Impact of oral contraceptive use and menstrual phases on patellar tendon morphology, biochemical composition, and biomechanical properties in female athletes

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Hansen M, Couppe C, Hansen CS, Skovgaard D, Kovanen V, Larsen JO, Aagaard P, Magnusson SP, Kjaer M. Impact of oral contraceptive use and menstrual phases on patellar tendon morphology, biochemical composition, and biomechanical properties in female athletes. *J Appl Physiol* 114: 998–1008, 2013. First published February 21, 2013; doi:10.1152/japplphysiol.01255.2012.—Sex differences exist with regards to ligament and tendon injuries. Lower collagen synthesis has been observed in exercising women vs. men, and in users of oral contraceptives (OC) vs. nonusers, but it is unknown if OC will influence tendon biomechanics of women undergoing regular training. Thirty female athletes (handball players, 18–30 yr) were recruited: 15 long-term users of OC (7.0 ± 0.6 yr) and 15 nonusers (>5 yr). Synchronized values of patellar tendon elongation (obtained by ultrasonography) and tendon force were sampled during ramped isometric knee extensor maximum voluntary contraction to estimate mechanical tendon properties. Furthermore, tendon cross-sectional area and length were measured from MRI images, and tendon biopsies were obtained for analysis of tendon fibril characteristics and collagen cross-linking. Overall, no difference in tendon biomechanical properties, tendon fibril characteristics, or collagen cross-linking was observed between the OC users and nonusers, or between the different phases of the menstrual cycle. In athletes, tendon cross-sectional area in the preferred jumping leg tended to be larger than that in the contralateral leg ($P = 0.09$), and a greater absolute ($P = 0.01$) and normalized tendon stiffness ($P = 0.02$), as well as a lower strain ($P = 0.04$), were observed in the jumping leg compared with the contralateral leg. The results indicate that long-term OC use or menstrual phases does not influence structure or mechanical properties of the patellar tendon in female team handball athletes.

estrogen; women; collagen; extracellular matrix; athletes

COMPARED WITH THEIR MALE counterparts, women display a greater risk of knee anterior cruciate ligament (ACL) tearing (2, 3, 31), but there is no monofactorial explanation for this connective tissue sex disparity. In addition to anatomical and neuromuscular explanations (32), sex differences in hormonal levels may be important. This notion is based on the fact that estrogen receptors have been identified in the human ACL (19, 43, 67), and women who are chronically exposed to high levels of estrogen, i.e., users of oral contraceptives (OC), may have altered collagen content of tendons and ligaments (27), which may change the biomechanical properties. In support of an acute effect of estrogen on connective tissue, the risk of ACL injury has been found to be higher in the preovulatory phase of the menstrual cycle (73), when circulating estrogen is peaking. Likewise, a greater joint laxity has been observed during the ovulation phase of the menstrual cycle (16, 33, 58, 62, 70), although there are contradictory result as well (5, 8, 10, 11, 61, 77).

Use of OC suppresses endogenous secretion of female hormones and thereby the naturally occurring cyclic hormonal variations. Use of OC has been associated with a greater risk of Achilles tendinopathy (37), persistent pelvic pain, pelvic joint instability (66), and lower back pain (76, 82), whereas others have reported a lower risk of traumatic injuries (55) or no difference in the risk of lower back pain (9) and ACL injuries (1, 63) compared with non-OC (NOC) users. The knowledge about the effect of OC on tendon and ligament biomechanical properties is sparse. Bryant et al. (8) observed a reduced strain of the triceps surae aponeurosis during maximal isometric plantar flexion in OC users, and Martineau et al. (51) showed a lower passive anterior translation of the tibia as measured by KT1000 in OC users compared with NOC. However, there appears to be no effect of OC on passive peripheral joint laxity (60).

Endogenous or exogenous estrogen may influence the risk of injuries by changing the structural composition of ligaments and tendons and thereby introduces changes in tendon mechanical properties. Nevertheless, equivocal data exist in the literature. Animal studies have reported no effect of estrogen on the mechanical properties of sheep knee ligaments (75), whereas ACL from rabbits ruptures at lower stress loads after 30 days of estrogen administration (74). In contrast, administration of synthetic estradiol and progesterone (ethinyl estradiol and levonorgestrel) as in OC strengthen the ACL in rats within 12 hormonal cycles (81). Human data have shown a lower normalized tendon stiffness (Young's modulus) in ovariectomized women using estrogen replacement therapy (ERT) compared with postmenopausal controls (25). In the latter study, tendon collagen fractional synthesis rate and the density of small-diameter tendon fibrils were higher in ERT users, which indicate that estrogen may enhance tendon collagen turnover. In contrast, tendon collagen fractional synthesis rate is reduced in young OC users compared with controls (27). Similar to the

animal findings, this indicates a differential effect between estrogen (estradiol) and synthetic estrogen (ethinyl estradiol). A lower tendon collagen synthesis rate and overall lower tendon collagen turnover may enhance the possibility for introducing intra- and intermolecular collagen cross-links and thereby increase tendon stiffness and resistance against ruptures. However, in perspective to the risk of sports injuries, it is interesting to note that both in vitro (41, 42) and in vivo observations (26) indicate a negative interaction between mechanical tendon loading and estrogen (estrogen or OC) abundance on collagen synthesis.

Our aim was to test mechanical properties of the patellar tendon in NOC and OC users at two different time points during the menstrual cycle and pill cycle, respectively. In addition to assessing in vivo tendon biomechanical properties, a patellar tendon biopsy was obtained from each participant for histological and biochemical analyses of the tendon structure to link cellular tendon properties to the overall biomechanical properties of the patella tendon in vivo.

MATERIALS AND METHODS

Design

The project was designed as a cross-sectional study comparing female athletes (handball players), who were either chronic OC users or NOC. The experiment consisted of a screening protocol followed by the experimental day, which was planned in the OC users in either *week 3* of the pill cycle or the nonpill week and in the NOC in the menstrual phase or in the 1–3 days after the day of a positive ovulation test. On the experimental day, the biomechanical properties of the patellar tendon in each leg were assessed during a ramped maximal voluntary isometric contraction (13, 14). Afterwards, tendon biopsies were obtained from the patellar tendons, which were analyzed for cross-links and collagen concentration and used for fibril characterization (29, 38). The study complied with the Declaration of Helsinki and was approved by the local ethics committees in Copenhagen (H-C-2009-025). All subjects gave their informed consent to participate before the experiments.

