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LOMA LINDA UNIVERSITY
School of Allied Health Professions
in conjunction with the
Faculty of Graduate Studies

Anterior Cruciate Ligament Elasticity and Force for Knee Flexion
during the Menstrual Cycle in Women

by

Haneul Lee

A Dissertation submitted in partial satisfaction of
the requirements for the degree
Doctor of Science in Physical Therapy

June 2013

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Each person whose signature appears below certifies that this dissertation in his/her opinion is adequate, in scope and quality, as a dissertation for the degree Doctor of Science.

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ABBREVIATIONS

MC	Menstrual Cycle
OCP	Oral Contraceptive Pills
E2	Estradiol
M	Menstruation
F	Follicular
EF	Early Follicular
LF	Late Follicular
O	Ovulation
L	Luteal
EL	Early Luteal
ML	Middle Luteal
LL	Late Luteal
ACL	Anterior Cruciate Ligament
ATD	Anterior Tibial Displacement
FFK	Force to Flex the Knee
KFEH	Knee Flexion-Extension Hysteresis
SD	Standard Deviation
ANOVA	Analysis of Variance

ABSTRACT OF THE DISSERTATION

Anterior Cruciate Ligament Elasticity and Force for Knee Flexion during the Menstrual Cycle in Oral Contraceptive Users and Non-oral Contraceptive Users

by

Haneul Lee

Doctor of Science, Graduate Program in Physical Therapy
Loma Linda University, June 2013
Dr. Jerrold Petrofsky, Chairperson

Numerous studies have been conducted on changes of knee ligament laxity during the menstrual cycle (MC) since there are more injuries in certain phases. Some researchers believe that since estrogen receptor β exists in ligaments and tendons in the knee, estrogen may modulate towards a state of laxity. However, increased tissue temperature also observed during the MC can predispose ligament and tendon laxness.

The number of women using oral contraceptive pills (OCP) has constantly increased in the United States. This exogenous source of synthetic forms of steroid hormones prevents ovulation by maintaining more consistent daily hormone levels than occurs. Since the estrogen receptor β exists on human connective tissue, OCP might have an impact on tendon and ligament synthesis, structure, and biomechanical properties. Therefore, the purpose of this study was to assess in women the relationship between Estradiol (E2) serum concentrations and tissue temperature during the MC and their combined effect on ACL elasticity, force to flex the knee (FFK), and knee flexion-extension hysteresis (KFEH) in OCP users and non-OCP users.

Nineteen non-athletic young healthy females were divided into two groups; OCP users and non-OCP users. E2 serum concentrations, ACL elasticity, FFK, and KFEH were assessed both at ambient temperature (22 °C) and after 38 °C warming of the leg to stabilize the normal temperature during the MC.

The result of this investigation showed that ACL elasticity was significantly lower and FFK and KFEH were significantly higher during ovulation compared to menstruation ($p < 0.05$) but after 38 °C warming of the leg, there were no differences in KKF and KFEH in non-OCP users. Also, ACL elasticity was significantly lower and FFK and KFEH were significantly higher OCP users than non-OCP users ($p < 0.05$) at ambient temperature. But, no significant difference in FFK and FFEH between the two groups was found after warming to 38 °C.

In conclusion, OCP users have more knee stiffness and effect of heat on FFK and KFEH when their legs were heated compared to non-OCP users. In addition, ACL elasticity, FFK, and KFEH were affected not only by estrogen but tissue temperature during the menstrual cycle.

CHAPTER ONE

INTRODUCTION

Knee injuries occur most frequently in the anterior cruciate ligament (ACL); 80% of ACL injuries are non-contact ACL injuries ¹. Due to an increase in women's sports participation, many researchers have looked at injuries occurring during sports activities and found that ACL injuries are more common in women compared to men who participate in the same activities ²⁻⁵. The differences are commonly assumed to be differences in the anatomical, neuromuscular, behavior patterns, and hormones that make women more susceptible to ACL injuries than men ^{6,7}. Regarding hormonal influences, many previous investigators have examined the relationship between female reproductive hormones and structure and mechanical properties of the ACL ⁸⁻¹¹. The role of estrogen in possibly causing ACL injuries has recently been studied at the cellular level and the presence of 17- beta estradiol receptors in the human ACL and muscle have been investigated ¹⁰⁻¹³. The constant changes in estrogen concentration in blood during the menstrual cycle have been investigated to see if sex hormones are related to ligament laxity ¹⁰⁻¹³. Human tendon, muscle, and ligament are composed of collagen fibers closely packed together. Due to the decrease of collagen formation and fibroblast proliferation with an increased estrogen serum concentration, the decreased collagen synthesis is believed to cause ligament laxness, and muscle and tendon flexibility all of which makes the ACL more susceptible to injuries ^{8, 10, 12}.

Estradiol is the estrogen in women that is most commonly measured in young non-pregnant females ¹⁴. Since most ACL injuries occur in young healthy women, most investigators have used estradiol as the major estrogen biomarker. Estradiol serum concentration fluctuates throughout the menstrual cycle but in women who are taking oral contraceptive pills (OCP), it does not vary during the menstrual cycle ^{15,16}. Eighty two percent of sexually active women aged 15-44 have used the OCP in the United States and 14% of women have used OCP for non-contraceptive purposes such as cycle regulation, treatment of dysmenorrhea and amenorrhea ^{17,18}. OCP consists of combined estrogen and progesterone as synthetic steroids and it plays a role in suppressing pituitary FSH and LH production and preventing ovulation from occurring ^{18,19}. This exogenous source of synthetic forms of the hormone prevents ovulation by maintaining more constant hormone levels ²⁰. Since estrogen is stabilized throughout the entire menstrual cycle, the response may be different in women on OCP. Therefore, OCP may be another factor that affect on tendon and ligament synthesis, structure, and biomechanical properties. Martineau and colleagues have shown that OCP users have less ACL laxity than non-OCP users ²¹.

However, there has been controversy as to the ACL laxity changes across the menstrual cycle. A systemic review on the effects of the menstrual cycle on anterior knee laxity by Zazulak and colleague showed that only three of the nine studies reported a significant difference on ACL laxity during the menstrual cycle ²². Among 3 studies saying there was a significant difference in ACL elasticity during the menstrual cycle, the results are different. Heitz and colleague showed that there was a greater knee laxity in the ovulation while Shultz and colleague found a greater knee laxity in the early follicular

phase^{23,24}. Although Zazulak and colleague found limited evidence of the correlation between ACL laxity and sex hormones, they claimed that cycle phases might have an effect on anterior knee laxity. These researchers suggested that future investigators should consider the limitation in present methodologies that they had discovered²². They also point out confounding variables that might affect anterior knee elasticity during the menstrual cycle.

Temperature is one of the factors that affect elasticity in connective tissues, such that, an increase in tissue temperature, by doing warm-up exercises will increase muscle and tendon extensibility^{25,26}. In healthy young adults, normal core temperature is maintained between 36-37.5°C and it changes approximately 0.5°C throughout the day²⁷.²⁸ In women with a regular menstrual cycle, their core temperature fluctuates not only throughout the day but also during the menstrual cycle²⁹. It is at a peak during the middle luteal phases while in the early follicular and the ovulation it is relatively low²⁹⁻³¹. However, skin temperature of the arms and legs is commonly measured around 6°C below the core, 30-31.5°C since human legs and arm tissues are used as a radiator to remove excess heat from the core^{32,33}. Due to the core temperature changes throughout the menstrual cycle, skin temperature can also be altered because of the core temperature changes. To increase core temperature, arm and leg temperature is reduced at first to conserve heat³⁴. To lower core temperature, arm and leg temperature increases to dissipate heat. Since, ligament, muscle, and tendon extensibility will decrease and increase with decreased or increased tissue temperature, it is of no surprise that the menstrual cycle has dramatic effects on tissue extensibility. Although tissue extensibility decreases with decreased tissue temperature, many studies have reported that anterior

knee laxity is greater in the ovulation phases where the core temperature is comparatively lower than other phases^{23,35}. However, core temperature increases after ovulation. To keep core temperature lower at ovulation, the shell must be dissipating more heat to keep the core cooler²⁹. Therefore, the shell must be warmer after ovulation. Thus ligament elasticity change during the menstrual cycle may be a consequence of estrogen fluctuations alone during the menstrual cycle. However, since sex hormones do not change in OCP users, their core temperature may change little during the menstrual cycle. In addition, Rogers and Baker have shown that OCP users have higher core temperature compared to non-OCP users due to exogenous forms of progesterone in OCP's³⁶. Thus, the effect of estrogen on ACL elasticity and muscle and tendon flexibility is probably a complex interaction between hormones and tissue temperature.

Many previous researchers examined biomechanical variables related to knee mechanical properties throughout the menstrual cycle^{24,37,38}. The effect of the menstrual cycle on knee flexibility measured by force to flex the knee (FFK) and knee flexion-extension hysteresis (KFEH) has not been investigated. Restricted knee extension affects normal leg movement and this could lead to more non-contact ACL injuries³⁹. The quadriceps muscle is one of the knee extensor muscles, which is crucial to stabilize and create range of motion of the knee⁴⁰. KFEH is the difference between the FFK and force to extend the knee (FEK). Elastic energy is stored during loading the knee (flexion) and is released during unloading (extension); thus, flexing the knee requires greater force than extending the knee. It represents stored elastic energy in the muscles and tendons, and lower elastic hysteresis values indicate more elasticity of muscle and tendon⁴¹.

Therefore, we will interpret quadriceps muscle and tendon flexibility by measuring FFK and KFEH.

In the present study, two series of experiments were conducted to examine the relationship ACL elasticity, FFK, and KFEH during the menstrual cycle. In series 1 (Chapter 2), we examined the relationship between estradiol serum concentration and ACL elasticity, FFK, and KFEH and how this relates to tissue temperature in young healthy women with a regular menstrual cycle. In series 2 (Chapter 3), we investigated the differences in ACL elasticity, FFK and KFEH between OCP users and non-OCP users and its relationship to tissue temperature during the menstrual cycle.

