contradictory.

The biological actions of androgens once inside the cell are mediated by the androgen receptor (AR). The AR complexes interact with various factors (e.g. coactivators or corepressors) to modulate transcription of androgen target genes via binding to specific DNA sequences and resulting in protein synthesis as an adaptation process to training stimuli. Androgens may also regulate cellular activity via a more rapid non-genomic mechanism involving membrane receptors and/or cytosolic receptors. These steroid receptors are able to activate intracellular signaling molecules, such as the mitogen-activated protein kinase 1 (MAPK1), by transcription-independent mechanisms (Enea, Boisseau, Fargeas-Gluck, Diaz & Dugue, 2011).

Apart from the effects of androgens, the more pronounced increase in muscle strength and muscle diameter in FT compared to LT may also be explained by alterations of the ovarian hormones throughout the menstrual cycle. It has long been demonstrated that the ovarian hormones fluctuate during the menstrual cycle (Oosthyse & Bosch, 2010, Reilly, 2000). E2 peaks prior to ovulation and during LP, while P4 reaches its highest values during LP after ovulation (Van Look & Baird, 1980). The ovarian hormones are known to have a noticeable influence on protein metabolism at rest and during exercise, which is often seen as increased catabolism in the LP. It appears that progesterone is responsible for the consistent finding of

increased protein catabolism in the LP, while estrogen may reduce protein catabolism (Oosthuyse & Bosch, 2010).

To check for ovarian hormone fluctuation in our study, we analyzed E2 and P4 on day 11 (pre-ovulation) and on day 25 (luteal phase).
On these days, both hormones showed high interindividual variations. P4 clearly increased in all subjects in the luteal phase, indicating that ovulation had occurred in all of them and that the training period had not induced any severe alteration in menstrual cycle integrity such as anovulation or luteal phase insufficiency. The similar concentrations in E2 on days 11 and 25 prior to the training period are probably due to the fact that day 11 represents a phase prior to ovulation, when E2 is already elevated compared to early and middle FP (Van Lock & Baird, 1980). The increase in E2 after LT may be due to exercise- and training-induced changes in menstrual cycle physiology, including alterations in feedback regulation of steroid hormones. Recently, serum estradiol and progesterone were shown to increase after a single bout of resistance exercise in healthy young women in the mid-luteal phase, but not in the early follicular phase, indicating that the responses of anabolic hormones to acute resistance exercise are different among the menstrual cycle states in young women (Nakamura, Aizawa, Imai, Kono & Mesaki, 2011). The authors concluded that menstrual cycle state may influence the exercise training-induced skeletal muscular adaptation, and that it would be possible for training programs for eumenorrheic women to be timed in accordance with the menstrual cycle in order to maximize anabolic effects. Our study suggests that acute effects of anabolic hormones on skeletal muscle adaptation at the beginning of a strength training period might interfere with the more chronic effects of the repetitive training stimulus on feedback regulation of the hormones of the hypothalamic-pituitary-ovarian axis.

This study is the first to investigate muscle fiber parameters after two types of menstrual-cycle-based strength training. As only 9 of the 20 subjects agreed in muscle biopsy results have to be interpreted carefully. We did

not find any changes in the proportion of type I and type II fibers after the two training periodization protocols. This is in line with other studies suggesting that most muscle fiber transformation after strength training occurs in type II fiber subtypes rather than between type I and type II fibers (Adams et al. 1993, Howald 1982, Wang et al. 1993). Unfortunately we were not able to differentiate between type IIa and type IIx fiber subtypes due to ATPase-staining problems, so that no information about changes in type II subtype fiber characteristics can be provided.

A remarkable finding of our study was the significant increase in type II fiber diameter after FT (delta: 6.7 µm, $p<0.01$) compared to only a tendency towards increase in type II fiber diameter after LT (delta: 5.1 µm, $p=0.045$). Resistance training leads to an increase in the volumes of myofibrils, interfibrillar space, mitochondria, and lipid droplets in females (Wang et al. 1993). An increase in myofibril number and/or size requires an increase in specific protein biosynthesis, whose degree is dependent on anabolic agents such as testosterone and estrogens. Therefore, the slightly higher increase in cell diameter of type II fibers after FT compared to LT in our study is again in line with the higher increase in muscle strength and muscle diameter after FT compared to LT, and menstrual cycle-dependent alterations in anabolic hormones.

Myonuclei-to-fiber ratios were analyzed by means of HE staining. Therefore, no fiber type-specific data are available. Interestingly, nuclei-to-fiber ratio increased after FT and remained unaffected after LT. Adult muscle fibers contain hundreds of myonuclei, where each myonucleus sustains the protein synthesis over a finite volume of cytoplasm. In this respect, significant enlargement of muscle fibers is accompanied by a significant increase in the myonuclear number. Existing myonuclei are able to support a certain level of fiber hypertrophy. However, when the transcriptional activity of existing myonuclei reaches its maximum, the enhancement of the number of myonuclei is thought to become involved in the enhancement of protein synthesis (Kadi, 2008). A substantial increase in the size of myofibers in the muscles requires the availability of satellite

cells that can provide additional myonuclei to support hypertrophy (Adams, 2006). While normally quiescent in adult skeletal muscle, in response to myofiber injury or overload, satellite cells re-enter the cell cycle, where they proliferate and differentiate to provide muscle-specific proteins needed for skeletal muscle growth and regeneration (Enns & Tiidus, 2010). A variety of alterations in the surrounding environment of the satellite cell, including mechanical and growth factors, as well as hormonal signaling including testosterone could regulate the activation and proliferation of satellite cells (Kadi, 2008). Furthermore, sex-mediated differences in muscle-fiber regeneration and satellite-cell numbers may be directly attributed to estrogenic influence, and estrogen may exert its influence on post-exercise muscle-satellite cell populations through events upstream of satellite-cell activation (Enns & Tiidus, 2008). Taken together, our results underpin a possible role of hormonal alterations, both of testosterone and estrogens, throughout the menstrual cycle in the process of satellite-cell incorporation-induced muscle hypertrophy.

1.6. Conclusion

In conclusion, follicular phase-based strength training induced a slightly higher effect on muscle strength, muscle and type II fiber diameter and nuclei-to-fiber ratio compared to luteal phase-based strength training. We recommend that moderately trained eumenorrheic females without oral contraception base the periodization of strength training on their menstrual cycle.

2. STUDY 2: EFFECT OF MENSTUAL PHASE-BASED STRENGTH TRAINING IN ORAL CONTRACEPTIVE

ABSTRACT

PURPOSE: The aim of this study was to investigate effects of quasi-follicular phase-based (qFT, day 1-14 of the menstrual cycle) in comparison to quasi-luteal phase-based strength training (qLT, day 15-28 of the menstrual cycle) on muscle strength, muscle volume and microscopic parameters in users of oral contraceptives (OC).

METHODS: Seventeen healthy women using oral contraception completed a strength training program on a Leg Press for 3 menstrual cycles. They trained one leg mainly in the quasi-follicular phase (qFP) and the other leg mainly in the quasi-luteal phase (qLP). Concentration of estradiol (E2), progesterone (P4), total testosterone (T), free testosterone (free T) and DHEA-s were analyzed during qFP and qLP. Maximum isometric force (F_{max}), muscle diameter (Mdm), muscle fiber composition (No), fiber diameter (Fdm) and cell nuclei-to-fiber ratio (N/F) were analyzed before and after training.

RESULTS: Prior to training E2, P4, DHEA-s and T were not significantly different between the two phases, while free T was lower in qLP compared to qFP. After three months of strength training, P4, DHEA-s and T became higher in qFP compared to qLP ($P < 0.05$), while the difference in free T was no longer detectable. F_{max} and Mdm increased significantly after qFT and qLT without any differences between the two types of training periodization. OC pills with or without androgenicity did not have any influence on the development of F_{max} and Mdm. Number of fiber type II tended to increase after qFT, however remained the same after qLT, while the other muscle cell parameters were unaffected by any training periodization.

CONCLUSION: Both, qFT and qLT showed significant effects on muscle strength and muscle hypertrophy after three months of strength training, without any differences between the two training periodizations or without any effect of androgenicity of the OC pill. This is in contrast to findings in

non-users of OC, who clearly showed higher increases in F_{max} and Mdm after follicular phase-based training as compared to luteal phase-based training. We therefore conclude that untrained or moderately trained OC users can perform their strength training independently from the phases of their menstrual cycle and that they can take any monophasic OC pill without caring for the type of progestin in the pill.

2.1. INTRODUCTION

Worldwide millions of women regularly use OC. Female athletes not only use OC for birth control but also for reasons of menstrual cycle control, management of premenstrual symptoms, dysmenorrhea, reduction of menstrual blood loss, lower risk of musculoskeletal injury and time-shifting of the menstrual cycle (Bennell, White & Crossley, 1999; Constantini, Dubnov & Lebrun, 2005; Wojtys, Huston, Boynton, Spindler & Lindenfeld, 2002). The most common used monophasic OC consist of ethinylestradiol and progestin in fixed doses. This kind of OC is taken for 21 days (consumption phase), followed by 7 days of OC break (withdrawal phase). As a result, endogenous E2 and P4 are suppressed and, according to the oral intake of constant amounts, blood concentrations of E2 and P4 remain nearly constant during the 21 days of consumption phase (Rechichi, Dawson & Goodman, 2009). As sexual steroid hormones play a considerable role in training adaptation processes, OC-induced alterations in their blood concentrations might lead to alterations in the amount of training adaptation in OC users compared to non-OC users.