Subjects

Subelite female handball players (18–30 yr) were recruited from 12 local handball clubs in Copenhagen. Female Team handball athletes were recruited since they are known to chronically load their tendons and ligaments, and they participate in a sport where ligament and tendon injuries are frequent (6, 56, 57). The subjects were nonsmokers, nulliparous, on no medication (except OC in the OC users), and absent of any orthopedic and medical conditions, as judged by history and routine medical examination. Women with a cycle length outside the typical range of 21–35 days were excluded (17). Additional exclusion criteria were parental predisposition for diabetes, connective tissue diseases, previous or present pregnancy, and use of an intrauterine device with hormones or use of minipills.

Fifteen OC users were included. They had used OC for a duration of 7 ± 1 yr (range 3–10 yr). They used different types of OC containing four different kinds of synthetic progesterone and different amount of ethinyl estradiol: *1*) 30 μg ethinyl estradiol and 0.15 mg levonorgestrel ($n = 1$); *2*) 20 or 30 μg ethinyl estradiol and 0.075 mg gestoden ($n = 5$); *3*) 20 or 30 μg ethinyl estradiol and 0.15 mg desogestrel ($n = 6$); and *4*) 30 μg ethinyl estradiol and 3 mg drospirenon ($n = 3$). Seven of the OC users were tested in the last week of the 3-wk pill period (*days 15–21*), and the remaining eight OC users were tested in the off-pill week (*days 2–5*).

As a control group, 15 NOC users participated. None of them had used OC for at least 5 yr (<2 mo in total). They were tested in either the follicular phase (FP, $n = 7$, *days 1–6* of menstrual cycle) or the luteal phase (LP) of the menstrual cycle (NOC LP, $n = 8$, *days 1–4* after a positive ovulation urine test; Wondfo One step ovulation urine test, Guangzhou Wondfo Biotechnology, Guangzhou, China). Blood samples analyzed for estradiol, progesterone, follicular stimulating hormone (FSH) and luteinizing hormone (LH) were used to confirm the menstrual phases.

Screening

All participants visited the laboratory at least 1 wk before the experimental day to receive detailed information about the experiment and went through a health examination and blood sampling. The blood samples were analyzed for plasma C-reactive protein, plasma creatine kinase, plasma calcium, plasma creatinine, plasma alkaline phosphatase, and hemoglobin A1c (HbA1c) (%). All results were within the normal range within the included women. Moreover, the two groups were similar with regard to age, height, weight, body mass index, and body composition, as measured by the sum of four skinfold measurements (18) (Table 1).

A criterion for participation was physical training at least three times a week. The OC users had been training and playing handball regularly for, on average, 13 ys (range 6–19 yr), which was comparable to NOC users (average 12 yr, range 4–22 yr). Similarly, no significant difference in training status between OC and NOC was observed (Table 1). Training status was tested in two different ways: *1*) daily physical activity level determined by use of a validated questionnaire (1); and *2*) maximal oxygen uptake ($\dot{V}o_{2 \, max}$) per kilogram body weight per minute determined in an incremental treadmill test (Table 1). Protocol for the $\dot{V}o_{2 \, max}$ test is as follows. After 5-min warm-up, the subjects were running for 2 min before the slope of the treadmill was raised 2%. Thereafter, the slope of the treadmill was raised 2% each 90 s until voluntary exhaustion. Respiratory variables (averaged for each 15-s period) were measured continuously through a mouthpiece connected to an automated metabolic cart (AMIS 2001, Innovision, Odense, Denmark). The mean of the three highest 15-s values was recorded as $\dot{V}o_{2 \, max}$. Heart rate (HR) was measured continuously by a wireless HR monitor (Polar Sport Tester, Polar Electro OY, Kempele, Finland). To ensure that a true $\dot{V}o_{2 \, max}$ was attained, at least two of the following three criteria had to be fulfilled: *1*) a $\dot{V}o_{2 \, max}$ plateau was reached; *2*) a HR was within ± 5 beats/min of the age-adjusted maximal HR; and *3*) CO_2 production (l/min)/O_2 uptake (l/min) > 1.1.

Patellar Tendon Cross-Sectional Area and Length

Patellar tendon cross-sectional area (CSA) and length were determined using magnetic resonance imaging (MRI), as previously described (39). MRI was performed before the biomechanical test and biopsy procedure. Patellar tendon CSA was measured *1*) just distal to the patellar insertion; *2*) just proximal to the tibia insertion; and *3*) midway between the two sites (39). The patellar tendon length was

Table 1. *Subject characteristics*

	NOC	OC
Age, yr	22 ± 1	23 ± 1
Height, m	1.70 ± 0.02	1.69 ± 0.02
Weight, kg	69 ± 3	68 ± 2
BMI, kg/m^2	24 ± 1	23 ± 1
Body fat, %	29 ± 1	32 ± 1
Fitness level, O$_2$ ml·min^{-1}·kg^{-1}	46 ± 1	45 ± 1
$\dot{V}o_{2max}$, O$_2$ l/min	3.2 ± 0.1	3.0 ± 0.1
Physical activity level (MET/24t)	1.9 ± 0.1	1.8 ± 0.1
Use of OC, yr	0 ± 0	7.0 ± 0.6

Values are means $\pm$ SE; $n = 15$ (NOC), $n = 15$ (OC users). BMI, body mass index; $\dot{V}o_{2max}$, maximal oxidative uptake of oxygen; MET, metabolic equivalents; OC, oral contraceptives; NOC, nonusers of oral contraceptives. No significant differences between groups except for duration of OC use.

obtained by measuring the distance from the dorsal insertion at the patella apex to the dorsal insertion on the tibia (39). The patellar tendon CSA and patellar tendon length were manually outlined using the software program Osiris 4.19 (http://www.softpedia.com). Tendon CSA and length were measured using gray-scale image display. The mean value of three measurements of the same image was used for analysis of the Patellar tendon CSA at each anatomical position. An average of the proximal, midway, and distal images was used for the calculation of normalized biomechanical properties. The MRI assessment investigator was blinded with regards to subject grouping. Intraindividual coefficient of variation (CV%) during the three measurements of each specific site was 1.3%. To compare tendon dimensions between subjects of various body size, tendon CSA data were normalized to body weight and raised to the power of two-thirds (49).