CHAPTER TWO
ANTERIOR CRUCIATE LIGAMENT ELASTICITY AND FORCE FOR KNEE
FLEXION DURING THE MENSTRUAL CYCLE

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Abstract

Background – A high occurrence of knee injuries have been observed in women during the menstrual cycle (MC). As a result, numerous studies have been conducted regarding knee ligament elasticity during the MC. Some researchers believe that since estrogen receptor β exists in ligaments and tendons in the knee, estrogen may modulate towards a state of laxity. However, increased tissue temperature also observed during the MC can predispose ligament and tendon laxness. Therefore, the purpose of this study was to assess in women the relationship between Estradiol (E2) serum concentrations and tissue temperature during the MC and their combined effect on knee laxity.

Material and Methods– Ten non-athletic young healthy females, 18 to 30 years of age participated in the study. E2 serum concentrations, anterior cruciate ligament (ACL) elasticity, and force to flex the knee (FFK), knee flexion-extension hysteresis (KFEH) were assessed both at ambient temperature (22 °C) and after 38 °C warming. Testing was performed multiple times during the participant's MC, for one full MC.

Results: ACL elasticity was significantly higher ($P < 0.01$) and FFK and KFEH were significantly lower ($p < 0.05$) during ovulation when E2 levels were highest. ACL elasticity was still higher during ovulation after warming to 38 °C. But, the effects of MC on FFK and KFEH were reduced by tissue warming.

Conclusions: ACL elasticity, FFK, and KFEH was affected not only by E2 but also tissue temperature. However, E2 had more impact on ACL elasticity while tissue temperature had more impact on FFK and KFEH at 38 °C warming.

Key words: menstrual cycle, ACL elasticity, muscle and tendon flexibility, temperature

Introduction

In the adult population, numbers of knee injuries arise every year during sports activities and exercise ¹. The most common injuries are ligament sprains, muscle strains, and contusions ². In the annually recorded 150,000 injuries affecting the knee, the Anterior Cruciate Ligament (ACL) injuries are the most frequently occurring injury.

According to the Sports Medicine Media Guide for 2011, the cost of ACL injuries has been estimated at half a billion dollar annually. Additionally, people who have a history of ACL injury are at a higher risk of potential arthritis later in their life and will cost an additional millions of dollars ³. Previous studies have indicated that women have a 2 to 8 times higher risk factor of knee injuries than men, specifically, involving the ACL ⁴⁻⁸. There has been ongoing debate on why women are more susceptible.

The differences are commonly assumed to be difference by anatomical, neuromuscular, behavior patterns, and hormonal influences that make women more susceptible to ACL injuries ^{8,9}. Recently investigators have examined the association between female reproductive hormones and structure material and mechanical properties of the ACL. There has been a common theory of the presence of 17-beta estradiol receptor in ACL for decades. The constant changes in the levels of estrogen during the menstrual cycle have been investigated to see if sex hormones are related to ligament laxity ¹⁰⁻¹³. Human tendon, muscle, and ligament are composed of collagen fibers closely packed together. Due to the decrease of collagen formation and fibroblast proliferation with an increased estrogen serum concentration, the decreased collagen synthesis causes ligament looseness, weak muscle strength and tendon elasticity all of which makes the ACL more susceptible to injuries ^{10, 11, 14}. Estradiol is one of the estrogens in women and

is the most commonly measured estrogen in young non-pregnant females ¹⁵. Since most ACL injuries occur in young healthy women, most investigators have used estradiol as the major estrogen biomarker.

However, there has been controversy on the result of ACL laxity changes across the menstrual cycle. A systemic review on the effects of the menstrual cycle on anterior knee laxity by Zazulak and colleague showed that only three of the nine studies reported a significant difference on ACL laxity during the menstrual cycle ⁶. Although this study found limited evidence of the correlation between ACL laxity and sex hormones, Zazulak and colleague claimed that cycle phases might have an effect on anterior knee laxity. These researchers suggested that future investigators should consider the limitation in present methodologies that they had discovered ⁶. They also point out confounding variables that might affect anterior knee elasticity during the menstrual cycle.

Temperature is one of the factors that affect elasticity in muscles, such that, an increase in tissue temperature by doing a warm-up exercise will increase muscle and tendon extensibility ^{16,17}. Normal core temperature is maintained between 36-37.5°C ^{18,19}. The core temperature shows a fluctuation across the menstrual cycle; it is at a peak during the middle luteal phases while in the early follicular and the ovulation it is relatively low ²⁰⁻²². Skin temperature of the arms and legs is commonly measured around 6°C below the core, 30-31.5°C since human legs and arms tissues are used as a radiator to remove excess heat from the core ^{23,24}. Due to the core temperature changes throughout the menstrual cycle, skin temperature can also be altered because of the core temperature changes. To increase core temperature, arm and leg temperature is reduced at first to conserve heat. To lower core temperature, arm and leg temperature increases to

dissipate heat ²⁵. Since, ligament, muscle, and tendon extensibility will decrease and increase with decreased or increased tissue temperature, it is of no surprise that the menstrual cycle has dramatic effects on tissue extensibility. Although tissue extensibility decreases with decreased tissue temperature, many studies have reported that anterior knee laxity is greater in the ovulation phases where the core temperature is comparatively lower than other phases ^{26,27}. However, core temperature increases after ovulation. To keep core temperature lower at ovulation, the shell must be dissipating more heat to keep the core cooler ²¹. Therefore, shell must be warmer after ovulation. These findings may explain that anterior knee laxity changes are a consequence of estrogen fluctuations alone during the menstrual cycle. The reported change may be temperature related to and not a direct effect of estradiol. Thus, the aim of the present study is to examine the relationship between estradiol serum concentration and ACL elasticity, knee flexibility and how this relates to tissue temperature in young healthy women with a regular menstrual cycle.

Materials and Methods

Participants

Ten non-athletic young healthy females with a regular menstrual cycle participated in this study. They were recruited by word of mouth and selected by their self-reported menstrual cycle. Participants were 18 to 30 years of age with a body mass index (BMI) between 15 and 30 who had a regular menstrual cycle for at least one year (Table 1). Participants had no history of pregnancy, neuropathy, myopathy or knee injury, and were not taking any medication that could alter sex hormones. The study was approved by Institutional Review Board of Loma Linda University and all participants signed an informed consent.

Table1. Mean (SD) of General characteristic in women with a regular menstrual cycle (N=10)

	Age (years)	Height (cm)	Weight (kg)	BMI	Cycle length (d)
Mean (SD)	24.7 (2.0)	164.6 (3.4)	57.1 (5.0)	21.0 (1.3)	29.5 (3.2)

Abbreviations: SD, standard deviation

Methods

Trained researchers measured all outcome variables throughout the experiments. The outcome variables assessed were ACL elasticity, force to flex the knee (FFK), knee flexion-extension hysteresis (KFEH), leg skin temperature, and estradiol serum concentration.

Estradiol Serum Concentration

Two certified phlebotomists drew 4cc of venous blood samples from each participant's antecubital area with 23-gauge needles to determine estradiol serum concentration. The serum was separated from plasma by centrifuging the blood. The centrifuge was set at 3000 rpm for 10 minutes (Beckman Coulter Inc., Brea, CA). The serum was kept at a temperature of -80 °C. After all samples of serum were collected, Estradiol serum concentration was assessed using the TOHSO Estradiol ST AIA assay (Fisher Healthcare Inc. Houston, TX).

Leg Skin Temperature

Leg skin temperature was measured using the forward-looking infrared 660 IR Camera (FLIR systems, Danderyd, Sweden). This device was validated to accurately

detect the temperature of the skin ²⁸. To acquire best images, the readings were at a perpendicular angle and at a distance of one meter away from the skin. Dark colored rooms were preferred to minimize infrared interference so that test was performed in the room with uniform Light Emitting Diode (LED) ²⁹. Baseline leg skin temperature was taken before ACL laxity and quadriceps elasticity measurements after stabilizing leg temperature in a neutral environment for 20 minutes and leg skin temperature was measured immediately when the heat pads at 38 °C were removed.

Anterior Ligament Elasticity

Knee elasticity of ACL was measured by a KT-2000 knee arthrometer (MED metric Corp, San Diego, California). This device had been validated for over twenty years in both clinical and research areas ^{30,31}. First, the knee was placed in a predetermined position while the participant lays supine. With the knee at 90 degrees, a strain gauge was used to measure the force necessary to generate an anterior glide of the proximal end of the tibia on the femoral condyles. This generated a force curve of elasticity in the anterior cruciate ligaments. The process was accomplished by supporting both limbs with a firm, comfortable platform placed proximal to the popliteal space. This helped keep the patient's knee flexion angle constant for the duration of the test. Along with this device, a foot support accessory supplied with the arthrometer, positioned the feet symmetrically allowing the leg position to be optimal for the test while reducing external rotation of the tibia. For the most comfortable position during the flexion angle test, knee flexion angle was held at 25 degrees (Figure 1). A thigh strap, provided, controlled hip external rotation while offering support allows patients to be most relaxed.

Force used for the experiment was applied at 30 lbs. (133N) of force. The output of ACL elasticity was displaced on an X-Y plotter. A vernier caliper was used to interpret anterior tibial displacement (ATD) graph. A single trained investigator measured ACL laxity measures throughout the study to minimize potential inter investigation variation.