A number of studies were carried out to find possible effects of OC on muscle strength. Elliott et al. (Elliott, Cable & Reilly, 2005) examined the blood concentrations of E2 and P4 and muscle strength on days 7 and 14 of the OC consumption phase in OC users and they did not find any differences in E2 and P4 between the two days. Moreover, they did not find any difference in muscle strength between the two phases. Phillips et al. (Phillips, Sanderson, Birch, Bruce & Woledge, 1996) also reported no significant change in muscle strength during the first two weeks of OC intake. Other studies also failed to detect significant differences in muscle strength during OC phases (Peters & Burrows, 2006; Sarwar, Niclos & Rutherford, 1996; Wirth & Lohman, 1982), indicating that a possible effects of OC use on muscle strength and performance is no more than minimal. Furthermore, the only available study on androgenicity of the progestin in oral contraceptive pills has failed to show any significant effect on maximal leg strength during different phases of the pill cycle (Peters et al. 2006).

In a preceded study we could demonstrate that follicular phase-based strength training showed more pronounced effects on muscle strength compared to luteal phase-based resistance training in eumenorrheic non-OC users (Sung et al. 2012), which was probably due to the specific hormonal milieu during each phase of the cycle. In contrast to this investigation in non-OC users, there are no training intervention studies available in OC users that have differentially assessed the trainability of strength in the two respective phases of OC use. Additionally, the possible influences of other interacting anabolic hormones like T and DHEA-s in training adaptation processes in OC users are not clear until now.

2.2. AIMS

The aim of this study was to investigate the effects of longer-lasting quasi follicular phase-based strength training on macroscopic and microscopic parameters of skeletal muscle adaptations compared to quasi luteal phase-based strength training in an in vivo controlled training intervention study in healthy young females, and to analyze for possible differences of OC pills with or without androgenicity.

2.3. METHODS

2.3.1. Subjects

Seventeen healthy women, with a mean (± SD) age of 22.5 ± 2.4 yr, height of 167.1 ± 6.6 cm and weight of 62.9 ± 9.4 kg volunteered to participate in this study. Subjects were untrained or moderately trained and they were currently not performing resistance training. Moreover they had been taking monophasic combined OC for at least one year prior to participation in this study and had no history of any endocrine disorders. Monophasic combined OC are taken for 21 days followed by a 7 day pill–free interval when a hormone-withdrawal bleeding occurs. The 21 pills contain constant concentrations of synthetic estrogen (20–35 µg of ethinylestradiol) and gestagen (100–250 and 2000–3000 µg of gestagen depending on brands), which inhibit fertility. The kind and number of preparation used by the subjects of this study including values of their assumed androgenic effects is given in Table 2-1. Prior to the study, participants were informed about the purpose, procedures and risks of the study and written informed consent was obtained from each participant. Approval for the experimental protocol was obtained from the Ethics Committee of the Ruhr-University Bochum, Germany.

TABLE 2-1: Monophasic oral contraceptive pills used by the subjects of this study including doses of ethinylestradiol and gestagen and their possible androgenicity index

Dose of Ethinylestradiol and Gestagen in OC					
Name of Trades	Ethinylestradiol (µg)	Gestagen (µg)	Type of Progestin	*Androgenicity*	Number of subjects
Aida	20	3000	Drospirenone	0	1
Belara	30	2000	Chlormadinone acetate	0	3
Cilest	30	2000	Norgestimate	3.8	1
Femigoa	30	150	Levonorgestrel	1.25	1
Leios	20	100	Levonorgestrel	0.83	2
Minisiston	30	125	Levonorgestrel	1.04	1
Petibelle	30	2000	Drospirenone	0	1
Vallete	30	2000	Dienogest	0	3
Yasmin	30	3000	Drospirenone	0	3
Yasminelle	30	3000	Drospirenone	0	1

2.3.2. Experimental design

Participants performed a strength training program of the left and right knee extension muscle groups, separately for each leg, on a leg press machine over a period of three menstrual cycles each. Subjects were randomly divided to two groups according to single-leg muscle strength in order to reduce effects of leg preference: one group (N = 9) mainly trained the right leg during the quasi - follicular phase (qFT), while the left leg was mainly trained during the quasi - luteal phase (qLT). The other group (N = 8) mainly trained the left leg during qFP (qFT), while the right leg was trained during qLP (qLT). For further analysis, both follicular phase-trained legs and both luteal phase-trained legs were taken together in the qFT- or qLT-trained leg group, respectively.

2.3.3. Study schedule

The entire study took four menstrual cycles (1 control cycle followed by 3 training cycles), equivalent to 112 days considering that one menstrual cycle always took 28 days. The first day of menstrual bleeding in the withdrawal phase was defined as day 1 of the cycle. Day 1 to day 14 was defined as qFP, and day 15 to the first day of the following menstrual bleeding as qLP, oriented on the terminology of menstrual phase classification in eumenorrheic women.

In the control cycle, blood samples for hormone analysis were taken from a cubital vein on day 11 (late qFP) and on day 25 (late qLP) of the menstrual cycle. Additionally, maximum isometric strength of the knee extension muscles (F_{max}) was determined on the same days. Furthermore, the diameter (Mdm) of three single muscles of the quadriceps muscle was measured on day 25, and muscle biopsies were taken from the vastus lateralis muscle on day 27 (late qLP) of the control cycle.

During the three training cycles, F_{max} was repeatedly measured during each cycle on day 25 in qLP. During the third training cycle, venous blood samples were taken again on days 11 and 25, Mdm was determined on day 25, and muscle biopsies were taken on day 27.

2.3.3.1. Strength training program

The subjects completed three cycles of a one-leg strength training program with different training quantities of the right and left leg in qFP and qLP, respectively, while the total number of single-leg training sessions in one menstrual cycle remained the same in qFP and qLP. In principle, the training was performed four times a week: three times a week (typically on Monday, Wednesday and Friday) under supervision on a leg press machine and once a week (typically on Saturday) at home with the subject's own body weight (one-leg squats). On the days on which both legs had to be trained separately, subjects performed exercises for both legs one after the other in randomized order. On the leg press, subjects performed a sub-maximum strength training (80% of maximum

strength of the respective leg) with three sets of 8-12 repetitions until exhaustion and with 3-5 min of recovery between sets.
The respective weight on the leg press machine was increased by 10 kg in the following training session if the subject was able to perform more than 12 repetitions during the last of the three sets. Training load of each individual one-leg training session on the leg press machine was documented. At home subjects performed three sets of 15-20 one-leg squats with 3-5 min recovery between sets.

The respective weight on the leg press machine was increased by 10 kg in the following training session if the subject was able to perform more than 12 repetitions during the last of the three sets. Training load of each individual one-leg training session on the leg press machine was documented. At home they performed three sets of 15-20 one-leg squats with 3-5 min recovery between sets.
One leg was mainly trained in qFP (qFT) and the other leg mainly in qLP (qLT). In qFT, subjects trained eight times in qFP and just twice in qLP. In qLT, they trained eight times in qLP and just twice in qFP.

2.3.3.2. Hormone analysis

Venous blood was centrifuged after blood clotting, and the serum was kept frozen at -80° C until analysis. Each sample was analyzed for E2, P4, total testosterone (T) and free T, and dehydrotestosterone-sulfate (DHEA-s). E2, P4, T, and DHEA-s were assayed by immunochemistry (Elecsys® 1010 System, Roche Diagnostics GmbH), and free T was assayed by radioimmunoassay (Multi-Crystal LB 2111 gamma counter, Berthold Technologies GmbH & Co. KG).

2.3.3.3. Measurement of isometric muscle strength

F_{max} of the right and left leg was measured separately once in late qFP (day 11) and once in the late qLP (day 25) in the control cycle and in each training cycle. F_{max} was determined on a leg press machine (Medizinische Sequenzgeräte, Compass, Germany) using a combined force and load cell (GSV-2ASD, ME-Messsysteme GmbH, Hennigsdorf, Germany). The intraclass correlation coefficient of repeated measurements (ICC) was 0.998, indicating a high internal consistency (reliability) of the system. Prior to testing the subjects underwent a 10-min warm-up period of aerobic, low-resistance ergometer cycling and were then familiarized with the test procedure and the testing position (knee angle: 90°, ankle angle: 90°) on the leg press. Each measurement was repeated three times with 30 s of rest between the tests. The best result was selected for data analysis. For the determination of the increase in F_{max} over time, strength values of the different tests during the training cycles were compared with the mean of both measurements in the control cycle.