Mechanical Properties of the Patellar Tendon

Details of the measurement, including the reliability of the method in our laboratory, have been reported previously (28). Within-day correlation coefficient and typical error percent for repeated measures were 0.95 and 9.9% for tendon stiffness, 0.97 and 5.5% for tendon strain, and 0.94 and 9.4% for Young's modulus. Subjects performed a 5-min warm-up on a stationary bike to secure proper preconditioning of the tendon before testing. Subsequently, the subjects were seated in a custom-made rigid chair with both hips and knees flexed to an angle of 90°. A leg cuff, which was connected to a strain gauge (Bofors KRG-4, Bofors, Sweden) through a rigid steel rod perpendicular to the lower leg, was mounted on the leg just above the medial malleolus. An ultrasound probe (7.5 MHz, linear array B-mode; Sonoline Sienna, Siemens, Erlangen, Germany) was fitted into a custom-made rigid cast that was secured to the skin above the patellar tendon in the sagittal plane. The ultrasound probe and cast were positioned so that the distal patella, the entire patellar tendon, and the proximal tibia were all visible within the viewing field throughout the isometric ramped contractions performed.

Ultrasound S-VHS video images obtained during the ramp trials were sampled at 50 Hz on a personal computer using frame-by-frame capturing software (Matrox Marvel G400-TV, Dorval, Quebec, Canada) (14). Force was sampled on two separate personal computers at 50 Hz via a 12-bit analog-to-digital converter (dt2810A; Data Translation). The two computers were interconnected to permit synchronous sampling of all data using a custom-built trigger device (7). The subjects performed four to five slow isometric knee extensions ramps by applying gradually increasing force until maximum over a 10-s period during which patellar tendon displacement and knee extension force were measured simultaneously. Each ramp was separated by a 2-min rest period. All measurements were performed on one side, randomized to either the right or left knee. During the ramp contractions, force was sampled at 50 Hz and subsequently digitally low-pass filtered (1.0-Hz cutoff frequency) using a fourth-order zero-lag Butterworth filter (14).

Tendon force was calculated by dividing the measured knee extension moment by the internal moment arm, the latter estimated from individually measured femur lengths (78). Tendon stress was calculated by dividing tendon force with the average patellar tendon CSA determined at the three length levels (proximal, mid, and distal) by MRI. Tendon deformation was defined as the change in distance between the patellar apex and the tibia (28, 46). Tendon strain was calculated as the change in length normalized to resting tendon length. Each single force-deformation curve was fitted to a second- or third-order polynomial fit ($r^2 > 0.98$). Tendon stiffness [change (Δ) in force/Δdeformation] and Young's modulus (Δstress/Δstrain) based on largest common force were calculated in the final 20% of the force-deformation and stress-strain curves, respectively (46). Mechanical properties of the patellar tendon were analyzed both at the same common force (2,500 N) for both groups (group common force) and at the maximal individual common force (comparing the two legs in each subject) to take into account nonsignificant differences in absolute and normalized force between the groups and the legs.

Tendon Biopsies

Tendon biopsies were obtained randomly from either the jumping leg or contralateral leg, according to procedures described in detail elsewhere (29, 38). In brief, after sterilization of the insertion site, the skin was injected with local anesthetic (1% lidocaine), and a 3- to 5-mm-long incision was made distally to the patella apex. Tendon biopsies were obtained by using a 16-G Monopty biopsy instrument (Bard, Covington, GA) with a disposable core biopsy needle (14 gauge). The biopsy needle was inserted into the tendon surface at an ~30° angle and fired, securing a tissue sample of approximately 8–10 mg. Biopsies were cleared of external adipose tissue and blood. The piece used for analysis of collagen cross-links was snap-frozen in liquid nitrogen and stored at −80°C for subsequent analysis. The other part of the tendon biopsy was used for transmission electron microscopy (TEM). The biopsy specimens for TEM were fixed in a 2% gluteraldehyde in 0.05 M sodium phosphate buffer (pH 7.2) and stored at 4°C until subsequent tissue processing. The procedure for TEM and the following measurements of collagen fibril diameter have previously been described in details (48).

Biochemical analysis of collagen cross-links. Freeze-dried tendon samples were hydrolyzed in 6 M HCl (+108°C, 24 h) and evaporated into dryness and dissolved in H_2O. Hydroxyproline, the collagen-specific amino acid, was measured spectrophotometrically to quantify collagen protein (15). Hydroxylysyl pyridinoline (HP), lysyl pyridinoline (LyP), and pentosidine were analyzed via a single reversed-phase high-performance liquid chromatography (HPLC) run and detected on the basis of their natural fluorescence (4). At 0–16 min, the wavelength for HP and LyP fluorescence was 400 nm for emission and 295 nm for excitation. The wavelengths were changed at 16–60 min to 328/378 nm to measure pentosidine. For the elution of the cross-links, a gradient was built up to contain 17% *eluent B* (75% acetonitrile with 0.13% heptafluorobutyric anhydride) at 0 min and 25% *eluent B* at 30 min. *Eluent A* was 0.13% heptafluorobutyric anhydride. Flow rate was 1 ml/min. HP was eluted at 12 min, LyP at 13.5 min, and pentosidine at 23 min. The HPLC system used included Quaternary Gradient Pump unit, PU-2089 Plus, Intelligent AutosamplerAS-2057 Plus, and Intelligent Fluorescence Detector FP-2020 by Jasco. Data processing software was Jasco Chrompass. The LiChroCART 125–4 column was from Merck Hitachi. The HP, LyP, and pentosidine concentrations were calculated according to the external standard method using pure HP, LyP, and pentosidine compounds at four different concentrations in each HPLC run. The intra-assay CV% based on duplicates within a run was 2.6, 3.7, and 3.9% for HP, LyP, and pentosidine, respectively. The detection limit for HP and LyP is 0.4 pmol and 0.05 pmol for pentosidine.