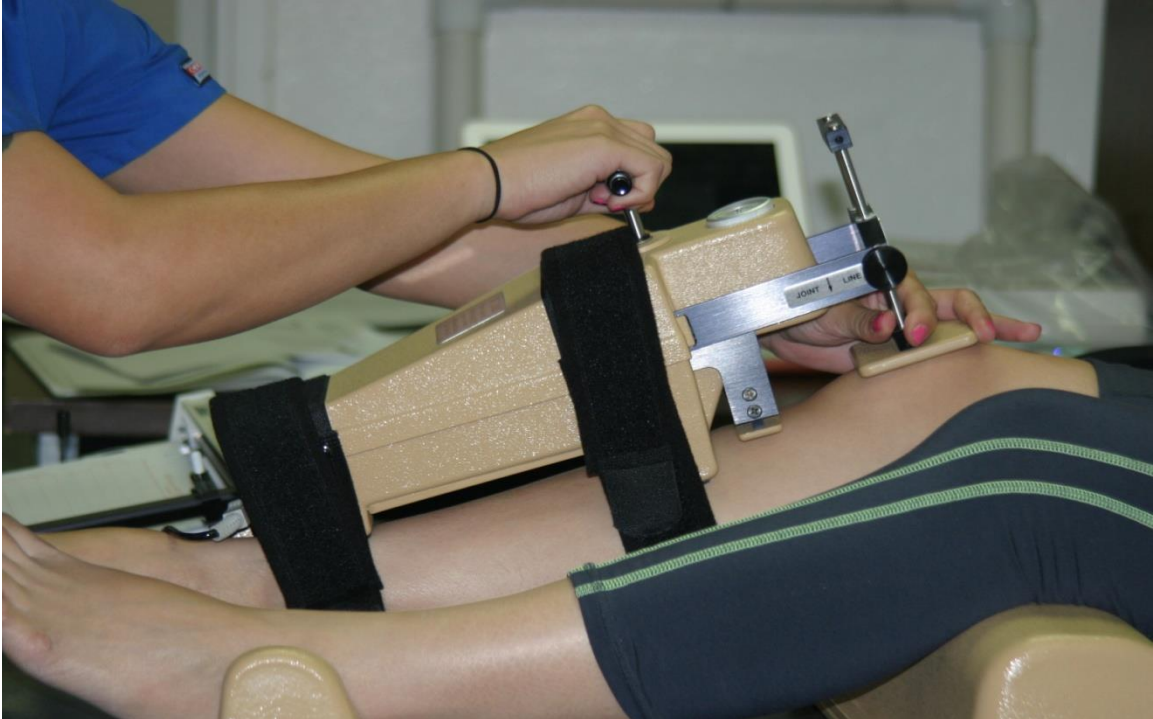


Figure1. Experimental setup for measuring ACL elasticity, the subject was positioned supine with their knee flexed to 25 degrees.

Muscle and Tendon Flexibility

Muscle and tendon flexibility was measured by a continuous passive motion device (CPM). Participants were asked to sit on the edge of the table while the leg hung freely. The knee was moved through 35 degrees of motion from 90 to 125 of knee flexion by CPM. The FFK and KFEH measured the flexibility of muscle and tendon. An electronic goniometer was placed on lateral side of the thigh leg to measure the degrees

of knee flexion while passively moving the knee. The goniometer used a ruby bearing 360 degree 5000 ohm potentiometer (Figure2). The output was digitized on Bio Pac MP 150 data collection system (Bio Pac System, Goleta, CA). The force needed to move the leg at 115 degrees of knee flexion was used to assess difference in FFK and KFEH.



Figure2. Experimental setup for measuring FFK and FEK of the knee, the subject was positioned sit with their hip and knee flexed to 90 degrees.

The elastic hysteresis curve, which is the difference between the FFK and force to extend the knee (FEK), was also measured by CPM (Figure 3). Elastic energy stored during the loading the knee (flexion) is greater than that of unloading (extension); thus,

flexing the knee requires greater force than extending the knee ³². A lower elastic hysteresis value indicates more elasticity of the muscle and tendon.

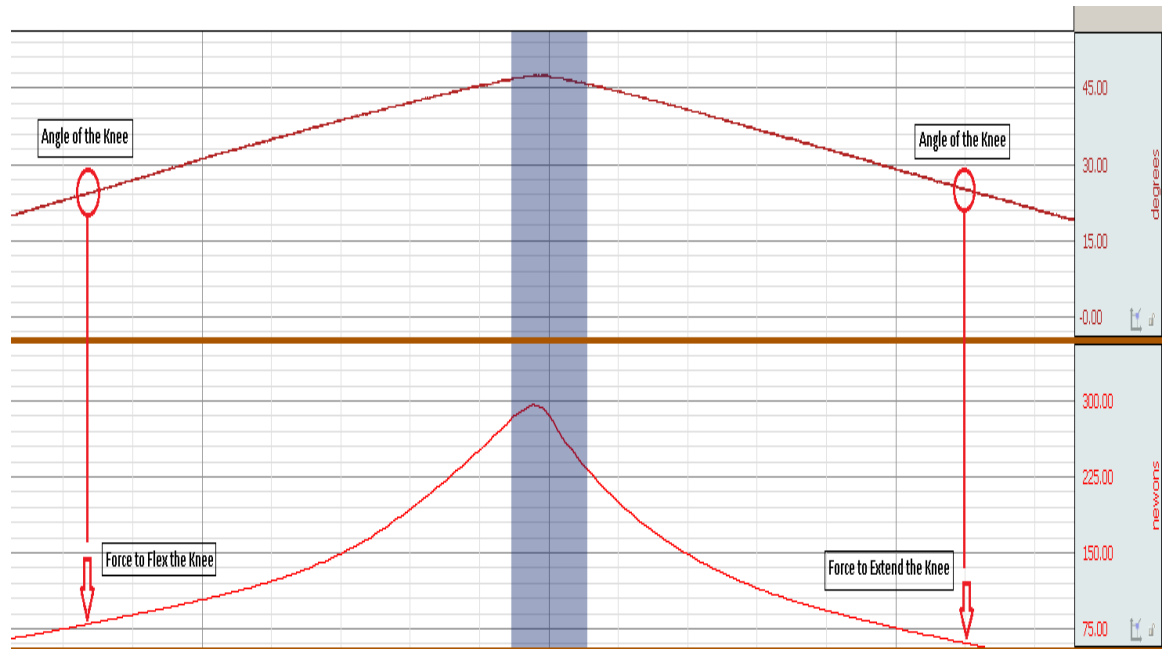


Figure3. The elastic hysteresis curve, which is the difference between the FFK and FEK, as measured by CPM.

Control of Quadriceps and Knee Temperature

Quadriceps and knee temperature were controlled with Berg's polar care therapy heat exchange unit (Berg Inc. East Carlsbad, CA). Water temperature of the bath was maintained 40°C by a water bath. A heating water bath circulator (Fisher scientific Inc. Pittsburgh, PA) was attached to the water bath to control the water bath temperature. Since water temperature dropped about 2 °C from the water bath to the therapy pad. Water temperature was kept at 40°C. This way each participant was given 38°C heat on their knee and quadriceps.

Procedures

Participants signed an informed consent form prior to their involvement. All tests were performed seven times depending on each participant's menstrual phases throughout one full menstrual cycle. Since the estradiol serum concentration in blood and body temperature fluctuates during the day, the tests were performed at the same time each experiment day.

On the first day, physical characteristics were measured including height, weight, BMI, and self-reporting menstrual cycle. Upon arriving to the examination area, participants rested comfortably in a regulated ambient temperature of 22 °C for 20 minutes to stabilize their body temperature in a neutral environment. First, 4cc of blood samples were taken to analyze estradiol serum concentration before measuring leg skin temperature. Next, ACL elasticity, FFK, and KFEH were measured. After this, two 38°C heat pads were placed on each participant's quadriceps and knee for 20 minutes to control their leg temperature, skin temperatures, ACL elasticity, FFK, and KFEH measurements were repeated.

Data Analysis

Kolmogorov-Smirnov test was conducted to examine the distribution of estradiol serum concentration, leg skin temperature, ACL elasticity, FFK, and KFEH. One-way repeated measures analysis of variance (ANOVA) was used to examine mean estradiol serum concentration, leg skin temperature, ACL elasticity, and the FFK at seven different phases in the menstrual cycle at ambient temperature. LSD pairwise comparisons test for

multiple comparisons was used to compare means of variables between any two different testing phases. A mixed factorial ANOVA was conducted to compare cycle phases with respect to the effect of estradiol on ACL elasticity, FFK, and KFEH at ambient temperature and 38°C warming. The level of significance was set at $p < 0.05$.

Results

The distribution of estradiol serum concentration, leg skin temperature, ACL elasticity, FFK, and KFEH was approximately normal ($p > 0.05$). Mean and Standard Deviation (SD) of number of days, estradiol serum concentration, and leg skin temperature in seven different phases of the menstrual cycle is listed in Table 2. The Participant's phases were determined by their self-report and estradiol serum concentration. Mean (SD) of the Anterior tibial displacement (ATD), FFK and KFEH at ambient temperature and 38°C warming in seven different testing phases during the menstrual cycle are shown in Table 3.