2.3.3.4. Determination of muscle diameter

Mdm of rectus femoris, vastus intermedius and vastus lateralis muscle of the right and left leg was measured by real-time ultrasound imaging prior to and after training at day 25 in qLP of the control cycle and the third training cycle analyzing the distances between the outer and inner muscle fasciae. Previous studies showed that muscle cross-sectional area might reliably be measured using real-time ultrasound imaging (Martinson & Stokes, 1991). We used a Vivid I CE 0344 ultrasound device (GE Medical System, Solingen, Germany) with a parallel scanner (8L–RS, 4.0-13.3 MHz), which provides 10 cm penetration depth of the sound wave and enables high quality analysis of deeper lying muscles. Subjects prevented long-lasting static muscular tension for at least 30 minutes prior to the measurement in order to avoid alterations in Mdm (Reimer, 2004). All subjects lay supine with outstretched legs on an examination table without any pad, cushion or pillow underneath. Ultrasound images were obtained

exactly half-way between the spina iliaca anterior superior and the upper margin of the patella.

The transducer was placed gently on the skin to avoid compression and distortion of the underlying tissue (Reimer, 2004). The transducer was held at angles of 90° towards the skin and towards the longitudinal direction of the muscles to ensure a clear cross-sectional image. The images were frozen on the screen to measure muscle diameter. The position of the transducer was recorded for each muscle to reproduce the exact position after training intervention. The mean of three measurements of each of the three analyzed muscles was taken for both legs and the sum of the 3 Mdm was calculated for both sides of the body. Reliability analysis was performed for Mdm determination. The obtained ICC was 0.997, indicating a high reliability of the ultrasound imaging of Mdm used in this study.

2.3.3.5. Histochemical analysis of muscle samples

Six subjects volunteered to participate in muscle needle biopsies taken on day 27 of the control cycle and of the third training cycle. After local anesthesia with 1% lidocaine and incision of the skin and fascia, percutaneous muscle biopsy samples (70–300 mg) were obtained from the vastus lateralis muscle of both the right and left leg by a standard needle biopsy technique (Bergstrom et al., 1976). Directly after sampling, the tissue was removed from the needle, mounted cross-sectionally in a Tissue-TEK® embedding medium, frozen in isopentane, put into an aluminium container, cooled further with liquid nitrogen, and stored at -80°C for subsequent analysis.

Thin sections (10 µm) of the frozen tissue were cut in a cryostat at -20°C and mounted on cover glasses for further staining. Histochemical analysis for the determination of muscle fiber types (types I and II) was performed with adenosine-triphosphatase (ATPase) staining procedures using an alkaline pre-incubation at pH 4.3 and 9.6 (Brooke & Kaiser, 1970). Fiber type counting and measurements were performed on photographs by two

investigators to standardize the procedure. All fibers of one sample were counted and measured twice and the average of the two counts was taken for statistical analysis. If the variation between the two counts or measurements was grater than 1%, fibers were counted a third time and the average of the two counts with the smaller variation was used for analysis. For muscle fiber type classification an average of 288 fibers from each sample was counted, the fiber type (Type I or Type II) identified, and the percentage of each type was calculated. For the determination of muscle fiber diameters (Fdm) an average of 62 fibers (range 20–119) from each fiber type was selected. Cellular diameters were determined using the cell life science documentation software (Olympus Life and Material Science Europe GmbH, Germany).

2.3.4. Statistical Analysis

Data are presented as mean values with SD. Normality of distributions was proved by the Kolmogorov-Smirnov test. A one-sided paired *t*-test was used to evaluate differences in training workload, F_{max}, Mdm, fiber composition, fiber diameter and muscle nuclei-to-fiber-ratio between values before (pre) and after the training intervention (post) (see below: a, b) and between qFT and qLT (see below: c), respectively. In all cases, P values < 0.025 were taken to indicate statistical significance. Statistics were tested with a hierarchical procedure: a) qFT_{post} better than qFT_{pre}; b) qLT_{post} better than qLT_{pre}; c) if a) significant: ΔqFT better than ΔqLT; if b) significant: ΔqLT better than ΔqFT (ΔqFT: absolute difference between qFT_{pre} and qFT_{post}, ΔqLT: absolute difference between qLT_{pre} and qLT_{post}).
A one-sided independent *t*-test was used to compare differences of OC pills with or without androgenicity in all parameters. In all cases, P values < 0.025 were taken to indicate statistical significance.
A two-sided paired *t*-test was used to compare hormone concentration between qFP and qLP and between pre- and post-training, and to compare training units between qFT und qLT for three training cycles. In these cases significance was defined as P < 0.05. A one-sided independent t-test was used to compare differences of OC pills with or

without androgenicity in all parameters. In all cases, P values < 0.025 were taken to indicate statistical significance. The intraclass correlation coefficient of repeated measurements (ICC) (McGraw et al. 1996) was determined to evaluate reliability of the determination of F_{max} and Mdm.

2.4. RESULTS

2.4.1. Number of training sessions

The total number of single-leg training sessions was 28 sessions per leg and did not different between qFT and qLT (qFT: N = 28.0 ± 0.0; qLT: N = 28.0 ± 0.0).

2.4.2. Training load

Mean training load did not differ between qFT and qLT at the beginning of the training period (qFT: 87.1 ± 16.1 kg; qLT: 85.9 ± 18.0 kg, P > 0.05). Training load was elevated continuously according to the increase in muscle strength from the beginning of the training period to the last training session in qFT and qLT. Due to a comparable increase in muscle strength, the training load was similar at the end of qFT and qLT (qFT: 115.3 ± 17.0 kg; qLT: 114.7 ± 16.2 kg, P > 0.05), and mean training load over the whole three months training period was also similar during qFT and qLT (qFT: 102.2 ± 19.4 kg; qLT: 101.6 ± 19.9 kg, P > 0.05).

2.4.3. Hormone concentrations

Prior to training E2, P4, DHEA-s and T were not significantly different between the two phases, while free T was lower in qLP compared to qFP. After three months of strength training, P4, DHEA-s and T became higher in qFP compared to qLP ($P < 0.05$) while the difference in free T was no longer detectable because of a significant decline in free T in qFP (Table 2-2).

TABLE 2-2: Serum concentrations of E2, P4, DHEA-s, T and free T in the quasi-follicular phase (qFP, day 11) and the quasi-luteal phase (qLP, day 25) before and after strength training (N = 17)

	Pre-Training		Post-Training	
	qFP	qLP	qFP	qLP
E2 (pg/ml)	16.3 ± 15.4	12.5 ± 9.2	21.1 ± 23.4	13.5 ± 9.6
P4 (ng/ml)	0.65 ± 0.53	0.46 ± 0.32	0.55 ± 0.26	0.45 ± 0.20 †
DHEA-s (ug/ml)	1.64 ± 0.49	1.48 ± 0.56	1.76 ± 0.56	1.38 ± 0.44 †
T (ng/ml)	0.22 ± 0.10	0.22 ± 0.14	0.24 ± 0.15	0.16 ± 0.09 *†
Free T (pg/ml)	1.79 ± 0.51	1.45 ± 0.39 †	1.50 ± 0.44 *	1.34 ± 0.47

E2: estradiol, P4: progesterone, T: testosterone, pre/post-training: before/after three months of strength training, qFP: quasi follicular phase, qLP quasi luteal phase, *: $P < 0.05$ post training vs. pre training, †: $P < 0.05$ qLP vs. qFP

2.4.4. Maximum isometric muscle strength

F_{max} of knee extension muscles increased continuously and significantly ($P < 0.025$) during both types of training periodization without any difference between qFT and qLT (Figure 2-1). Absolute increases in F_{max} after qFT and qLT also did not differ between both training periodization ($P > 0.025$, Figure 2-2). Furthermore, F_{max} in the subjects taking OC pills without any

androgenicity increased in about the same size as in the subjects taking OC pills with known androgenicity (Figure 2-3).

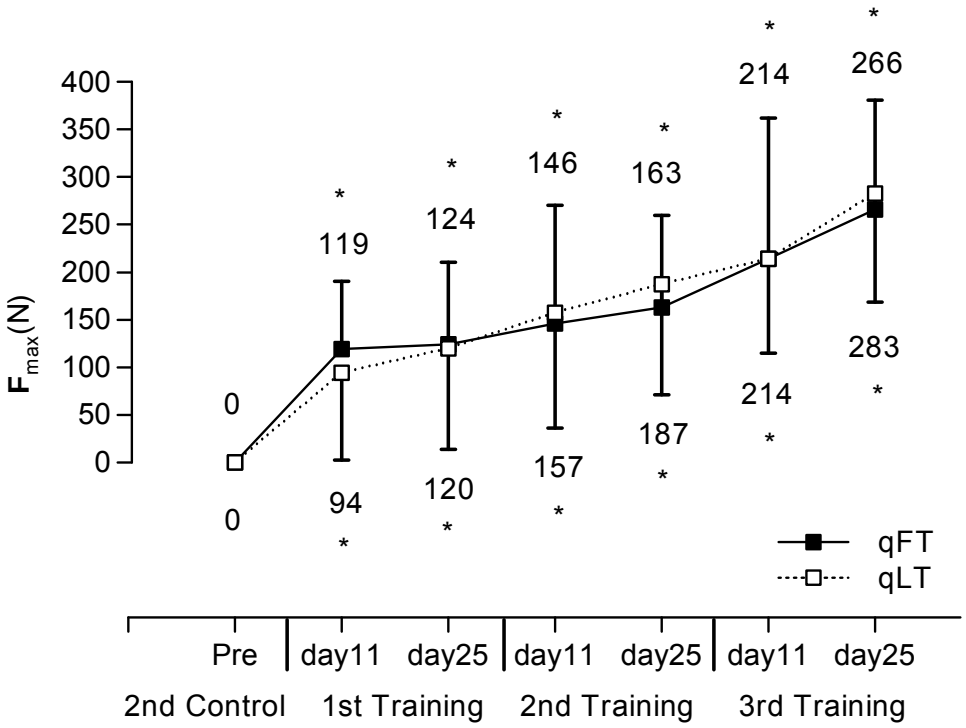

FIGURE 2-1: Increase in F_{max} compared to the pre-training value during quasi-follicular phase-based (qFT) or quasi-luteal phase-based (qLT) strength training (N = 17)
Pre: before training, Control: control cycle, Training: training cycle, *: P < 0.25 compared to pre-training