Stereology. A simple, random sample of 10 digitized electron microscopy images were obtained from each biopsy cross section to obtain an estimation of the volume fraction of collagen fibrils and their density (number per CSA) and size distribution (diameter). The analyses were carried out on a computer monitor onto which the digitized electron microscopy image was merged with a graphic representation of the stereological test systems (C.A.S.T.-grid software, The International Stereology Center at Olympus). Each TEM image was examined with 16 uniformly positioned points (for estimation of the volume faction) and 16 uniformly positioned unbiased counting frames (23) for estimation of the density of fibrils and to obtain a number-weighted sample of fibrils for measuring the diameter distribution. Each counting frame had an area of 0.0426 μm^2 and was positioned in a fixed position relative to the image. The counting frames covered 15% of the area of the TEM images. On average, 446 $\pm$ 42 fibrils (range 303–865) were analyzed per biopsy cross section in the NOC specimens, and 384 $\pm$ 25 from each of the OC specimens (range 270–514). All measurements were performed in a blinded fashion.

Blood Analyses

All blood samples were taken from an antecubital vein. Serum estradiol was determined by chemiluminescent competitive immunoassay (estradiol, Diagnostic Product, Los Angeles, CA). Plasma progesterone was determined by a microparticle enzyme immunoassay (progesterone, Abbott Diagnostics, Wiesbaden, Germany). Testosterone was analyzed by liquid chromatography mass spectrometry using an atmospheric pressure chemical ionization interface (CV% < 15%) at Statens Serum Institute, Denmark. Serum LH and FSH were determined by an electrochemiluminescent immunoassay at Hvidovre Hospital, Denmark. Blood glycated HbA1c was determined by ion exchange HPLC at Bispebjerg Hospital. Insulin-like growth factor I (IGF-I) was determined by time-resolved immunofluorometric assays after acid-ethanol extraction, as previously described (21). Relaxin was determined by an enzyme-linked-immunosorbent assay (ALPCO Diagnostics, Salem, MA). For each variable, all samples were measured in the same assay run (intra-assay CV% < 5%).

Statistical Analysis

Statistical analyses for differences between OC and NOC in subject characteristics (Table 1), fibril characteristics (see Table 5) and tendon content of collagen and cross-links (Fig. 3) were performed using Student's unpaired t-tests. One-way ANOVA was used to test for differences between groups (NOC FP, NOC LP, OC pill period, and OC off-pill period) in serum estradiol, testosterone, FSH, and IGF-I and HbA1c since these data passed normality criterions (Shapiro-Wilk). Post hoc tests were performed when statistical significance was observed (all pairwise multiple comparison procedures, Holm-Sidak method). Data for progesterone, LH, and relaxin did not follow a normal (Gaussian) distribution, and Mann-Whitney rank sum tests, therefore, were performed to test for differences between groups. A two-way ANOVA (one-factor repetition: jumping leg vs. contralateral leg) was used to test for difference in tendon CSA and tendon biomechanical properties caused by training load or use of OC. Pearson product-moment correlation analyses were performed to test for correlation between blood parameters and tendon biomechanical properties (individual common force data) and between HbA1c and tendon cross-links (HP, LyP, and pentosidine). The level of significance was set at $P \leq 0.05$. The statistical analyses were performed using the statistical software package Sigma Plot version 11.0.

RESULTS

Blood Parameters

The concentrations of 17β-estradiol and progesterone were higher in the NOC group tested in the days after ovulation (NOC LP) compared with both OC users and the NOC group tested in

the days after start of menstruation (NOC FP). Estradiol was below the detection level (0.1 nmol/l) in five of seven OC users in the pill period and three of the eight OC users in the off-pill period. IGF-I was lower in the OC group tested in the pill period compared with the other groups. No significant difference in testosterone, HbA1c, or relaxin was detected between groups. HbA1c was within normal range in all individuals (range 4.9–5.6%). FSH and LH were within normal range for menstrual phases. LH was significantly higher in women tested within the LP compared with women tested in FP or in the OC pill period. LH and FSH concentrations were suppressed to below the detection levels (<0.1 internal unit/l for both FSH and LH) in two OC users in the pill period and one OC users in the off-pill period. All blood data are shown in Table 2.

Tendon CSA

Tendon CSA (mm^2) did not differ between the different phases of the menstrual cycle and pill cycle (data not shown) in the NOC or OC groups, respectively. Consequently, data from the different phases within the OC groups and NOC groups was pooled in the comparison between OC and NOC.

Tendon CSA adjusted for body weight (mm^2/kg$^{2/3}$) tended to be significantly greater in the jumping leg compared with the contralateral leg at the distal level of the patellar tendon ($P = 0.09$). Furthermore, there was a tendency toward a significant interaction between training (jumping leg vs. contralateral leg) and treatment (OC vs. NOC) ($P = 0.08$). Post hoc analysis revealed greater patellar tendon CSA (mm^2/kg$^{2/3}$) at the distal tendon in the jumping leg compared with the contralateral leg in OC users ($P = 0.05$), but not in NOC users ($P = 0.98$). No difference in tendon CSA adjusted for body weight was observed at the proximal and medial recording in the patellar tendon between OC and NOC or between the legs (Fig. 1).

Mechanical Properties of the Patellar Tendon

No difference in mechanical properties of the patellar tendon was observed between the two NOC groups tested in different menstrual phases or between the OC groups tested in either the pill period or in the off-pill period (data not shown). Therefore, in the subsequent analyses for an effect of OC use and training load on mechanical properties of the patellar tendon, data were collapsed across the different time phases.

No difference in tendon biomechanical properties (group common force and individual common force) was observed between

Table 2. Blood parameters

	NOC		OC	
	Follicular	Luteal	Pill−	Pill+
n	7	8	8	7
Estradiol, nmol/l	0.14 ± 0.02	0.40 ± 0.07*	0.09 ± 0.03	0.05 ± 0.03
Progesterone, nmol/l	1.3 ± 0.3	21.3 ± 6.3*	2.9 ± 2.0	1.8 ± 0.5
FSH, int. unit/l	6.4 ± 0.6	4.6 ± 0.7	5.4 ± 1.1	2.8 ± 1.0
LH, int. unit/l	4.8 ± 0.4	6.9 ± 1.6†	5.6 ± 2.0	2.0 ± 0.4
Testosterone, nmol/l	1.0 ± 0.1	1.3 ± 0.2	1.4 ± 0.2	1.3 ± 0.2
IGF-I, μg/l	389 ± 25	348 ± 25	317 ± 21	228 ± 16‡
HbA1c, %	5.4 ± 0.1	5.3 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
Relaxin, ng/l	98 ± 40	152 ± 55	63 ± 18	56 ± 27