Table2. Summary of Outcome Variables in 7 Different Test Phases during the Menstrual Cycle (N=10)

Outcome Measure	M	EF	LF	O	EL	ML	LL	p- value
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
Number of days	2.2 (0.8)	6.3 (1.38)	10.2 (1.28)	14.2 (2.2)	17.8 (2.7)	23.4 (2.4)	28.1 (3.2)	<0.001
Estradiol, pg/ml	51.4 (9.0)	68.3 (14.3)	82.5 (17.6)	175.8 (45.9)	103.9 (26.3)	130.3 (38.8)	68.7 (17.0)	<0 001
Knee skin temperature, °C	31.8 (0.7)	31.4 (0.4)	31.7 (1.0)	31.5 (0.8)	31.9 [#] (0.8)	31.8 [#] (0.5)	31.8 [#] (0.6)	0.39
Quadriceps skin temperature, °C	31.8 (0.9)	31.5 (0.5)	31.8 (1.1)	31.6 (0.7)	32.1 ^{#*} (1.0)	31.9 [#] (0.7)	31.9 (0.98)	0.29

Abbreviations: SD, Standard deviation; M, Menstruation; EF, Early Follicular; LF, Late Follicular; O, Ovulation; EL, Early Luteal; ML, Middle Luteal; LL, Late Luteal

[^] Significant changes from menstruation

[#] Significant changes from early follicular phase

^{*} Significant changes from ovulation phase

Table3. Summary of Biomechanical Variables in 7 Different Test Phases during the Menstrual Cycle at Ambient temperature and 38°C warming (N=10)

Phase	ATD, mm			FFK, N			FKEH, N		
	Pre Heat Mean(SD)	Post Heat Mean(SD)	p-value ^a	Pre Heat Mean(SD)	Post Heat Mean(SD)	p-value ^a	Pre Heat Mean(SD)	Post Heat Mean(SD)	p-value ^a
M	5.1(1.5)*	5.5(1.6)* [^]	<0.01	126.0(35.4)* [^]	113.2(23.8)	0.04	34.4(9.1)* [^]	28.4(8.0)	<0.01
EF	5.4(1.5)* [^]	5.5(1.8)*	0.43	123.9(34.2)	112.0(31.3)	0.05	30.0(8.7)*	26.9 (10.4)	0.07
LF	5.4(1.7)*	5.6(1.7)	0.24	116.6(38.0)	110.9(34.6)	0.01	28.9 (7.4) [^]	25.0 (9.2)	0.03
O	5.9(1.7) [^]	6.0(1.8)	0.41	111.2(24.6)	109.0(25.9)	0.36	27.0(6.0)	24.2(8.7)	0.44
EL	5.4(1.6)*	5.8(1.7)	0.02	122.4(31.9)	111.5(31.2)	<0.01	30.6 (7.2)*	25.5(7.8)	0.05
ML	5.7(1.7) [^]	5.8(1.6)	0.66	115.2(18.4)	108.9(30.2)	0.2	30.0(7.0)*	25.6(5.3)	0.02
LL	5.2(1.2)*	5.4(1.0)	0.26	122.1(27.7)	112.0(27.4)	0.02	30.9 (9.1)	24.9(6.8)	<0.01
p-value ^b	0.03	0.35		0.2	0.82		0.06	0.71	

Abbreviations: SD, Standard deviation; M, Menstruation; EF, Early Follicular; LF, Late Follicular; O, Ovulation; EL Early Luteal; ML, Middle Luteal; LL, Late Luteal

[^] Significant changes from menstruation

[#] Significant changes from early follicular phase

^{*} Significant changes from ovulation phase

^a p- values for the null hypothesis that there is a no difference between pre and post heat

^b p- values for the null hypothesis that there is a no difference across 7 phases of the menstrual cycle

Estradiol Serum Concentration

As shown in Figure 4, estradiol concentrations significantly changed throughout the menstrual cycle ($p < 0.001$). The lowest estradiol concentration was found during menstruation (51.4 ± 9.0 pg/ml) and the peak of estradiol concentration was found during ovulation (175.8 ± 45.9 pg/ml, Table 2).

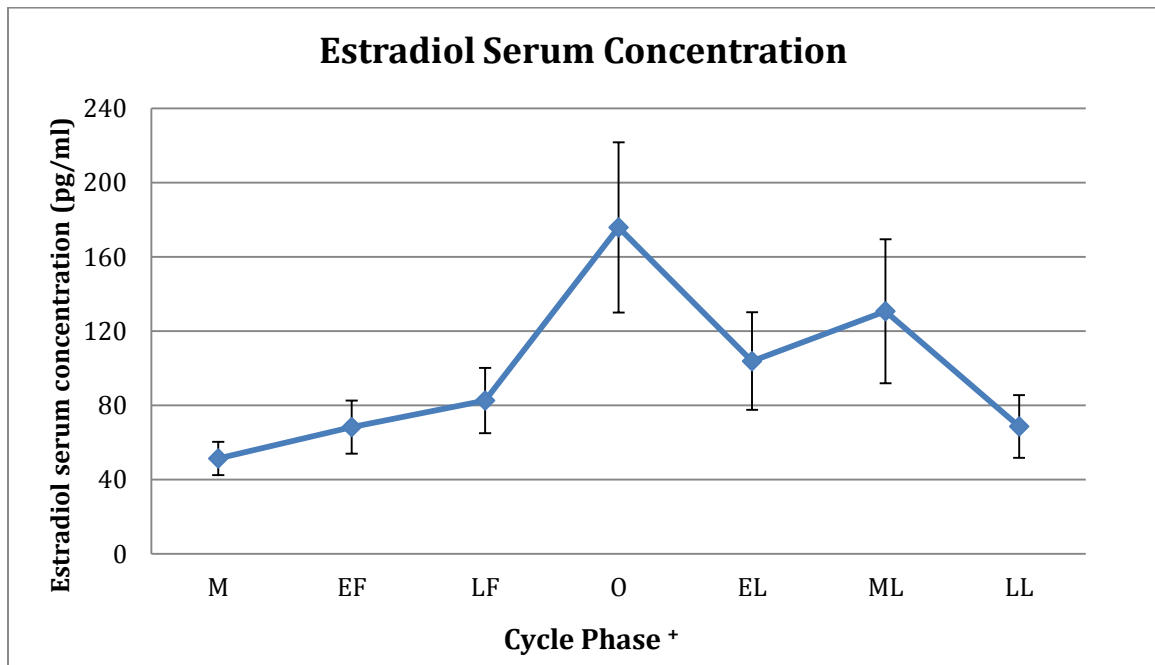


Figure 4. Mean \pm SD of estradiol serum concentration measured in 7 different phases during the menstrual cycle.

⁺Abbreviations: M, Menstruation; EF, Early Follicular; LF, Late Follicular; O, Ovulation; EL, Early Luteal; ML, Middle Luteal; LL, Late Luteal

Leg Skin Temperature

Results indicated that there were no significant differences in knee and quadriceps skin temperatures during the menstrual cycle at ambient temperature ($p > 0.05$, Table 2). However, there was a significant difference in knee skin temperature between the early

follicular and the early luteal phase (31.4 ± 0.4 °C vs. 31.9 ± 0.8 °C, $p=0.01$). The quadriceps skin temperature was also significantly higher in the early luteal phase than early follicular phases (32.3 ± 1.0 °C vs. 31.5 ± 0.5 °C, $p=0.04$). Leg skin temperature significantly increased and held at 38 °C warming when two heat pads were applied on the knee and quadriceps.

Anterior Knee Ligament Elasticity

Anterior knee ligament elasticity was a measure of ATD. The greatest ATD during ovulation occurred both at ambient temperature and after 38 °C warming (Figure 5). ATD during ovulation was significantly higher than any other phase at ambient temperature ($p=0.03$). There was also a significant difference in ATD between menstruation and ovulation as well as between early follicular and menstruation at 38 °C warming ($p<0.05$, Table 3). The greatest ATD was observed when estradiol peaks while the opposite occurred with lower estradiol concentration across the seven phases during the menstrual cycle. There were significant differences in ADT between ambient temperature and 38 °C warming during menstruation and early luteal phases (Table 3).

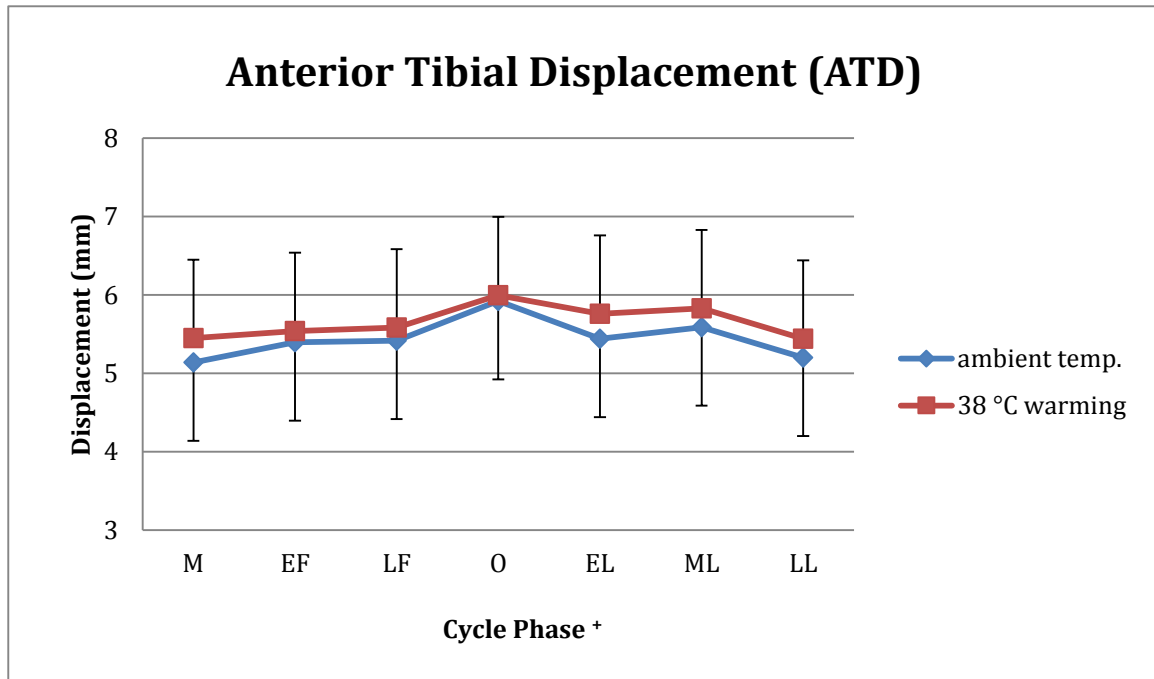


Figure 5. Mean \pm SD of the ATD measured in 7 different phases during the menstrual cycle at ambient temperature and 38°C warming.

+Abbreviations: M, Menstruation; EF, Early Follicular; LF, Late Follicular; O, Ovulation; EL, Early Luteal; ML, Middle Luteal; LL, Late Luteal

Measurement of Muscle and Tendon Flexibility

Force to Flex the Knee (FFK)

The FFK decreased 15% from menstruation to ovulation at ambient temperature ($p=0.4$, Table 3). However, no significant changes in FFK across the menstrual phases at 38 °C warming were detected ($p>0.05$, Table 3). Also, a significant difference was found in the FFK in between the ambient temperature and 38°C warming all the phases except the ovulation and the middle luteal phase in which estradiol concentrations were relatively higher than other phases (Table 3, Figure 6).