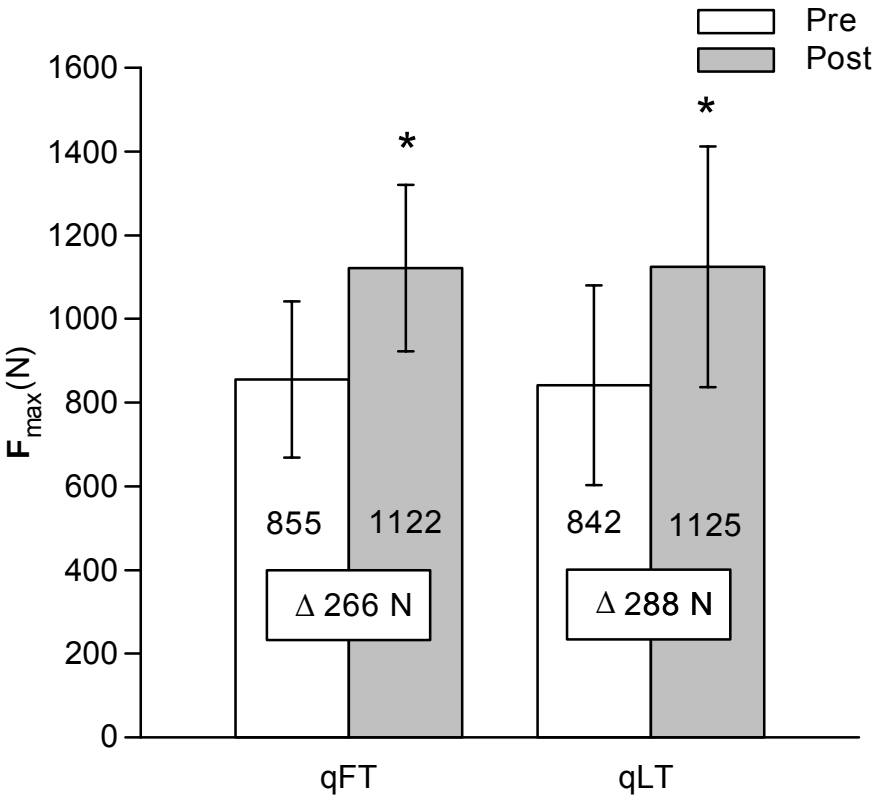

FIGURE 2-2: F_{max} before and after three months of quasi-follicular phase-based (qFT) or quasi-luteal phase-based (qLT) strength training (N = 17)

Pre: before training, Post: after training, *: P < 0.025 post training vs. pre training

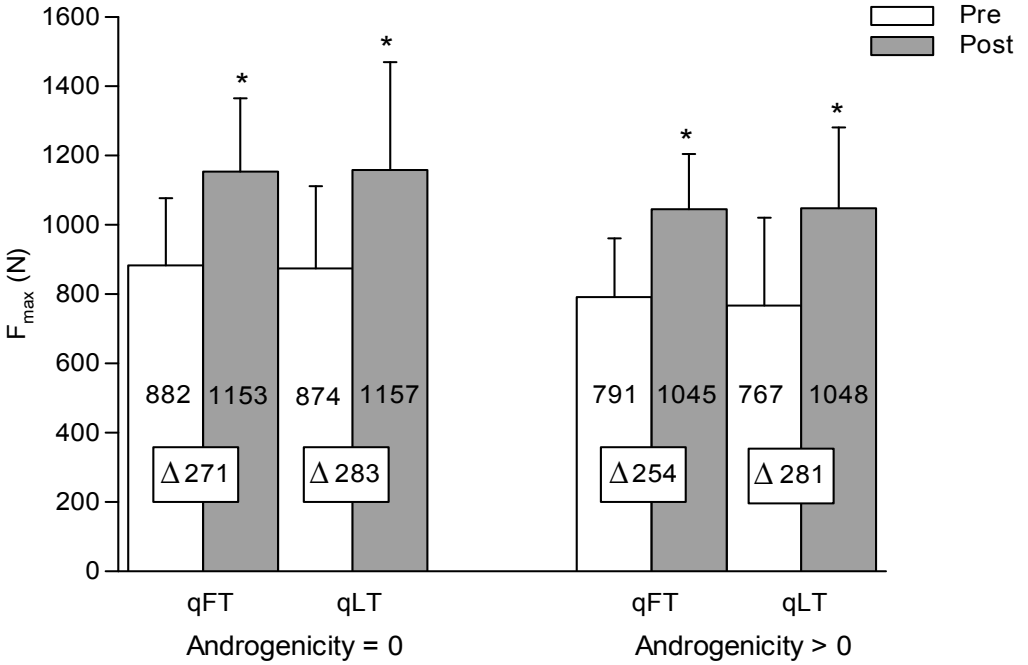

FIGURE 2-3: F_{max} before and after three months of quasi-follicular phase-based (qFT) or quasi-luteal phase-based (qLT) strength training in two groups of subjects taking OC without any androgenicity (N = 12) or with known androgenicity (N = 5)

Pre: before training, Post: after training, *: P < 0.025 post training vs. pre training

2.4.5. Muscle diameter

The sum of Mdm of the three muscles increased significantly ($P < 0.025$) after both types of training periodization compared to the pre-training level. Absolute increase in Mdm was not significantly different between qFT and qLT ($P > 0.025$, Figure 2-4). Mdm in the subjects taking OC pills without any androgenicity increased in about the same size as in the subjects taking OC pills with known androgenicity (Figure 2-5).

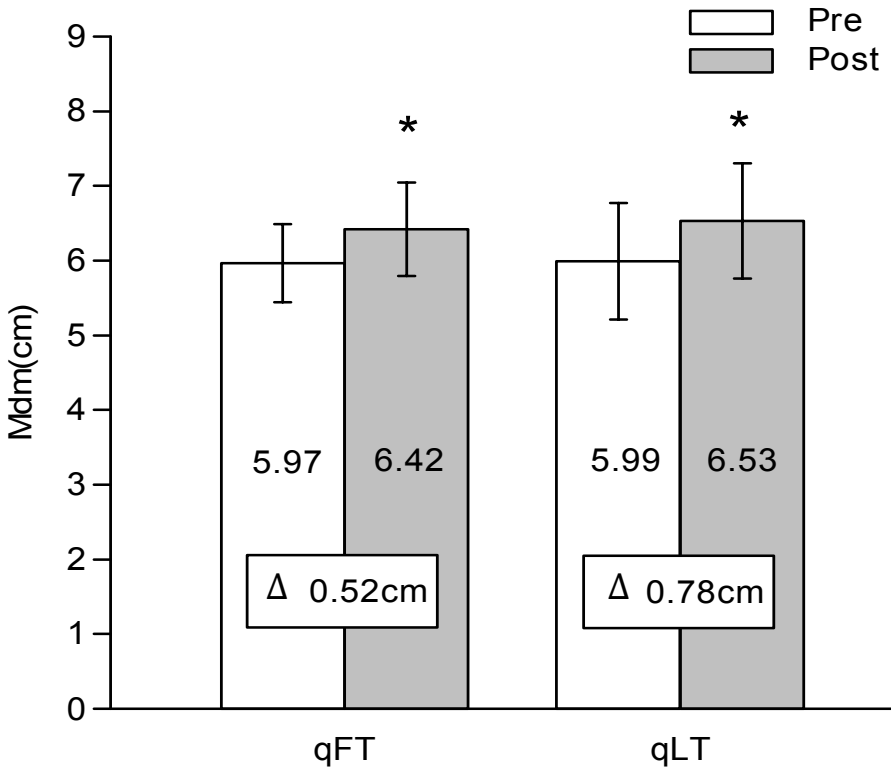

FIGURE 2-4: Sum of the diameters of rectus femoris, vastus intermedius and vastus lateralis muscle before and after three months of quasi follicular phase-based (qFT) or quasi luteal phase-based (qLT) strength training (N = 17); Pre: before training, Post: after training, *: $P < 0.025$ post training vs. pre training