Values are means ± SE; n, no. of subjects. FSH, follicle stimulating hormone; LH, lutenizing hormone; IGF-I, insulin-like growth factor I; int. unit, internal unit (Hvidovre Hospital, Copenhagen); HbA1c, hemoglobin A1c. *Significant higher compared with the other groups ($P < 0.001$). †Significant higher than pill+ ($P = 0.001$) or follicular phase ($P = 0.002$). ‡Significantly suppressed compared with the other groups.
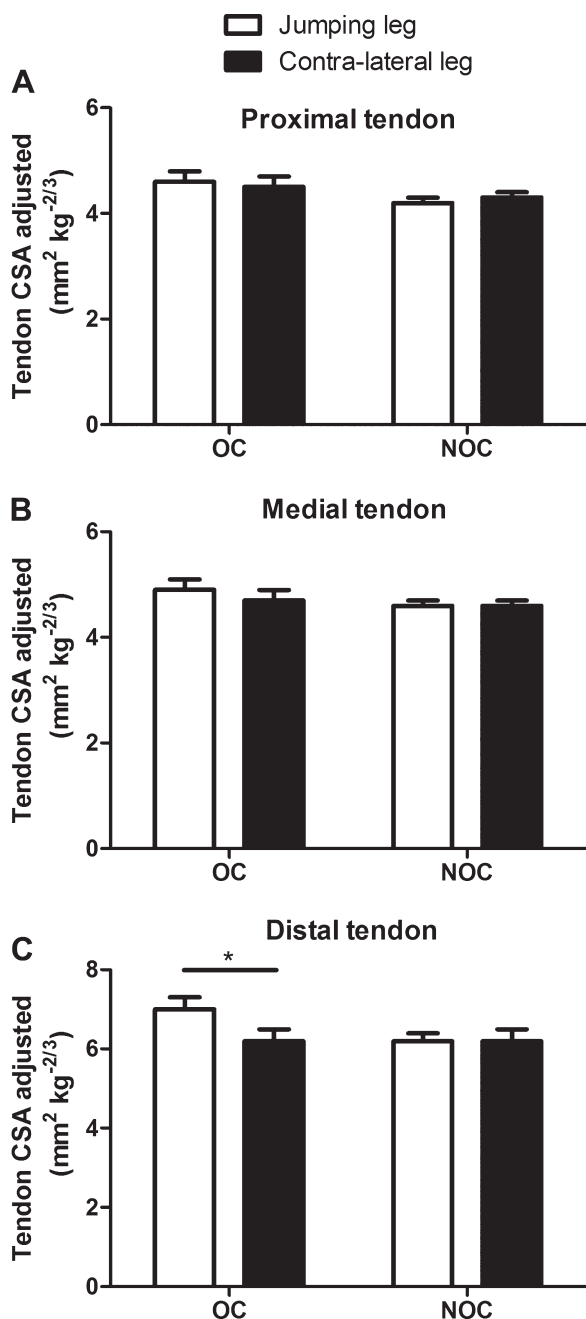
Fig. 1. Tendon cross-sectional area (CSA) (mm²/kg²/³) in the jumping leg and contralateral leg in oral contraceptive users (OC; $n = 14$) and nonusers of OC (NOC; $n = 15$). Values are means ± SE. Two-way ANOVA (one-factor repetition) results are as follows. *A*: proximal [treatment (OC vs. NOC), $P = 0.21$, training (jumping vs. contralateral leg), $P = 0.84$, interaction ($P = 0.20$)]. *B*: medial (treatment $P = 0.22$, training $P = 0.42$, interaction $P = 0.27$). *C*: distal (treatment $P = 0.41$, training $P = 0.09$, interaction $P = 0.08$). *$P = 0.05$, significant effect of training (jumping leg compared with the contralateral leg) in the OC users (Student's paired t-test).

OC and NOC. Similarly, no significant interaction was observed between treatment (OC vs. NOC) and training load (jumping leg vs. contralateral leg) for any of the biomechanical tendon parameters examined (Tables 3 and 4). However, a tendency toward a significant interaction between treatment and training load was observed for stress (individual common force, N/mm², $P = 0.06$).

When comparing the tendon biomechanical properties between the legs, significant differences were observed for several parameters. For the jumping leg at the maximal group common force level (2,500 N), a significant reduced (-11%) strain ($P = 0.04$), greater (15%) absolute stiffness ($P = 0.01$), and greater (14%) normalized stiffness (Young's modulus, $P = 0.02$) were observed compared with the contralateral leg. Furthermore, deformation tended to be lower (-9%) in the jumping leg compared with the contralateral leg ($P = 0.06$). When analyzing the data from the jumping leg at the same individual common force level, tendon deformation was smaller (-11%) compared with that from the contralateral leg ($P = 0.03$). Furthermore, Young's modulus tended to be higher (12%) in the jumping leg compared with the contralateral leg when analyzing the data at the same individual common force level ($P = 0.11$).

An inverse relationship between serum estradiol and tendon stiffness was observed for all subjects ($r = -0.49$, $P = 0.02$). Furthermore, correlations between serum estradiol and tendon deformation ($r = 0.41$, $P = 0.07$) and strain ($r = 0.41$, $P = 0.07$) also tended to be statistically significant (Fig. 2). Serum estradiol did not correlate with any of the tendon biomechanical data when the data from the OC groups were analyzed separately, whereas serum estradiol within the NOC groups correlated negatively with tendon stiffness ($r = -0.53$, $P = 0.04$) and tended to correlate positively with tendon deformation ($r = 0.45$, $P = 0.09$) and tendon strain ($r = 0.49$, $P = 0.06$), respectively. No tendon biomechanical parameters were correlated with circulating testosterone or relaxin, but a positive correlation between serum progesterone and strain was observed ($r = 0.52$, $P = 0.04$).

HbA1c correlated negatively with tendon deformation ($r = -0.615$, $P < 0.001$), tendon strain ($r = -0.544$, $P < 0.01$), and toe region strain ($r = -0.60$, $P < 0.001$). Furthermore, HbA1c tended to correlate positively with the content of cross-links (LyP, $r = 0.37$, $P = 0.07$). The latter finding was even more pronounced when analyzing the data from the NOC groups separately (HP, $r = 0.59$, $P = 0.04$; LyP, $r = 0.79$, $P = 0.001$).