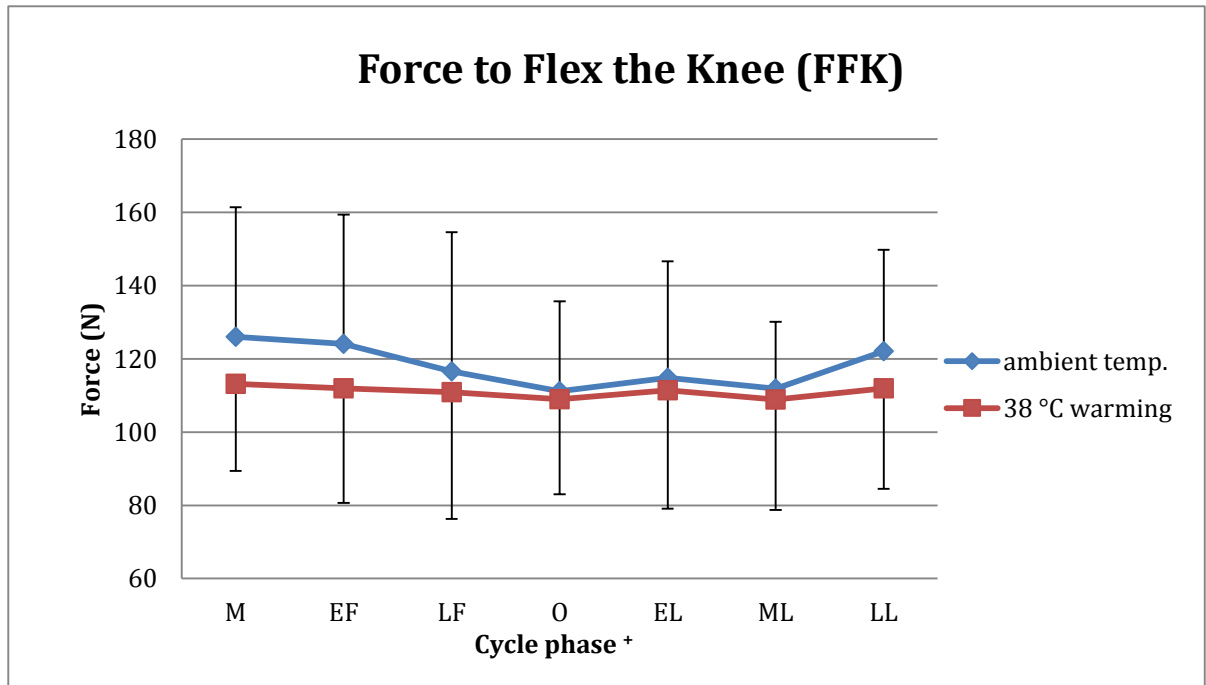


Figure 6. Mean \pm SD of the FFK measured in 7 different phases during the menstrual cycle at ambient temperature and 38°C warming.

⁺Abbreviations: M, Menstruation; EF, Early Follicular; LF, Late Follicular; O, Ovulation; EL, Early Luteal; ML, Middle Luteal; LL, Late Luteal

Knee Flexion-Extension Hysteresis (KFEH)

Figure 4 shows the KFEH curve across the seven phases. As shown in Figure 7, there were significant differences of KFEH over the phases at ambient temperature. The KFEH increased 25% from ovulation to menstruation (26.0 ± 6.0 N vs. 34.5 ± 9.1 N, $p < 0.01$), 15% from ovulation to the early luteal (26.0 ± 6.0 N vs. 30.63 ± 7.23 N, $p = 0.04$), and 13% from ovulation to the middle luteal phases (26.0 ± 6.0 N vs. 29.97 ± 6.98 N, $p = 0.03$). However, there were no significant changes in the KFEH over the phases at 38 °C warming ($p > 0.05$). The KFEH was significantly higher at 38 °C warming as compared to that at ambient temperature in all phases except the ovulation ($P < 0.05$, Figure 6).

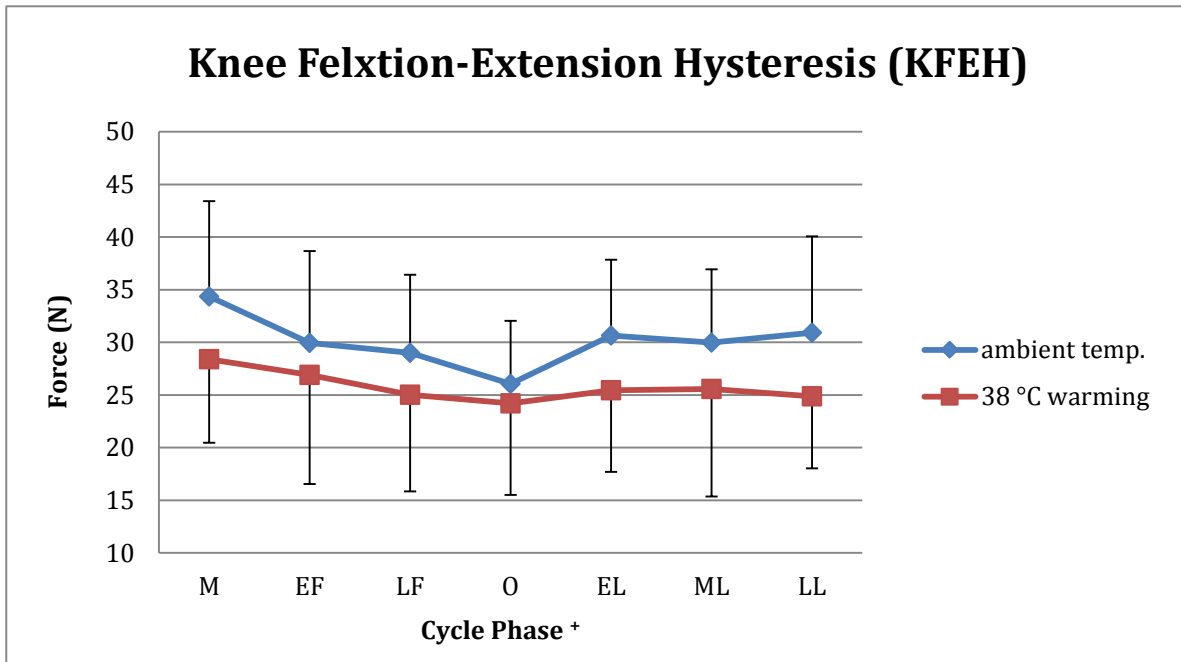


Figure 7. Mean \pm SD of the KFEH measured across the 7 different phases during the menstrual cycle at ambient temperature and 38°C warming

+Abbreviations: M, Menstruation; EF, Early Follicular; LF, Late Follicular; O, Ovulation; EL, Early Luteal; ML, Middle Luteal; LL, Late Luteal

Discussion

ACL injury is more common in women compared to men who participate in the same activities. Numerous studies have been conducted on changes of knee ligament elasticity during the menstrual cycle to examine the correlation between sex hormones, which fluctuate over the menstrual cycle, and ACL laxity^{33,34}. In spite of that, researchers are still debating on the results of these studies because the study methods and procedures were vastly different. Increased tissue temperature could be one factor that causes ligament and tendon laxness¹⁶. In this investigation, ACL elasticity, FFK, and KFEH were measured during seven different phases of the menstrual cycle at

ambient temperature and after warming the leg to 38 °C in young healthy women with a regular menstrual cycle.

The most important finding of the present study was a significantly greater ACL elasticity, less FFK, and KFEH during ovulation where estradiol concentration peaks compared to the menstruation where estradiol level is lowest during the menstrual cycle. The relationship between sex hormones, especially, estrogen and knee ligament elasticity as well as between estrogen and musculotendinous stiffness in young women with a regular menstrual cycle, has been demonstrated^{13, 35-37}. However, this study is the first to examine the relationship between estradiol serum concentration and knee elasticity and its relationship to tissue temperature in young healthy women with a regular menstrual cycle. Greater ACL elasticity was found to occur during ovulation. There are two factors that may cause this: 1) estrogen and 2) tissue temperature. Heat is generally used to increase the elasticity and flexibility of soft tissues³⁸. Ligaments must have more elasticity with relatively higher temperature since these are elastic structures⁴³. In this investigation, skin temperature was comparably lower during ovulation than menstruation and the early follicular phase. But the only important temperature is the temperature of deep tissue. There are very few papers on deep shell tissue temperature in deep tissues during the menstrual cycle²². These investigations found that muscle temperature was significantly elevated during ovulation and the well documented increase in core temperature. This is because heat has to be conserved by skin to get increased body temperature. Heat goes through the muscle and leaves the skin by radiation, convection, conduction, and evaporation. Skin needs to be cooler than the core temperature to aid in

heat loss or conserve heat ²⁵. Therefore, to keep the body cool at ovulation, the shell needs to remove more heat; hence muscle temperature increases at ovulation.

When the knee was heated to 38 °C, there was still difference in ACL elasticity at ovulation when compared to other phases of the menstrual cycle. Therefore, while the temperature change in deep tissues causes increased laxness, so must estradiol. Both are synergistic at ovulation in increasing ACL elasticity. However, previous studies examining ACL elasticity did not control tissue temperature. Therefore, if it was a cool day, the cool temperature of the shell would increase ACL stiffness and its effect would be cancelled. Clothing and ambient temperature become important to consider in studies and may be the reason the results were so varied in different studies. No previous study has even had subjects acclimatized to room temperature before measuring measurements.