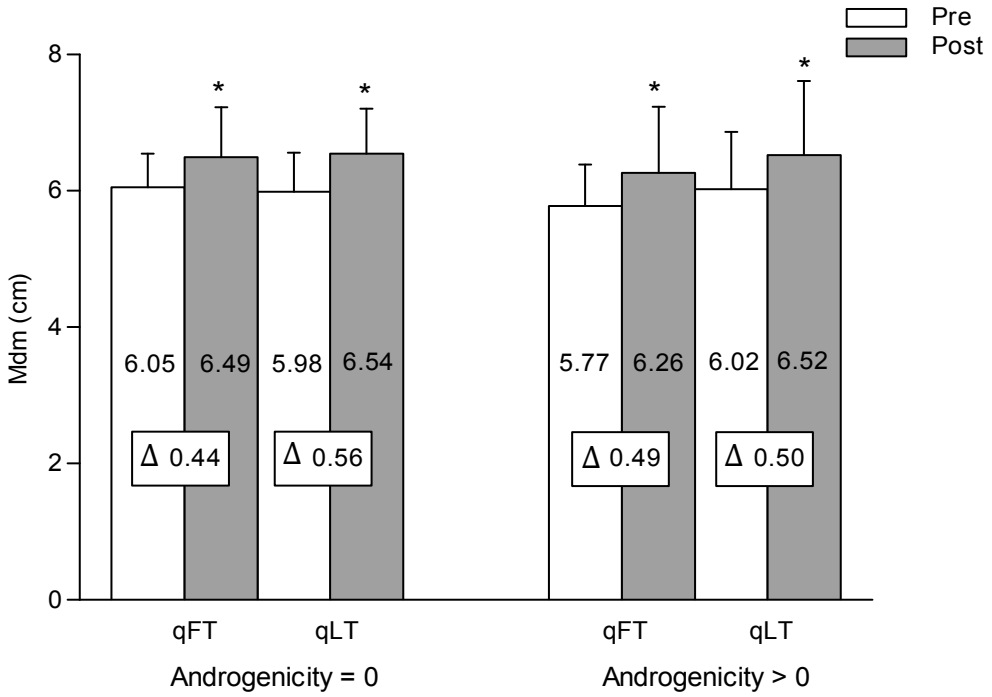

FIGURE 2-5: Sum of the diameters of rectus femoris, vastus intermedius and vastus lateralis muscle before and after three months of quasi-follicular phase-based (qFT) or quasi-luteal phase-based (qLT) strength training in two groups of subjects taking OC without any androgenicity (N = 12) or with known androgenicity (N = 5)
Pre: before training, Post: after training, *: $P < 0.025$ post-training vs. pre-training

2.4.6. Muscle Cell Parameters

Freeze damage, created by freeze thawing during preparation, is a major artifact that affects morphological analysis in this type of study. Although the least damaged fibers in well-preserved regions were selected, there was still evidence of minor damage. The volume of artifacts varied between individuals, but pre- and post-training sample quality was similar so the results were not affected. All muscle cell parameters showed wide inter-individual variations. Number of fiber type II tended to increase after qFT, however remained the same after qLT ($P = 0.035$) (Table 2-3) Fiber diameter and nuclei-to-fiber ratio were unaffected by any training periodization ($P > 0.025$).

TABLE 2-3: Muscle fiber type distribution (No), fiber diameter (Fdm) and nuclei-to-fiber ratio (N/F) before and after three months of quasi follicular phase-based or quasi luteal phase-based strength training (N = 6)

	Pre-Training				Post-Training			
	qFT		qLT		qFT		qLT	
	Type I	Type II	Type I	Type II	Type I	Type II	Type I	Type II
No (%)	45.1 ± 20.1	54.9 ± 20.1	43.2 ± 9.5	56.8 ± 9.5	38.0 ± 13.9	62.0 ± 13.9	41.9 ±13.9	58.1 ± 13.9
Fdm (µm)	52.7 ± 6.1	53.7 ± 4.9	54.2 ± 7.0	50.0 ± 8.3	54.0 ± 6.1	55.4 ± 9.6	54.6 ± 6.4	56.4 ± 8.9
N/F	2.9 ± 0.3		3.5 ± 0.8		3.4 ± 0.6		3.3 ± 0.1	

qFT: quasi follicular phase-based training, qLT: quasi luteal phase-based training

2.5. DISCUSSION

This study is the first one about planning strength training with respect to hormonal fluctuations during the menstrual cycle in OC users. The main findings of the present investigation are comparable increments of F_{max} and Mdm after both, quasi-follicular phase-based and quasi-luteal phase-based strength training in OC users, and similar increments of F_{max} and Mdm in OC users taken preparations without any androgenic activity compared to preparations with known androgenic activity.

OC are the main form of birth control in the general population and with the introduction of low dose OC preparations, their use has increased in athletic women (Rechichi et al., 2009).

OC pill use in athletic women matches the prevalence of use within the general community. OC pill reduces cycle-length variability and provides a consistent 28-day cycle by systematically controlling concentrations of endogenous sex hormones, reducing the natural production of estrogens and progesterone through inhibition of the pituitary secretion of gonadotropins, thus inhibiting ovulation and preventing pregnancy. Monophasic pills provide the woman with fixed doses of estrogen and progestogen over 21 days, followed by 7 days of placebo (Bennell et al., 1999; Burrows & 2007; Sitruk-Ware, 2006).

Only one synthetic estrogen (ethinylestradiol) is found in today's monophasic OC pills, compared with one of several progestogens. Ethinylestradiol is hormonally effective by activating the estrogen receptor and thus is an estrogen. While ethinylestradiol is considered to be responsible for insulin resistance, progestins are associated with changes in the insulin half-life and increased insulin response to glucose (Sitruk-Ware & Nath, 2011). However, a review of studies in women without diabetes suggests limited effects of hormonal contraceptives on carbohydrate metabolism, indicating that there is no strong evidence of a diabetogenic effect of OC pills, but the few studies with limited sample size and poor reporting of methods are not conclusive (Lopez, Grimes & Schulz, 2009). The form of progestogen used in the OCP will oppose estrogen to varying levels depending on its potency and androgenicity.

Apart from binding to the progesterone receptor, most progestins according to their chemical structure could also interact with the androgen receptor, estrogen receptor, glucocorticoid receptor or mineralocorticoid receptor and by these mechanisms can influence metabolic parameters (LaGuardia, Schangold, Fischer, Friedmann & Kafrissen, 2003) and, consecutively, training adaptation processes. Androgenicity refers to the ability of the progestogen to produce masculine characteristics and is calculated by multiplying the progestogen dose within the OC pill by its androgenic activity. As the more androgenic progestogens oppose the estrogen effects, one would expect the OC pills containing progestogens with higher potency and androgenicity to have a more significant impact on performance then those OC pills containing progestogens with low potency and androgenicity (Burrows & Peters. 2007).

The plasticity of skeletal muscle is reflected in its ability to adapt to altered metabolic and functional demands. Resistance training results in an increase in muscle strength accompanied by an increase in neural adaptation and muscle size. This increased muscular size is due to muscle fiber hypertrophy. Women have the same physiological ability as males to tolerate and adapt to heavy resistance training (Wang, Hikida, Staron & Simoneau, 1993). However, only very few studies are concerned with the effects of the hormonal milieu throughout the menstrual cycle in OC users and the adaptation to strength training in these women. The data pertaining to the effect of OC use on muscular strength and performance is minimal and inconclusive (Rechichi et al., 2009), and interventional data on trainability of muscle strength in OC users depending on the phase of their menstrual cycle when the training stimuli are set is completely missing. Although suffering from problems of design and small sample size, the few studies on the variation of strength parameters in different phases of the menstrual cycle in users of new, low-dose monophasic OC failed to show significant differences of any strength-related parameter (Burrows & Peters, 2007; Rechichi & Dawson, 2009). Only one study is available analyzing the potential effect of androgenicity on muscle strength in female OC users. In this study, androgenicity of the progestin in oral contraceptive pills has failed to show

any significant effect on maximal leg strength during three phases of the pill cycle (Peters & Burrows, 2006).

In our investigation, subjects taking monophasic OC have been included. However, as we wanted to include a representative group of women taking different monophasic preparations into the study, we did not further reduce inclusion criteria to some specific monophasic OC preparations. Therefore, the type of monophasic preparation varied between subjects concerning both, the amount of ethinylestradiol (20 – 35 µg) and the type and amount of progestogen with their different levels of androgenicity (Table 2-1). After dividing the subjects into two groups either taking OC without any androgenicity (N = 12) or taking OC with proposed androgenic activity (N = 5), we could demonstrate that in both groups increments in F_{max} and Mdm were comparable after three month of one-leg strength training (Figure 2-3 and Figure 2-5). Although number of subjects in the one group is only small, we conclude that untrained or moderately trained women probably do not need to care for the type of monophasic OC when they want to optimize the effects of strength training.

The primary role of the combined pill is the suppression of the hypothalamic-pituitary system (including the endogenous production of estrogen and progesterone), which prevents the midcycle surge of gonadotrophins, inhibiting ovulation and subsequent pregnancy (Fotherby, 1996). Serum levels of ethinylestradiol peak approximately one hour after ingestion fall rapidly for the following six hours and then decline slowly. Approximately 24 hours after ingestion, 33% of ethinlyestradiol remains in circulation compared with about 20 to 25% of progestogens. However, ethinylestradiol is detectable for up to two days after discontinuation, while some progestogens are detectable for up to five days (Dooley & Brincat, 1994).

Therefore, early in the withdrawal phase both endogenous estrogen and progesterone continue to be suppressed, but later in the withdrawal phase endogenous estrogen levels may rise while progesterone levels stay suppressed (Rechichi et al., 2009).