Tendon Structure

Tendon biopsies were obtained in the jumping leg in seven OC users and in seven NOC users, whereas eight OC users and six NOC users had a tendon biopsy taken in the contralateral leg. No difference between phases in tendon fibril characteristics, collagen content, or collagen cross-linkings was observed when the two groups of OC users and two groups of NOC users were compared separately. Therefore, all data from the OC users and NOC users were merged in the subsequent comparisons between OC and NOC.

No difference between NOC and OC was observed in collagen volume fraction (%) ($P = 0.73$), fibril density ($P = 0.22$), mean fibril diameter ($P = 0.38$), %fibrils with a diameter between 0 and 60 mm ($P = 0.24$), between 61 and 90 mm ($P = 0.59$), and above 90 mm ($P = 0.47$) (Table 5). Likewise OC and NOC did not differ with respect to tendon collagen content ($P = 0.25$) and cross-links (HP, $P = 0.48$; LyP, $P = 0.69$; and pentosidine $P = 0.94$, Fig. 3).

Table 3. *Tendon biomechanical properties (group common force)*

	Jumping	Contralateral	P Value OC vs. NOC	P Value Training	P Value Interaction
Stress, MPa					
NOC	30.0 ± 0.8	29.4 ± 0.8	0.35	0.49	0.17
OC	28.0 ± 1.0	29.3 ± 1.1			
Deformation, mm					
NOC	1.3 ± 0.1	1.5 ± 0.1	0.35	0.06	0.14
OC	1.5 ± 0.1	1.6 ± 0.1			
Strain, %					
NOC	2.8 ± 0.2	3.2 ± 0.2	0.41	0.04*	0.68
OC	3.1 ± 0.2	3.4 ± 0.3			
Strain-toe region, %					
NOC	1.3 ± 0.1	1.5 ± 0.1	0.42	0.20	0.52
OC	1.5 ± 0.2	1.6 ± 0.2			
Stiffness, N/mm					
NOC	3,768 ± 293	3,243 ± 233	0.57	0.01*	0.63
OC	3,477 ± 338	3,062 ± 237			
Young's modulus, GPa/%					
NOC	2.2 ± 0.2	1.8 ± 0.1	0.46	0.02*	0.17
OC	1.9 ± 0.1	1.8 ± 0.2			

Values are mean values of both legs ± SE; $n = 14$ (OC users), $n = 15$ (NOC). The values are calculated based on the same common force (2,500 N) for both groups to adjust for individual differences in muscle strength. Description of the test parameters is given in MATERIALS AND METHODS. The results based on the jumping legs and contralateral legs are presented separately. P values show results from two-way ANOVA (one-factor repetition). *$P < 0.05$, significant effect of training (jumping leg vs contralateral leg).

DISCUSSION

Effect of OC

In the present study, no significant influence of regular use of OC on tendon morphology, major strength providing cross-links of collagen, or tendon biomechanical properties in female athletes was observed. The influence of OC on the risk of overuse injuries and acute injuries is still debatable (9, 37, 55, 66, 76, 82). Moreover, the effect of exogenous female hormones on tendon and ligament composition, tissue turnover, and biomechanical properties is inconsistent (8, 26, 27, 51, 81). In a previous study, we observed a lower collagen fractional synthesis rate in the patellar tendon of young OC users than NOC users, both at rest and in response to knee extensor exercise (26, 27). This observation was related to OC reduce bioavailability of IGF-I, which has a marked stimulating effect on tendon collagen synthesis (24). In the present study, tendon collagen content was not significantly reduced in OC users ($P = 0.25$), and the adaptation in tendon CSA to loading seems to be more pronounced in OC users than NOC users. This

Table 4. *Tendon biomechanical properties (individual common force)*

	Jumping	Contralateral	P Value OC vs. NOC	P Value Training	P Value Interaction
Stress, MPa					
NOC	46.1 ± 2.9	43.9 ± 2.8	0.69	0.53	0.06
OC	41.8 ± 3.2	45.9 ± 3.8			
Deformation, mm					
NOC	1.6 ± 0.1	1.7 ± 0.1	0.33	003*	0.83
OC	1.7 ± 0.1	2.0 ± 0.2			
Strain, %					
NOC	3.4 ± 0.2	3.9 ± 0.2	0.46	0.03	0.86
OC	3.7 ± 0.2	4.1 ± 0.3			
Strain-toe region, %					
NOC	1.4 ± 0.2	1.6 ± 0.2	0.15	0.29	0.54
OC	1.8 ± 0.1	1.9 ± 0.2			
Stiffness, N/mm					
NOC	4,423 ± 339	4,013 ± 363	0.43	0.37	0.68
OC	4,241 ± 432	3,781 ± 280			
Young's modulus, GPa/%					
NOC	2.7 ± 0.3	2.1 ± 0.2	0.80	0.11	0.17
OC	2.4 ± 0.2	2.4 ± 0.3			
Patellar force, N					
NOC	3,643 ± 220	3,799 ± 245	0.75	0.18	0.55
OC	3,479 ± 198	3,706 ± 220			