This study also measured the FFK and KFEH in knee flexion and extension. These measures examined muscle and tendon flexibility. Human muscles and tendons are soft tissues composed of collagen fibers, which may be also affected by estradiol concentration and temperature as explained above. Results of this study showed a significantly lower FFK and KFEH at ovulation compared to menstruation. This finding indicates that less muscle and tendon stiffness are shown in the ovulation than menstruation stage. In addition, this result may infer that less muscle flexibility occurs with increased estradiol concentration and lower skin temperature which may lead to higher muscle tissue temperature in young healthy women. However, for the knee, the probable effect of estradiol is different. There was more energy storage at ovulation showing increased laxity. When the leg was warmed to the core temperature, this effect disappeared. This result implies that the flexibility is more affected by temperature than

estrogen; although estrogen is responsible for the temperature fluctuation. Thus, using heat would be beneficial on increasing knee flexibility; the optimal time would be in the menstruation and early follicular phase, where the less flexibility occurs.

Knee joint stiffness is a major risk factor for non-contact ACL injuries ³⁹. It is so common for athletes to do warm-up stretching or use heat packs to decrease stiffness before exercise for injury prevention ⁴⁰. Women have greater knee stiffness due to estradiol and temperature effects during menstruation. However, this investigation did not examine how knee stiffness is related to injuries. Laxity may cause injuries or may not. Myklebust and colleague and Slatuterbeck and colleague reported that there are more ACL injuries during menstruation where less ACL elasticity was found ^{41,42}. On the other hand, Wojtys and colleague claimed that more ACL injuries occur during ovulation where greater ACL elasticity was found ⁴³. However, these studies did not control environment or tissue temperature. Temperature may be another factor that affects ACL injuries but previous studies did not examine ACL elasticity under the same condition such as controlling the effects of clothing or environment temperature. If women play sports during ovulation in a hot environment, both temperature and estrogen causes excessive knee laxity which can leads to more knee injuries. However, if women play sports during menstruation in cold environments, both temperature and estrogen cause excessive knee stiffness that can lead to more knee injuries. Thus, in further investigation, both estrogen and temperature should be considered as key risk factors that can cause ACL injuries during menstrual cycle.

Conclusions

ACL elasticity, FFK, and KFEH were affected not only by estradiol serum concentration but tissue temperature during the menstrual cycle. However, estradiol serum concentration had more impact on ACL laxity while tissue temperature had more impact on FFK and KFEH at 38 °C warming.

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CHAPTER THREE

DIFFERNECE IN ANTERIOR CRUCIATE LIGAMENT AND FORCE
FOR KNEE FLEXION IN WOMEN: ORAL CONTRACEPTIVE USERS
VERSUS NON-ORAL CONTRACEPTIVE USERS

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Abstract

Introduction: Eighty two percent of sexually active women aged 15-44 have used oral contraceptive pills (OCP) in the United States. Hormone changes during the menstrual cycle (MC) are believed to have an impact on anterior cruciate ligament (ACL) laxity due to estrogen. OCP may have a potent impact on tendon and ligament synthesis, structure, and biomechanical properties. Temperature has also been known to have an effect on tissue elasticity. Therefore, the purpose of this study was to investigate the differences in ACL elasticity, force to flex the knee (FFK), and knee flexion-extension hysteresis (KFEH) between OCP users and non-OCP users and its relationship to tissue temperature. **Methods:** Nineteen non-athletic young healthy females were divided into two groups; OCP users and non-OCP users. Blood for estradiol serum concentration (E2) was taken before beginning the tests. ACL elasticity, FFK, and KFEH were assessed both at ambient temperature (22 °C) and after 38 °C warming of the leg to stabilize the tissue temperature. Assessments were performed four times during the MC. **Results:** Throughout the MC, ACL elasticity, FFK, and KFEH fluctuated in non-OCP users but not in OCP users. At ambient temperature, ACL elasticity was significantly lower and FFK and KFEH were significantly higher in OCP users than non-OCP users ($p < 0.05$). But, no significant differences in FFK and KFEH between the two groups were found after warming to 38 °C.

Conclusions: The effect of MC on ACL is both hormonal and temperature related while the knee is temperature related.

Key words: Oral contraceptive pill, Knee elasticity, Muscle and tendon flexibility, and Tissue temperature

Introduction

Women have a greater incidence of Anterior Cruciate Ligament (ACL) injury than men ¹⁻⁴. This gender difference is commonly assumed to be due to anatomical, neuromuscular, movement patterns, in women as well as hormonal influences ^{4,5}. Regarding hormonal factors, numerous studies have found a correlation between reproductive hormones and ACL injury risk ¹⁻⁴. The role of estrogen in possibly causing ACL injuries has recently been studied at the cellular level and the presence of 17- beta estradiol receptors in human ACL and muscle has been investigated ⁶⁻⁹. Previous studies have examined the changes in the levels of estrogen in blood as related to ACL laxity during the menstrual cycle and a correlation between ACL laxity and estrogen was detected ⁶⁻⁹.

The number of women using OCP has constantly increased. Eighty two percent of sexually experienced women aged 15-44 have used the oral contraceptive pill (OCP) in the United States and 14% of women have used OCP for non-contraceptive purposes such as cycle regulation, treatment of dysmenorrhea and amenorrhea ¹⁰. OCP consists of combined estrogen and progesterone as synthetic steroids and it plays a role in suppressing pituitary FSH and LH production and preventing ovulation from occurring ^{10,11}. This exogenous source of synthetic forms of the hormone prevents ovulation by maintaining more consistent hormone levels ¹². The 17- beta estradiol receptors on human connective tissue is well known and therefore, the OCP may have an impact on tendon and ligament synthesis, structure, and biomechanical properties. Also, Martineau and colleagues have shown that OCP users have less ACL laxity than non-OCP users ¹³.

It is not surprising that many people do a warm-up exercise before sports activities to increase muscle and tendon extensibility to prevent injuries ¹⁴. Temperature is another factor that influences tendon and ligament of collagen synthesis. In healthy young adults, core temperature changes approximately 0.5°C throughout the day. In women with a regular menstrual cycle, their core temperature fluctuates not only throughout the day but also during the menstrual cycle ¹⁵. It peaks during the luteal phase while in the follicular and at ovulation it is relatively low ¹⁵⁻¹⁷. The elevated thermoregulatory set point during the luteal phase is the most accepted explanation for the observed change in core temperature ^{18,19}. Since, soft tissue extensibility changes with different levels of tissue temperature; the menstrual cycle has dramatic effects on tissue extensibility. However, because hormones do not change in OCP users, their core temperature may change little during the menstrual cycle. In addition, Rogers and Baker have shown that OCP users have higher core temperature compared to non-OCP users due to exogenous forms of progesterone in OCP's ²⁰.

Thus, the effect of estrogen on ACL elasticity and muscle and tendon flexibility is probably a complex interaction between the hormone and tissue temperature. Therefore, the purpose of this study was to investigate the differences in ACL elasticity, force to flex the knee (FFK) and knee flexion-extension hysteresis (KFEH) between OCP users and non-OCP users and its relationship to tissue temperature during the menstrual cycle.

Materials and Methods

Subjects

Twenty non-athletic young healthy females volunteered to participate in this

study. Subjects were divided into two groups: subjects with a regular menstrual cycle and those who are taking OCP according to their self-reporting. Subjects were 18 to 30 years of age with a Body Mass Index (BMI) between 15 and 30 who had a regular menstrual cycle or currently using low-dose OCP (<50 μ g-ethinyl estradiol) for at least six months. Subjects had no history of pregnancy, neuropathy, myopathy or knee injury, and were not taking any medication would affect sex hormones other than OCPs. All procedures and protocols were approved by Institutional Review Board of Loma Linda University and all subjects signed an informed consent.

Procedures

Subjects performed a total of four different tests throughout one full menstrual cycle. Subjects were asked to report the days of their cycle at the beginning of the study. OCP users were screened by questionnaire, which included questions regarding brand name, type, and the length of taking OCP. At the beginning of the study, subjects signed an informed consent before they participated in the study. Since the estradiol serum concentration in blood and body temperature fluctuates during the day, the various tests were performed at the same time each day.

On the first day, before beginning the experiment, height, weight, BMI, and self-reported menstrual cycle were measured. Upon arriving at the laboratory, subjects rested comfortably in a regulated temperature room at 22 °C for 20 minutes to stabilize their body temperature in a neutral environment. Blood samples (4cc) were taken to analyze estradiol serum concentration before measuring leg skin temperature. Next, ACL elasticity, FFK, and KFEH were measured. After this, two 38°C heat pads were placed on

the subject's quadriceps and knee for 20 minutes to stabilize their leg temperature. Skin temperature, ACL elasticity, FFK, and KFEH measurements were repeated.

Measurements

A trained researcher performed all measurements. The outcome variables assessed were ACL elasticity, force to flex the knee (FFK), knee flexion-extension hysteresis (KFEH), leg skin temperature, and estradiol serum concentration.

Estradiol (E2) Serum Concentration

Estradiol serum concentration was analyzed using the TOHSO E2 ST AIA assay (Fisher Healthcare, Inc., Houston, TX, USA). Four cc of blood was obtained from an antecubital vein with a 23-gauge needle and centrifuged for separating serum from plasma. The centrifuge rotated at 3000 rpm for 10 minutes (Beckman Coulter Inc., Brea, CA). Once serum was separated, it was stored at -80°C until all samples of serum were collected for the hormone analysis.

Leg Skin Temperature

Leg skin temperature was measured using the forward-looking infrared 660 IR Camera (FLIR systems, Danderyd, Sweden). This device has been validated to deliver accurate temperature measurements²¹. To acquire best images, the readings were at a perpendicular angle and at a distance of 1 meter away from the skin. Measurements were performed in the room illuminated with light emitting diode (LED) lights to minimize any infrared interference²¹. Baseline leg skin temperature was taken after stabilizing leg

temperature at a neutral environment for 20 minutes and after applying 38 °C heat immediately when the heat pads were removed.