We analyzed serum concentrations of sex steroids on day 11 (quasi follicular phase, early OC consumption phase) and on day 25 (quasi luteal phase, late OC consumption phase). Concentrations of endogenous E2 and P4 did not vary significantly between these two days indicating that suppression of endogenous sex steroid secretion was successful. Furthermore, DHEA-s and T did not differ between these days, while free T clearly declined on day 25 compared to day 11. This is probably due to OC induced increase in SHBG (Wiegratz, Jung-Hoffmann & Kuhl, 1995). As the free fraction of T mainly effects skeletal muscle cell adaptation one would expect higher training adaptation in the second phase of OC pill cycle (the quasi luteal phase) compared to the quasi-follicular phase-based training. In a study with subjects without OC use, strength training in FT induced higher increases in F_{max} compared to strength training in LT (Sung E., 2012). In this investigation with OC users, increments of F_{max} and Mdm, however, were independent from the type of periodization. We, therefore, assume that other effects probably were interacting with the anabolic effect of free T. After three month of one-leg strength training, DHEA-s and total T were lower in qLP compared to qFP, while free T declined in qFP compared to the value prior to training (Table 2-2). Therefore, chronic training might interfere with sex steroid metabolism in OC users. Serum estradiol and progesterone have been shown to increase after a single bout of resistance exercise in healthy young women without OC in the mid-luteal phase, but not in the early follicular phase, indicating that the responses of anabolic hormones to acute resistance exercise are different among the menstrual cycle states in young women (Nakamura, Aizawa, Imai, Kono & Mesaki, 2011). Furthermore, another interacting factor could be the negative influence of (non-androgenic) gestagens on muscle strength and Mdm during all 21 days of pill intake via negative effects on protein anabolism (Oosthuyse & Bosch, 2010).

This study is the first to investigate muscle fiber parameters depending on menstrual cycle phase-based strength training in OC users. Only six of 16 subjects volunteered to participate in needle muscle biopsies. Therefore, data have to be interpreted carefully. All muscle cell parameters showed broad inter-individual variation (Table 2-3). The tendency for an increase

in the number of type II fibers after qFT was not reflected by an expected similar decline in the number of type I fibers. We therefore assume that this tendency is rather due to artifact rather than real physiologic alterations. As Fdm and nuclei-to-fiber ratio also were unaffected by training periodization, we conclude that specific anabolic hormonal influence on skeletal muscle cell adaptation was either inconsistent or not sufficient.

2.6. CONCLUSION

To conclusion, quasi-follicular phase-based strength training induced the same increase in muscle strength and muscle diameter as quasi-luteal phase-based strength training in monophasic OC users. Furthermore, the androgenic properties of the OC pills did not have any significant effect on the amount of training adaptation. We therefore conclude that untrained or moderately trained OC users can perform their strength training independently from the phases of their menstrual cycle and that they can take any monophasic OC pill without caring for the type of progestin in the pill.

3. STUDY 3: EFFECTS OF MENSTRUAL PHASE-BASED STRENGTH TRAINING IN NON-OC USERS VERSUS IN OC USERS

ABSTRACT

PURPOSE: Hormonal fluctuation during the menstrual cycle may influence trainability of muscle strength. However oral contraception (OC) alters the profile of these hormones. We, therefore, compared effects of menstrual cycle-based strength training between eumenorrheic females (non-OC users) and oral contraceptive using females (OC users).

METHOD: Females (N = 37: non-OC users = 20, OC users = 17) completed one-leg strength training on a leg press for three menstrual cycles. They trained one leg mainly in the first half of the menstrual cycle (follicular phase training (FT) or quasi-follicular phase training (qFT)) and the other leg mainly in the second half of the cycle (luteal phase training (LT) or quasi-luteal phase training (qLT). Concentrations of 17-beta estradiol (E2), progesterone (P4), total testosterone (T), free testosterone (free T) and DHEA-s were analyzed in blood samples taken during follicular phase (FP)/ quasi-follicular phase (qFP) and luteal phase (LP)/ quasi-luteal phase (qLP). Maximum isometric muscle strength (F_{max}) and muscle diameter (Mdm) were analyzed before and after training.

RESULTS: Concentrations of E2, DHEA-s, T and free T were significantly ($p < 0.05$) higher in non-OC users compared to OC users. Absolute increase of F_{max} after training intervention was the lowest after LT in non-OC users (188 N) compared to FT (268 N) in non-OC users and qFT (266 N) and qLT (282 N) in OC users.

CONCLUSIONS: Although non OC users had higher anabolic hormone concentrations in FP and LP compared to qFP and qLP, increase of F_{max} after qFT and qLT was significantly higher than LT. Further studies with more subjects are needed in order to understand the underlying mechanisms of training adaptations between non-OC users and OC users.

3.1. INTRODUCTION

Fluctuation of hormones, such as estradiol (E2), progesterone (P4) and testosterone over the course of the menstrual cycle has been repeatedly reported in eumenorrheic females (non-OC users). 17-beta estradiol (E2) peaks prior to ovulation and during the luteal phase (LP), while progesterone (P4) reaches its highest values during LP after ovulation (Van Look et al. 1980). In both sexes, androgens are produced by the reproductive organs and the adrenals. The most important androgen secreted is testosterone; the adrenal glands and the ovaries produce very little testosterone but secrete weaker androgens. In particular, dehydroepiandrosterone (DHEA; and its sulfoconjugate) secreted by the adrenals, and androstenedione secreted by the adrenals and the ovaries are of physiological importance in women (Enea et al. 2011). Moreover, the levels of androstenedione and testosterone, for instance, reach their peaks prior to, or at the time of ovulation (Longcope 1986).

Oral contraceptive users (OC users) have different hormone concentrations compared to non-OC users due to the intake of fixed doses of synthetic E2 and progestin. The most common used monophasic combined OC consist of ethinylestradiol and progestin in fixed doses. This kind of OC is taken for 21 days (consumption phase), followed by 7 days of OC break (withdrawal phase). As a result, endogenous E2 and P4 are suppressed and, according to the oral intake of constant amouts, blood concentrations of E2 and P4 remain nearly constant during the 21 days of consumption phase. After 21 days of the consumption phase, no oral contraceptives are taken for 7 day (the withdrawal phase). The concentration of both hormones are continually suppressed in the early withdrawal phase and estrogen starts to increase in the late withdrawal phase, while progesterone remains further suppressed (Dooley & Brincat, 1994; Rechichi, Dawson & Goodman, 2008; Rechichi et al., 2009)

Due to this exogenous and endogenous hormonal regulation, levels of testosterone, free testosterone and dehydroepiandrosterone (DHEA; and its sulfoconjugate) decrease significantly with usage of oral contraceptives

(Graham, Bancroft, Doll, Greco & Tanner, 2007; Rickenlund et al., 2004). E2 was found as well to be significantly lower in OC users in comparison to non-OC users (Vaiksaar et al., 2011). Moreover, P4 remaines low in the quasi luteal phase in OC users, whereas it increases significantly in the luteal phase compared to the follicular phase in non-OC users (Vaiksaar et al., 2011).

The main sex hormones such as E2 and P4 are known to influence substrate metabolism during the exercise performance and the trainability of muscle strength (Constantini, Dubnov & Lebrun, 2005; Janse de Jonge, 20003; Lebrun, 1994). Most animal studies have demonstrated that female estrogen-supplemented rodents exhibit less skeletal muscle myofiber injury and inflammation following exercise-induced muscle injury. In addition, estrogen may also influence post-damage repair processes through activation and proliferation of satellite cells (Enns & Tiidus, 2010). The potential mechanism(s) underlying estrogenic action remain elusive. Among others, the discovery of three types of estrogen receptors (ERs) has led to the discovery that estrogen may govern the regulation of a number of downstream genes and molecular targets (Enns et al. 2010, Lowe et al. 2010). One recent study comparing postmenopausal females with or without HRT use reported that those women using HRT had significantly greater up-regulation of pro-anabolic gene expression both at rest and following eccentric exercise (Dieli-Conwright et al. 2009). Furthermore, it has recently been postulated that the beneficial effect of estrogens on muscle strength is accomplished by improving the intrinsic quality of skeletal muscle, whereby fibers are enabled to generate force, i.e., myosin strongly binds to actin during contraction (Lowe et al. 2010).

Only very few data exist on the physiological effects of P4 on the female skeletal muscle cell. Recent studies have consistently found amino acid oxidation and protein degradation to be greater in LP compared with the follicular phase (FP) at rest and during exercise. It appears that P4 is responsible for the consistent finding of increased protein catabolism in

LP, while estrogen may reduce protein catabolism (Oosthuyse et al. 2010).

Overall, the existing data indicate a more anabolic state in FP and the peri-ovulatory phase of the menstrual cycle as compared to a more catabolic state in LP. The only available strength training intervention study using the different hormonal milieu of FP and LP as modulators of training adaptability analyzed the possible divergent effects of training stimuli in either FP or LP on the amount of strength gain in healthy women (Reis et al. 1995).

Since estrogen, progesterone and other ovarian hormones are discussed to be main important factors for muscle strength and as sexual steroid hormones play a considerable role in training adaptation processes, OC-induced alterations in their blood concentrations might lead to alterations in the amount of training adaptation in OC users compared to non-OC users.