Values are means ± SE; $n = 14$ (OC users), $n = 15$ (NOC). The values are calculated based on individual common force. Description of the test parameters is given in MATERIALS AND METHODS. The results based on the jumping legs and contralateral legs are presented separately. P values show results from two-way ANOVA (one-factor repetition). *$P < 0.05$, significant effect of training (jumping leg vs. contralateral leg).
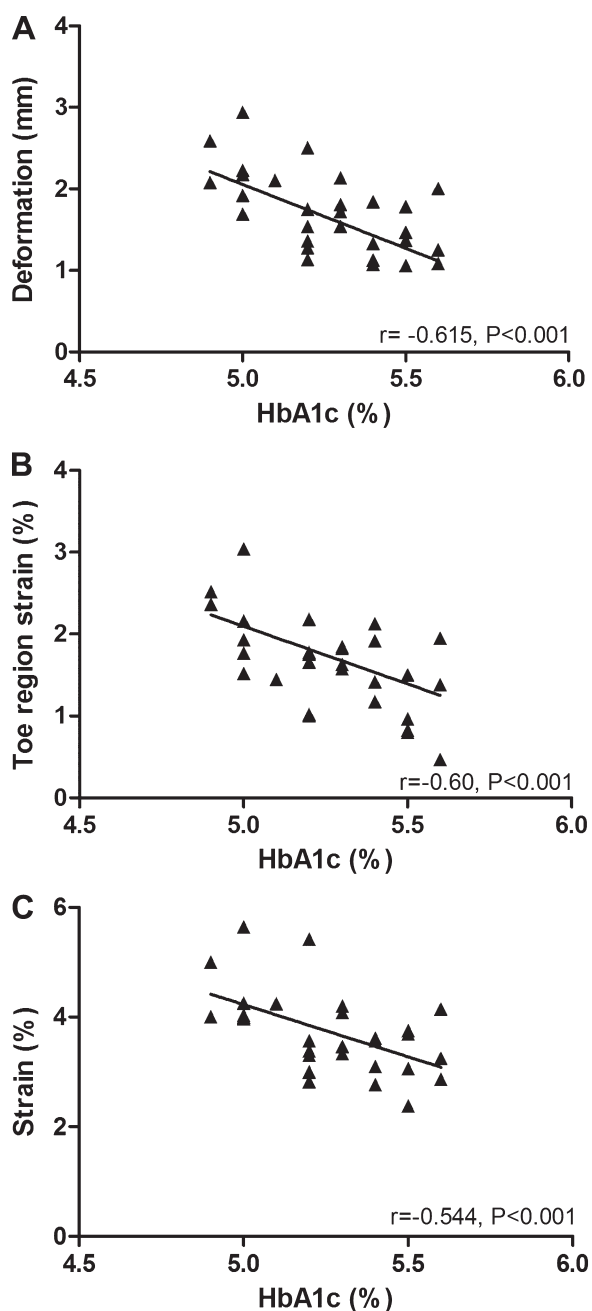
Fig. 2. Correlations between serum estradiol in NOC and biomechanical parameters. A: tendon stiffness. B: tendon deformation. C: tendon strain. HbA1c, hemoglobin A1c.

suggests that OC reduces tendon collagen turnover. However, this hypothesis awaits experimental verification.

Methods used in previous studies (8, 51, 60) to investigate the association between use of OC and biomechanical properties of the connective tissue differ from the methods used in the present study. Martineau et al. (51) observed reduced passive anterior translation of the tibia measured by KT1000 in OC users, but others have failed to reproduce these findings (60). Bryant et al. (8) observed a reduced strain of the triceps surae aponeurosis during maximal isometric plantar flexion in OC users, measuring deformation of both the Achilles tendon and the aponeourosis. Since the mechanical properties of the free tendon and aponerosis is divergent (20, 47), it is difficult to compare these data with the present data on the isolated effect of isometric contraction on the tendon. Moreover, use of an ultrasound head, which monitors the entire patellar tendon, as in the present study, is considered superior when quantifying deformation than single reference point, as in the study by Bryant et al. (8).

Effect of Menstrual Cycle Phases and Estrogen

OC prevent the mid- and late-cycle surges of estrogen, which have been associated with an enhanced knee laxity (16, 33, 62, 69, 70). Increased knee joint laxity influences landing biomechanics and leads to increased knee joint loads during athletic movements (59, 72) and enhanced risk of ACL injuries (73, 80). Nevertheless, knowledge about direct effects of estrogen on the collagen structure and biomechanical properties of the ACL is primarily based on in vitro studies or tests of ACL from animals (41, 44, 68, 74, 83, 84), since the ACL is not easily accessible in non-knee-injured subjects. Ligaments and tendons have specific functions and may adapt differently due to a potential differential composition of estrogen receptors (α and β) (52). However, due to morphological/physiological similarities between tendon and ligaments (collagen types, %collagen of the extracellular matrix, and estrogen receptor presence) (19, 32, 50), the effect of estrogen on the patellar tendon may be transferred to the ACL and other knee ligaments important for knee stability.

Differences in the mechanical properties of the patellar tendon across menstrual phases were nonsignificant in the present study. This may be due to different cyclic changes in laxity among women, which reduce the possibility to detect a difference between phases in a cross-sectional study (69, 71). Still, circulating estradiol in NOC correlated with tendon stiffness ($r = -0.53$, $P = 0.04$), tendon deformation ($r = 0.45$, $P = 0.09$), and strain ($r = 0.49$, $P = 0.06$) in the present study. Although the generalizability between the properties of the patellar tendon and the ACL can be questioned, our data on the patellar tendon support the assumption that knee laxity is increased if women are considerably exposed to estrogen (16, 62, 69, 70). However, the correlations presented showed that difference in estrogen explained only approximately 20–28% of the variations in the patellar tendon biomechanical properties. Based on this, it seems plausible to assume that the risk of ACL is multifactorial, and several other risk factors must be considered (35). The muscles serve as dynamic joint stabilizers, and the neuromuscular control of the knee flexors and

Table 5. *Tendon fibril characteristics*

	NOC	OC	P Value: OC vs. NOC
Volume fraction, %	0.59 ± 0.03	0.60 ± 0.03	0.73
Density, fibril/μm^2	85 ± 8	74 ± 4	0.22
Mean fibril diameter, nm	87 ± 4	92 ± 4	0.38
Mean fibril area, nm^2	7,325 ± 574	8,250 ± 684	0.28
%Fibrils			
Diameter 0–60 nm	32 ± 4	26 ± 2	0.24
Diameter 61–90 nm	29 ± 2	31 ± 3	0.59
Diameter >90 nm	39 ± 4	42 ± 3	0.47