Anterior Knee Ligament Elasticity

KT-2000 knee arthrometer (MED metric Corp, San Diego, California) was used to measure knee laxity of the anterior cruciate ligament. The measurement of the anterior knee ligament laxness was performed by one experienced examiner for consistency. Subjects were asked to lay supine in a predetermined position. A strain gauge was used to measure the force necessary to generate an anterior glide of the proximal end of the tibia on the femoral condyles. This generated a force curve of elasticity in the anterior cruciate ligaments. The process was accomplished by supporting both limbs with a firm, comfortable platform placed proximal to the popliteal space. This helped keep the subject's knee flexion angle constant for the duration of the test. Along with this device, a foot support accessory supplied with the arthrometer, positioned the feet symmetrically allowing the leg position to be optimal for the test while reducing external rotation. For the most comfortable position during the flexion angle test, knee flexion angle was held at 25 degrees (Figure 8). A thigh strap controlled hip external rotation while offering support in order to help relax the subject. Force used for the experiment was applied at 30 lbs. (133N) of force. The force displacement data was shown on an X-Y plotter. A venire caliper was used to measure anterior tibial displacement (ATD) graph.

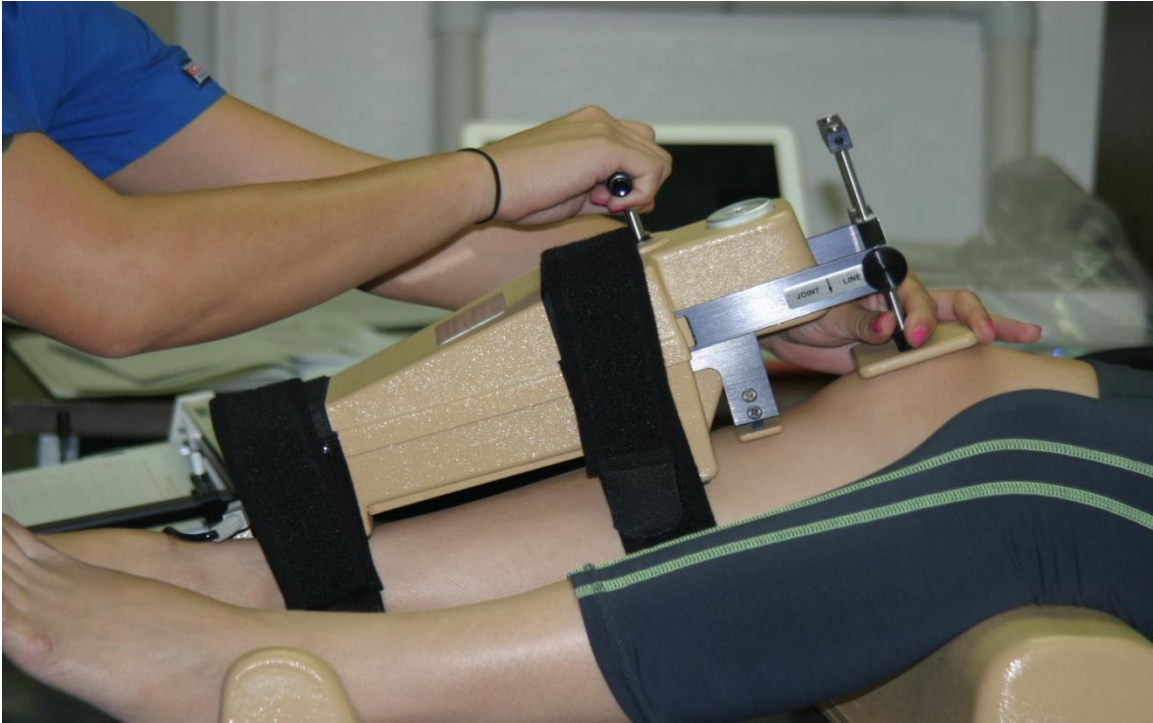


Figure8. Experimental setup for measuring ACL elasticity, the subject was positioned supine with their knee flexed to 25 degrees.

Muscle and Tendon Flexibility

The angle of the force measured to extend the knee was from 90 to 125 degrees. Elevated off the floor and in a seated position, the subject's leg hung at an initial angle of 90 degrees. To passively move the knee through 35 degrees of flexion and extension, an ankle strap was connected to a linear actuator (Figure 9). The measurement was recorded at a rate of movement of 45 degrees in 7.5 seconds. The force in flexion and extension was measured with a strain gage bridge amplified with a gain of 500(DAC 100, Bio Pac Systems, Goleta California). The output was then digitized at 2000 hertz with a resolution of 24 bits on an MP150 Bio Pac data acquisition system (Bio Pac Systems, Goleta California). The knee goniometer, using a ruby bearing 360 degree 5000 ohm potentiometer, measured the angle of the knee to calculate the force needed per degree



Figure9. Experimental setup for measuring FFK and FEK of the knee, the subject was positioned sitting with their hip and knee flexed to 90 degrees.

The difference between the FFK and force to extend the knee (FEK), called KFEH, was then calculated. Potential elastic energy stored during the loading (flexion) is greater than that of unloading (extension); thus, flexing the knee required greater force than extending the knee²². A lower elastic hysteresis value means more elasticity of the muscle and tendon (Figure 10).

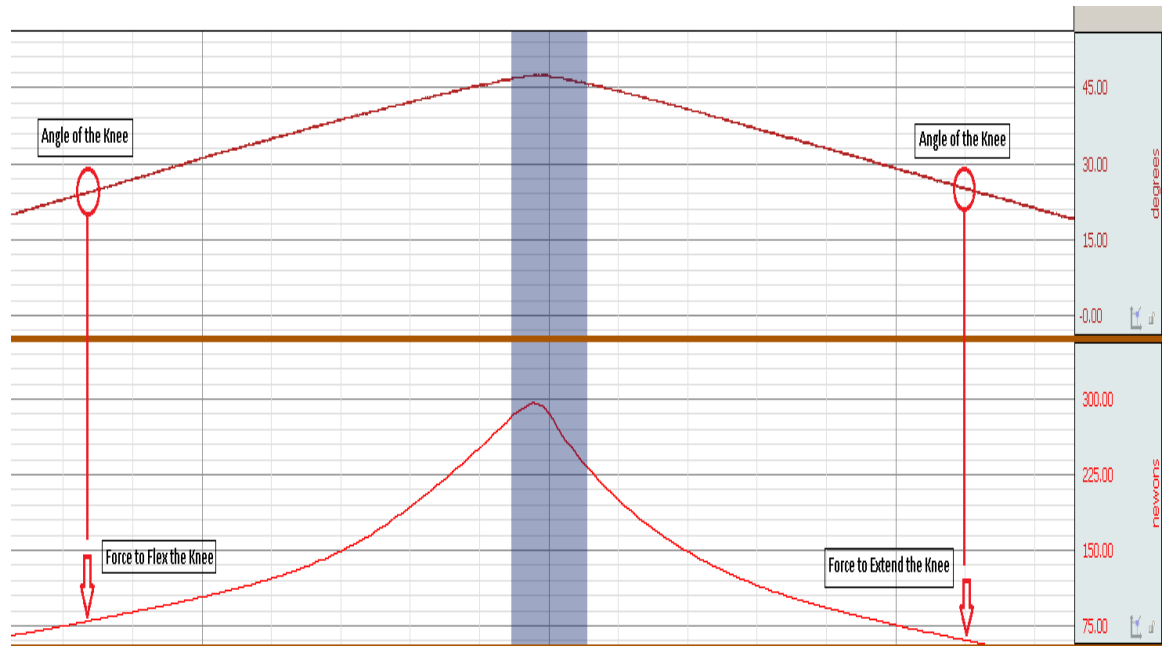


Figure10. The elastic hysteresis curve, which is the difference between the FFK and FEK, as measured by CPM.

Control of Quadriceps and Knee temperature

Quadriceps and knee temperature were controlled with a Berg's polar care therapy heat exchange unit (Berg Inc. East Carlsbad, CA) maintained at 40°C by a water bath. On the water bath, a heating water bath circulator (Fisher scientific Inc. Pittsburgh, PA) was attached to control temperature. Since water temperature dropped about 2 °C throughout the unit, water temperature was kept at 40°C to give 38°C heat on subject's knee and quadriceps.

Data Analysis

Data was summarized using means and standard deviation (SD). An independent t-test was conducted to compare general characteristics between OCP users and non-OCP users. One-way repeated measures analysis of variance (ANOVA) was used to examine changes in mean estradiol serum concentration, leg skin temperature, ACL elasticity, FFK, and KFEH at four different phases of the menstrual cycle. For significant findings, LSD pairwise comparisons test for multiple comparisons was used to compare means of variables between any two different phases. For all data collected over the phases within each test, a mixed factorial ANOVA was conducted to compare cycle phases with respect to the effect of estradiol concentration on ligament elasticity, FFK, and KFEH at room temperature and 38°C in each group. The level of significance was set at $p < 0.05$.

Results

Ten subjects were enrolled in each group. However, one subject from the OCP group was excluded because the estradiol level was similar to that of a normal menstrual cycle subject. There were no significant differences in mean age, weight (kg), height (cm), BMI (kg/m^2), and length of cycle ($p > 0.05$, Table 4). The subject's menstrual phase was determined by their self-reporting and estradiol serum concentrations. Mean (SD) of the ADT, FFK, and KFEH at ambient temperature and 38°C warming in four different phases during the menstrual cycle is shown in Table 6,5,7.