Nevertheless, to the authors' knowledge, there are no training interventional studies that have compared the trainability of strength capacity of menstrual cycles between OC users and non-OC users. Based on the findings, this study compared the sex hormone concentration of the two phases of menstrual cycle (follicular phase and luteal phase) between non-OC users and OC users. Moreover, effects of menstrual phase-based strength training on physiological and microscopic parameters were compared between non-OC users and OC users.

3.2. AIMS

To our knowledge, no field studies have been conducted that effect of strength after training intervention compared between non OC users and OC users who participated in a strength training program. The purpose of this study was to find which training group, such as non-OC users and OC users, has more strength improvement after 3 month training intervention.

3.3. METHODS

For the subjects, experimental design, the study schedule, the training program and analyzing methods please refer to Study 1 or Study 2

3.3.1. Statistical Analysis

Data are presented as mean values with SD. Normality of distributions was proved by the Kolmogorov-Smirnov test. A one-tailed paired *t*-test was used to evaluate differences in training workload, F_{max}, Mdm, fiber composition, fiber diameter and muscle nuclei-to-fiber-ratio between values before (pre) and after the training intervention (post) (see below: a, b) and between FT and LT (see below: c), respectively. In all cases, P values < 0.025 were taken to indicate statistical significance. Statistics were tested with a hierarchical procedure: a) (q)FT_{post} better than (q)FT_{pre}; b) (q)LT_{post} better than (q)LT_{pre}; c) if a) significant: Δ(q)FT better than Δ(q)LT; if b) significant: Δ(q)LT better than Δ(q)FT (Δ(q)FT: absolute difference between (q)FT_{pre} and (q)FT_{post}, Δ(q)LT: absolute difference between (q)LT_{pre} and (q)LT_{post}). A two-tailed paired *t*-test was used to compare hormone concentration between (q)FP and (q)LP and between prior to and after training and to compare training units between (q)FT und (q)LT for three training cycles. Significance was defined as $P < 0.05$. The intraclass correlation coefficient of repeated measurements (ICC) (McGraw et al. 1996) was determined to evaluate reliability of the determination of F_{max} and Mdm.

A one-sided *t*-test was used to examine if the training intervention lead to positive effects in parameters such as F_{max} and Mdm (non-OC users + OC users), muscle fiber composition and muscle nuclei (just non-OC users). Significance in this study was defined as $p \leq 0.025$.

Hormone concentration values were compared a) between two phases (FP vs. LP and qFP vs. qLP, a paired *t* -test) and b) between two groups (FP vs. qFP, FP vs. qLP, LP vs. qFP, LP vs. qLP, an independent *t* -test) by using a two-sided *t*-test. Significance was defined as $p \leq 0.05$.

3.4. RESULTS

3.4.1. Number of training sessions

The total number of single-leg training sessions by non-OC users was approx. 28 sessions per leg and was not different between FT and LT (FT: N = 28.6 ± 1.7; LT: N = 28.1 ± 1.9; P > 0.05) and by OC users was 28 sessions per leg and did not different between qFT and qLT (qFT: N = 28.0 ± 0.0; qLT: N = 28.0 ± 0.0).

3.4.2. Hormonal concentrations

The hormone values of before and after training were calculated together for each FP, LP, qFP and qLP to compare the concentrations between phases and between non-OC users and OC users independently from training effect. Levels of E2, DHEA-s, IGF-1, T and free T were significantly higher in non-OC users (FP and LP) as compared to in OC users (qFP und qLT). P4 level was the highest in LP than other phases ($p < 0.05$).

TABLE 3-1: Serum concentrations of E2, P4, DHEA-s, T and free T in the (quasi-) follicular phase (FP/qFP, day 11) and the (quasi-) luteal phase (LP/qLP, day 25)

	Non-OC users		OC users	
	FP	LP	qFP	qLP
E2 (pg/ml)	108 ± 71	128 ± 40	18.8 ± 18.4 †	13.0 ± 8.9
P4 (ng/ml)	0.80 ± 0.39 *	7.01 ± 2.47 §	0.60 ± 0.35 †	0.45 ± 0.22
DHEA-s (ug/ml)	2.60 ± 0.87	2.55 ± 0.71	1.70 ±0.45 †	1.43 ± 0.46 ** §
T (ng/ml)	0.40 ± 0.16	0.36 ± 0.15 ◊	0.23 ± 0.12 †	0.19 ± 0.11 §
Free T (pg/ml)	2.36 ± 0.66	2.02 ± 0.59 *	1.64 ± 0.42 †	1.41 ± 0.40 § ♦

*: P < 0.05 FP vs. LP, **: P < 0.05 qFP vs. qLP, †: P < 0.05 FP vs. qFP,
§: P < 0.05 LP vs. qLP, ◊: P = 0.058 FP vs. LP, ♦: P = 0.065 qFP vs. qLP.

3.4.3. Maximum isometric muscle strength (F_{max})

Figure 3-1 shows the comparison of the absolute increase (∆post-pre) F_{max} value of FT vs. qFT (268.6N vs. 266.4N) and FT vs. qLT (268.6N vs. 282.2N), there is not significant different between non-OC users and OC users. However, we found that comparing the absolute increase (∆post-pre) F_{max} value of LT vs. qFT (188.3N vs. 266.4N) and LT vs. qLT (188.3N vs. 282.2N) did differ significantly from each other.

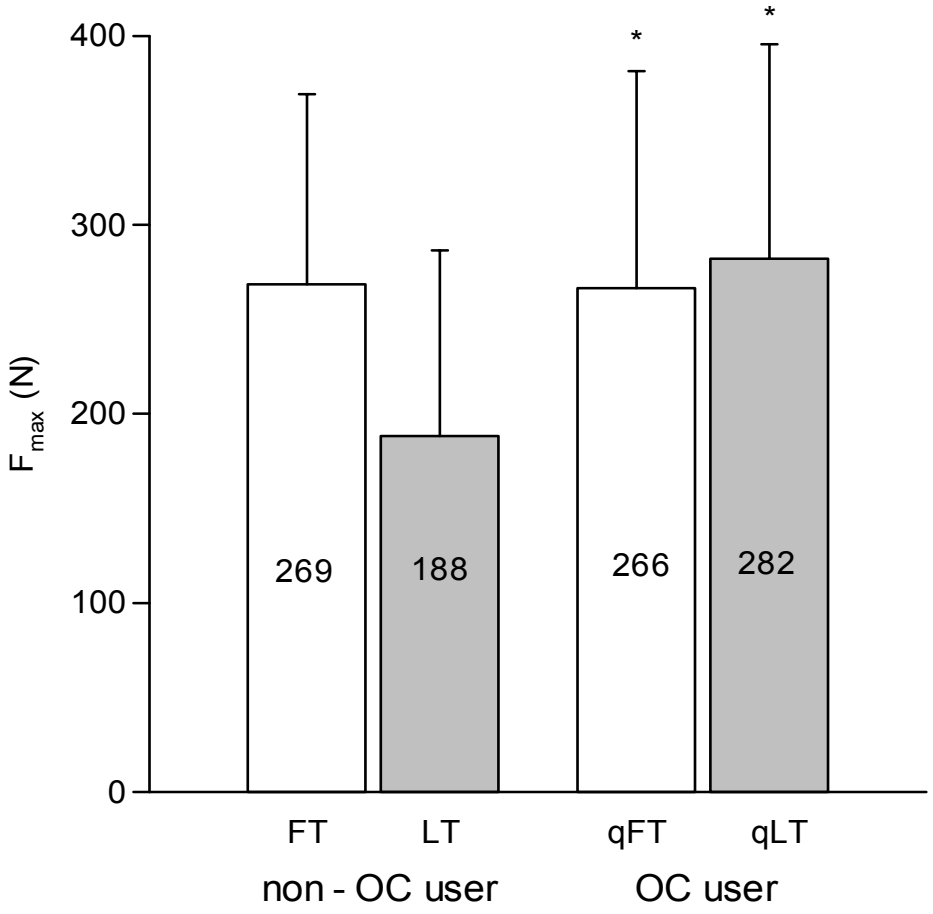

FIGURE 3-1: Absolute increase values (∆) F_{max} after three months of follicular phase-based (FT), luteal phase-based (LT), quasi-follicular phase-based (qFT) or quasi-luteal phase-based (qLT) strength training, *: P < 0.025 ΔLT vs. ΔqFT and ΔLT vs. ΔqLT.