Values are means ± SE; n = 14 (OC users), n = 13 (NOC). P values show results from Student's unpaired *t*-test.
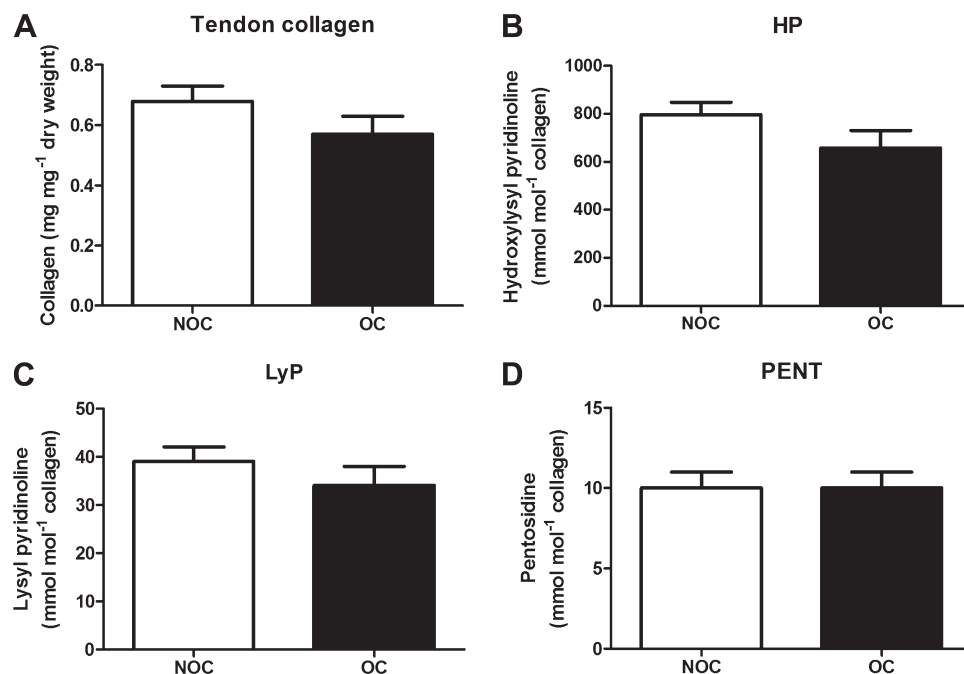
Fig. 3. Patellar tendon concentration of collagen ($P = 0.25$; *A*), hydroxylysyl pyridinoline (HP; $P = 0.48$; *B*), lysyl pyridinoline (LyP; $P = 0.69$; *C*), and pentosidine (PENT; $P = 0.94$; *D*). Values are means ± SE; OC users, $n = 14$; NOC, $n = 13$. *P* values are shown from Student's unpaired *t*-test for test of difference between groups.

extensors influences the risk of an ACL rupture (85). It has been suggested that female hormones may influence the neuromuscular joint control of the lower extremity (35, 72) and muscle fatigability (64) and thereby the risk of injuries, but these factors are not fully elucidated (12, 34, 40).

If high exposure to estrogen (average or peak level of estrogen) alters ligaments and tendon composition and thereby their biomechanical properties by influencing fibroblast proliferation (44, 83, 84), collagen synthesis (41, 44, 83, 84), and/or collagen degradation (22, 36, 54, 65), the effect of estrogen is probably not acute, but an accumulated effect over several cycles. In support, elderly women who had used ERT for ~17 yr were characterized by a higher tendon collagen synthesis rate, changed tendon fibril morphology, and a lower tendon stiffness compared with age-matched postmenopausal controls (25).

Effect of Training Load

Tendon CSA adjusted for body weight was greater in the jumping leg, which is an advantageous adaptation reducing tissue stress during loading. Young's modulus represents the biomechanical quality of the tendon, which takes into account differences in tendon CSA and differences in the absolute loading of the tendon during the stiffness assessment. For both groups, we observed a higher Young's modulus in the patellar tendon of the jumping leg, which has been exposed to a higher load during the years of training compared with the contralateral tendon. Furthermore, tendon stiffness was greater and tendon deformation was lower during loading in the jumping leg. Similarly, greater patellar tendon stiffness and CSA have been observed in elite male badminton players and fencers in the lead leg compared with the contralateral leg (14). Nevertheless, previous studies have indicated that the responsiveness of tendon tissue to mechanical loading and the ability to adapt to regular training was reduced in young women than in men (30, 45, 53, 79). Actually, no change in tendon biomechanical properties and CSA was observed in young women after 9 mo

of regular running training (30), and Westh et al. (79) observed no difference in weight-normalized Achilles and patellar tendon CSA between trained female runners (>40 km/wk for >5 yr) and untrained women, while CSA of the tendons was greater in trained men compared with both female groups. The apparent discrepancy between the present data and those reported previously (30, 79) may be owing to methodological differences across study designs. Strength of the present design was that the two legs within the same subject were compared instead of comparing different groups of individuals. Still, based on the present observations, we cannot exclude that women adapt less sensitively to a given training stimuli compared with men.

HbA1c

HbA1c, a screening marker for abnormal glucose regulation, was within normal range in all of the subjects. Nevertheless, inverse relationships between HbA1c and tendon deformation and strain were observed, which may be related to a higher content of cross-links. If glucose regulation influences tendon structure and biomechanical properties in healthy individual, the effect may be even more pronounced in diabetic populations.

Limitations

Differences observed between the jumping leg and contralateral leg underline the importance of taking into account not only training status of the individuals, but also limb preference, when comparing the biomechanical properties between different groups of subject. Training status ($\dot{V}O_{2\ max}$, physical activity level, training volume, and frequency) did not differ between OC and NOC. Still, we are aware that the cross-sectional design includes some inherent limitations besides the training status of the subjects. Furthermore, tendon mechanical properties were only assessed at a single time point. These

facts point to a small effect of OC or menstrual cycle on the tendon biomechanical properties may have been overlooked due to somewhat low statistical power.

Conclusion

No differences in patellar tendon structural composition, collagen cross-linking, and biomechanical properties were observed between users of OC and NOC. Serum estradiol was inversely correlated to patellar tendon stiffness in NOC users. If this finding on the patellar tendon is representative for the effect of estrogen on other tendons and ligaments, the present data support the findings in previous studies, linking high exposure to estrogen in young women to increased knee laxity and thus an elevated risk of an ACL injury.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.H., S.P.M., and M.K. conception and design of research; M.H., C.C., C.S.H., and D.S. performed experiments; M.H., C.C., C.S.H., V.K., J.O.L., and P.A. analyzed data; M.H., C.C., V.K., J.O.L., S.P.M., and M.K. interpreted results of experiments; M.H. prepared figures; M.H. drafted manuscript; M.H., C.C., C.S.H., D.S., V.K., J.O.L., P.A., S.P.M., and M.K. edited and revised manuscript; M.H., C.C., C.S.H., D.S., V.K., J.O.L., P.A., S.P.M., and M.K. approved final version of manuscript.

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