3.4.4. Muscle diameter

Figure 3-2 shows the comparison of the absolute value of Mdm increase between FT vs. qFT, FT vs. qLT, LT vs. qFT and LT vs. qLT, we could not find a significantly different between non-OC users and OC users. However, we observed, the absolute increase Mdm were lower in LU (0.39cm) than other phases (FT, qFT, qLT)

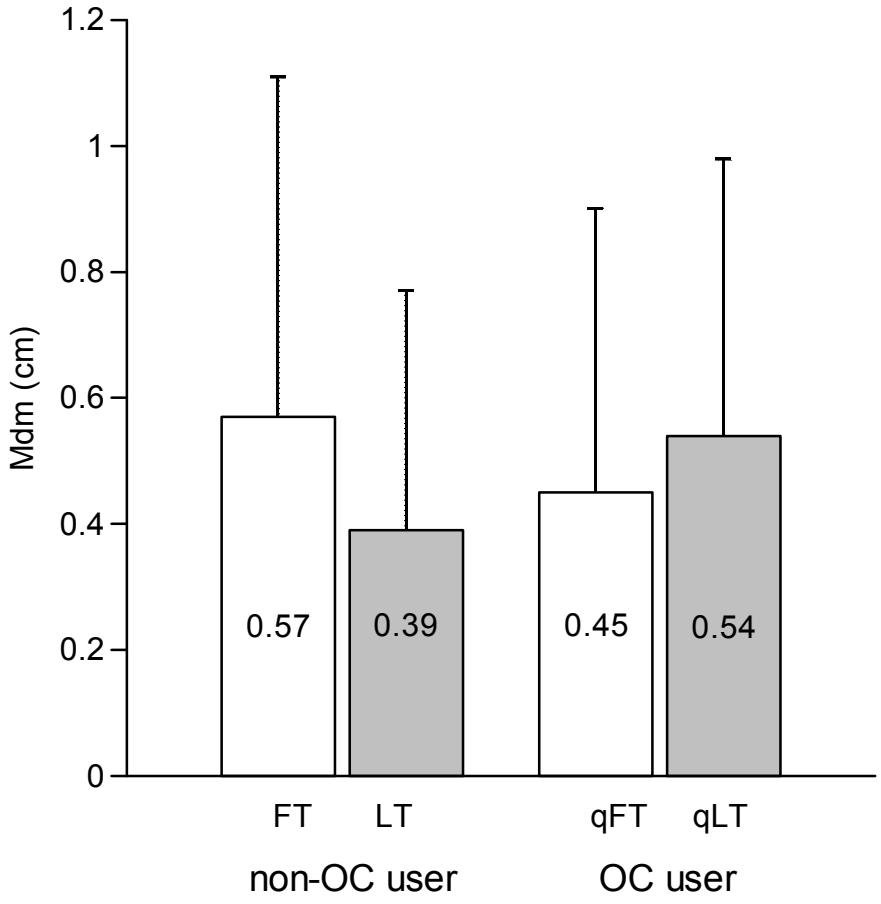

FIGURE 3-2: Absolute increase values (∆) of Mdm after three months of follicular phase-based (FT), luteal phase-based (LT, quasi-follicular phase-based (qFT) or quasi-luteal phase-based (qLT) strength training

3.5. DISCUSSION

This study is the first one about planning strength training with respect to hormonal fluctuation during the menstrual cycle and first to compare the muscle strength depending on menstrual cycle phased between non-OC users and in OC users. The main finding of the present investigation is clearly higher ($P < 0.05$) concentration of anabolic hormones in non-OC users in comparison with OC users and the lowest increase ($P < 0.025$) in F_{max} after LT as compared to FT, qFT and qLT. Moreover, LT showed the lowest increase in Mdm as well.

Ultimately, the differentia between non-OC users and OC users was hormone concentration during the menstrual cycle. In our present study, pronounced differences between non-OC users and OC users was significantly higher hormone concentration of E2 , DHEA-s, T and freeT in non-OC users as compared with OC users.

The biological actions of androgens once inside the cell are mediated by the androgen receptor (AR). The AR complexes interact with various factors (e.g. coactivators or corepressors) to modulate transcription of androgen target genes via binding to specific DNA sequences and resulting in protein synthesis as an adaptation process to training stimuli. Androgens may also regulate cellular activity via a more rapid non-genomic mechanism involving membrane receptors and/or cytosolic receptors. These steroid receptors are able to activate intracellular signaling molecules, such as the mitogen-activated protein kinase 1 (MAPK1), by transcription-independent mechanisms (Enea et al. 2011).
Apart from the effects of androgens, The ovarian hormones are known to have a noticeable influence on protein metabolism at rest and during exercise, which is often seen as increased catabolism in the LP. It appears that progesterone is responsible for the consistent finding of increased protein catabolism in the LP, while estrogen may reduce protein catabolism (Oosthuyse et al. 2010).

Despite of higher anabolic hormone concentration in LP as compared to qFP and qLP, increase of F_{max} and Mdm was lower after LT as compare to FT, qFT and qLT. Furthermore, concentration of P4 in LP was the highest ($P < 0.05$) as compare in FP, qFP and qLP.

For this reason, some researchers suggested that the ratio of E/P should be considered during the menstrual cycle because there are interactive effects between estrogen and progesterone on muscle capacity.
Sarwar et al. (Sarwar et al., 1996) found a significant increase of about 11% in quadriceps and handgrip strength, quadriceps contractile properties and fatiguability during the menstrual cycle by non-OC users and muscle was stronger, slower and more fatiguable in mid-cycle (corresponding to the ovulatory phase). This result was not seen in OC users and they suggested that levels of estrogen and progesterone are higher in the luteal phase as compared to the follicular phase. However, the highest estrogen level is observed just prior to ovulation and they assumed that high estrogen levels during mid-cycle (late follicular phase) would increase muscle strength. Moreover, they suggested that progesterone might inhibit the proposed strength-enhancing effect of estrogen (D'Eon et al., 2005; Oosthuyse, Bosch & Jackson, 2005). Furthermore, Phillips et al (Phillips et al., 1996) also suggested that estrogen has a strengthening effect on skeletal muscle and they found also an increase in maximal strength just during the follicular phase.
Both estrogen and progesterone concentrations are low during the early follicular phase and estrogen starts to increase through the follicular phase to reach a peak in the late-follicular phase and then sharply drop just prior to ovulation. After ovulation, both estrogen and progesterone increase until a plateau is reached during the mid-luteal phase. In the late luteal phase, estrogen and progesterone decrease again (Ferin M, 1993). This comparison of the increase in the estrogen concentration (E) relative to progesterone concentration (E/P) in the luteal phase should be considered in the study with women and it is supported by Janse de Jange who reported that consideration in research on the influence of the menstrual

cycle on exercise performance is the timing of the testing with respect to the menstrual cycle.
Moreover, Oosthuyse et al. (Oosthuyse & Bosch, 2010) suggested that future studies should consider the increase in estrogen relative to progesterone in FP and the absolute magnitude of increase in estrogen between follicular phase and luteal phase.

In our present study, E/P ratio in non-OC user was higher in the follicular phase (180 ± 163.9) as compare to the luteal phase (19.4 ± 4.9) and level of E/P ratio in OC user in both qFP (35.7 ± 25.7) and qLP (31.5 ± 19.6) war similar to LP.

We presumed that the higher value of E/P in FP can be related to increase of maximum isometric force and muscle diameter in non-OC users. Moreover, low concentration of P4 presented FP (0.8 ng/ml) prior to the ovulation, qFP (0.6 ng/ml) and qLP (0.5 ng/ml) and relatively higher concentration of P4 were observed in LP (7.0 ng/ml). This might suggest that the lowest increase of F_{max} after LT related to the lowest E/P ratio in the LP. Therefore, this hormonal milieu influenced negatively to improve muscle strength in LP. Since surge of estrogen is the highest around the ovulation without the antagonistic affects of progesterone (Bunt,1990; Pehrsson, Westberg, Landen & Ekman, 2007; Phillips et al., 1996) strength training might be optimized in the late FP and around the ovulation.

3.6. CONCLUSION

In conclusion, in this study OC users showed the strength trainability does not vary during the OC phase. They have benefit to perform strength training independently regardless of menstrual cycle because hormone levels remain constant and stable. In non-OC users, it is necessary to know the own menstrual cycle and there is more benefit to perform focused strength training between late follicular phase and ovulation than in the luteal phase. Since there is different training effect between menstrual phases, investigator should take this into consideration for the future research with women.

4. SUMMARY

This thesis demonstrated the different strength trainability between the follicular phase and the luteal phase in non-OC users, OC users and also non-OC users vs. OC users. After the three months of strength training, Firstly, we detected a significantly higher effect on muscle strength and on muscle diameter after FT as compared to LT. In addition, higher concentrations of E2, T, free T and DHEA-s were observed in the follicular phase as compared to the luteal phase in non-OC users Secondly, we were not able to find any significant difference in any parameters between quasi-follicular phase based training and quasi-luteal phase based training in OC users, which might be due to the intake of fixed dose of exogenous hormones (synthetic E2 and P4) in OC. Moreover, there was not any significant difference in hormone concentrations between quasi-follicular and quasi-luteal phase OC users.

Ultimately, the differentia between non-OC users and OC users was hormone concentration. OC users showed significantly lower anabolic hormone as compared to non-OC users

Although anabolic hormones, such as E2, DHEA-s, T and free T were significantly higher in LP compared to qFP and qLP, increase of F_{max} was significantly ($p < 0.025$) lower after LT as compare to after FT, qFT and qLT and as well as lower increase in Mdm. This result might be due to the low E/P ratio and higher level of P4 in LP in comparison to other phases.

Based on these results, we conclude that untrained or moderately trained OC users can perform their strength training independently from the phases of their menstrual cycle and that they can take any monophasic OC pill without caring for the type of progestin in the pill and we recommend non-OC users to perform strength training in the follicular phase.

Since there are different training effects between two menstrual phases in non-OC users, investigator should take this into consideration for the future research with women.

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