Table 4. Mean (SD) of general characteristics (N= 19)

	Non-OCP users	OCP users	p –value*
Age (years)	24.7 (2.0)	25.1(3.3)	0.74
Height (cm)	164.6 (3.4)	165.7(5. 1)	0.59
Weight (kg)	57.1 (5.0)	59.4(7.0)	0.35
BMI (kg/m ²)	21.0 (1.3)	21.6(4.1)	0.48
Cycle length (d)	29.5 (3.2)	28.6(1.1)	0.38

Abbreviation: SD, Standard deviation; OCP, Oral contraceptive pills

* Independent t-test

Table 5. Summary of Outcome Variables in 4 Different Test Phases during the Menstrual Cycle (N=19)

Outcome Measure	M Mean (SD)	F Mean (SD)	O Mean (SD)	L Mean (SD)	P-value
Number of days					
Non-OCP users	2.2(0.8)	10.2(1.3)	14.2(2.2)	23.4(2.4)	<0.001
OCP users	2.1(0.5)	9.1(0.9)	13.8(1.2)	22.9(1.6)	<0.001
Estradiol, pg/ml					
Non-OCP users	51.4 (8.9)	82.5 (17.6)	175.8 (45.9)	130.3 (38.8)	<0.001
OCP users	29.6 (5.3)	34.8 (11.1)	31.3 (9.7)	32.7 (9.9)	0.42
Knee skin temperature, °C					
Non-OCP users	31.8(0.7)	31.7(1.0)	31.5(0.8)	31.8(0.5) [#]	0.43
OCP users	31.8(0.3)	31.8(0.3)	31.7(0.3)	31.9(0.5)	0.74
Quadriceps skin temperature, °C					
Non-OCP users	31.8(0.9)	31.8(1.1)	31.6(0.7)	31.9(0.7) [#]	0.52
OCP users	31.9(0.7)	31.8(0.8)	32.0(1.3)	31.9(0.9)	0.88

Abbreviations: SD, Standard deviation; M, Menstruation; F, Follicular; O, Ovulation; L, Luteal; OCP, Oral contraceptive pills

[#] Significant changes from ovulation

Estradiol (E2) Serum Concentration

Mean and SD of estradiol serum concentrations in four different phases during the menstrual cycle is displayed in Table 5. There were significant changes in estradiol serum concentration found across the menstrual cycle in the OCP user group ($P < 0.001$), but not in OPC group (Figure 11, Table 2). The lowest estradiol concentration was found during menstruation (51.4 ± 9.0 pg/ml) and the highest estradiol concentration was found during ovulation (175.8 ± 45.9 pg/ml) in non-OCP user group (Table 5).

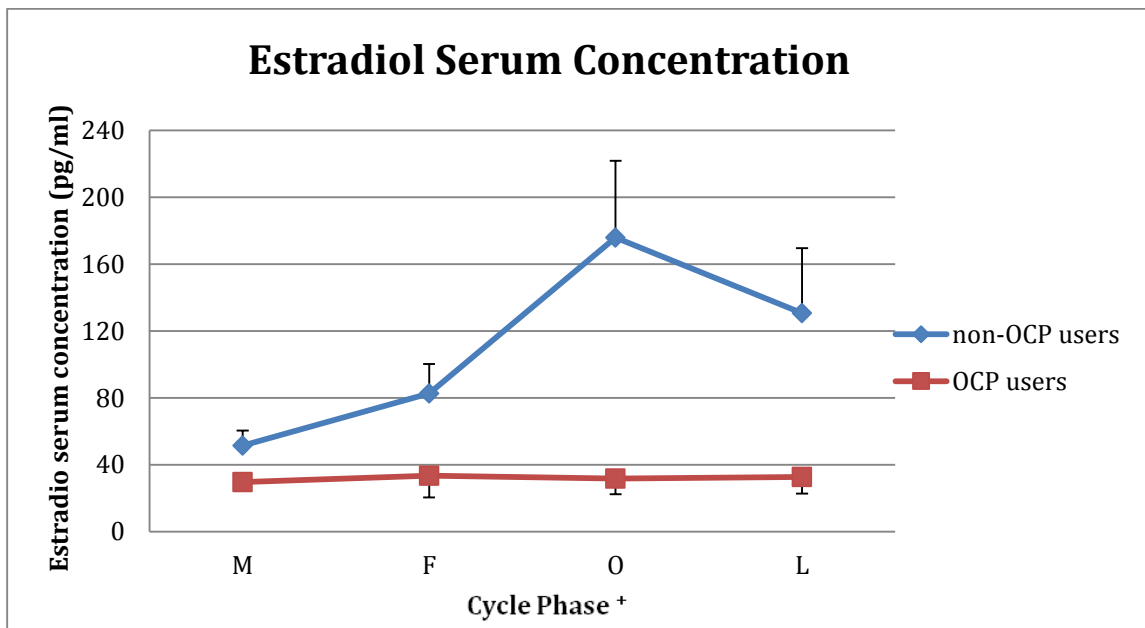


Figure 11. Mean \pm SD of estradiol concentration measured in 4 different phases of the menstrual cycle in non-OCP users and OCP users.

Abbreviations: M, Menstruation; F, Follicular; O, Ovulation; L, Luteal; OCP, Oral contraceptive pills

Leg Skin Temperature

Both knee and quadriceps skin temperatures were significantly lower during ovulation compared to luteal phase in non-OCP users (31.5 ± 0.8 vs. 31.8 ± 0.5 , $p = 0.04$,

31.6±0.7 vs. 31.9±0.7, p=0.04, Table 5). However, there were no significant changes during the menstrual cycle in non-OCP users (p>0.05, Table 5). Also, no significant differences in temperature were detected between OCP users and non-OCP users in all phases (p>0.05). When two heat pads were applied on the knee and quadriceps skin temperature significantly increased and held at 38 °C warming

Anterior Knee Ligament Elasticity

Anterior knee ligament elasticity was measured by ATD. The results of the ATD changes in four different phases during the menstrual cycle at ambient temperature and after 38°C warming in non-OCP and OCP users are shown in Figure 12. When comparing ATD in four different phases of the menstrual cycle, ATD was significantly lower in OCP users compared to non-OCP at both temperatures (Figure 12). The greatest ATD was found at ovulation and the least ATD was found at menstruation in non-OCP users (Table 6). At ambient temperature, the ATD decreased 10% from ovulation to menstruation and the ATD differences between ovulation and menstruation were not significant in OCP users (p>0.05, Table 6). There were significant differences in ATD between OCP users and non-OCP users (p>0.05) after applying 38 °C warming, There was a significant difference in ATD between ovulation and menstruation in OCP users (6.0 ± 1.8 mm vs. 5.6± 1.6 mm, p=0.01, Table 6, Figure 12).

Table 6. Mean (SD) of the ATD measured in 4 different phases of the menstrual cycle at ambient temperature and after 38°C warming in non-OCP users and OCP users (N=19).

Anterior Tibial Displacement, mm						
Group	Non-OCP users			OCP users		
Phase	Pre Heat Mean (SD)	Post Heat Mean (SD)	p-value ^a	Pre Heat Mean (SD)	Post Heat Mean (SD)	p- value ^a
M	5.1(1.5)	5.5(1.6)	<0.01	4.4(0.8)	4.5(1.0)	0.51
F	5.4(1.7)	5.6(1.7)	0.24	4.3(0.9)	4.5(0.9)	0.16
O	5.9(1.7) ^{*^}	6.0(1.8) [*]	0.41	4.4(0.9)	4.6(0.9)	0.08
L	5.7(1.7) [*]	5.8(1.6)	0.67	4.4(0.6)	4.6(0.8)	0.32
p-value ^b	<0.01	0.44		0.89	0.97	

Abbreviations: SD, Standard deviation; M, Menstruation; F, Follicular; O, Ovulation; L, Luteal; OCP, Oral contraceptive pills

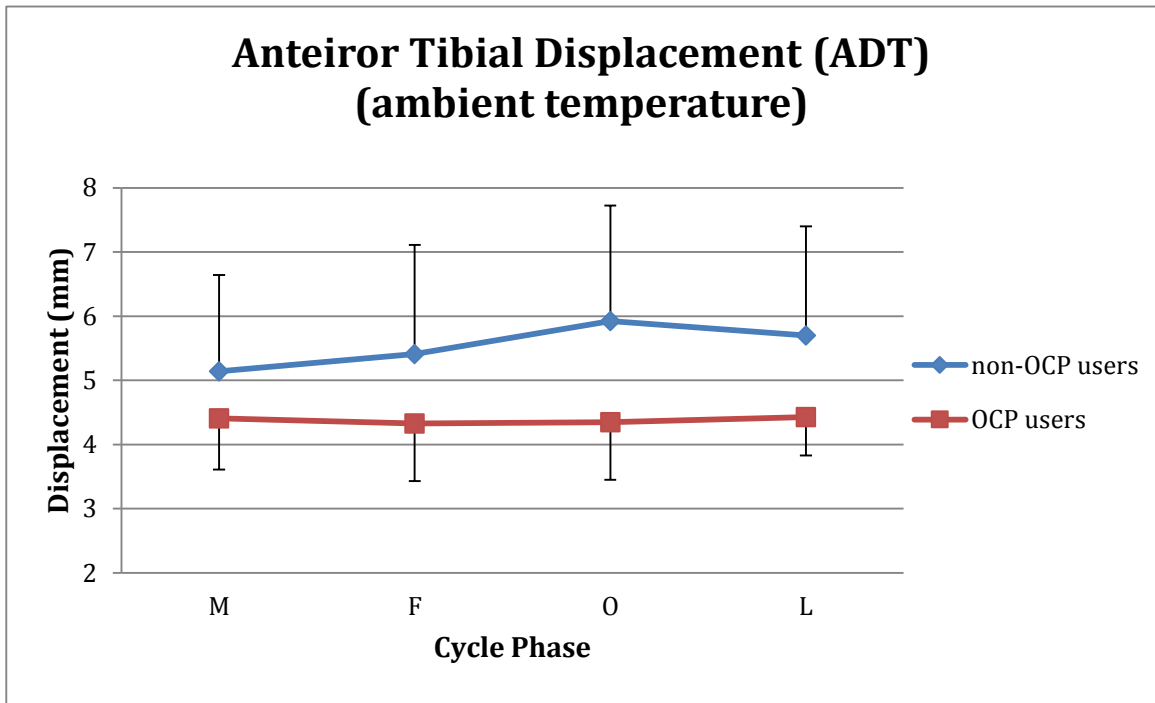
^{*} Significant changes from menstruation

[^] Significant changes from follicular phase

^a p- values for the null hypothesis that there is a no difference between pre and post heat

^b p- values for the null hypothesis that there is a no difference across 4 phases of the menstrual cycle

(A)



(B)

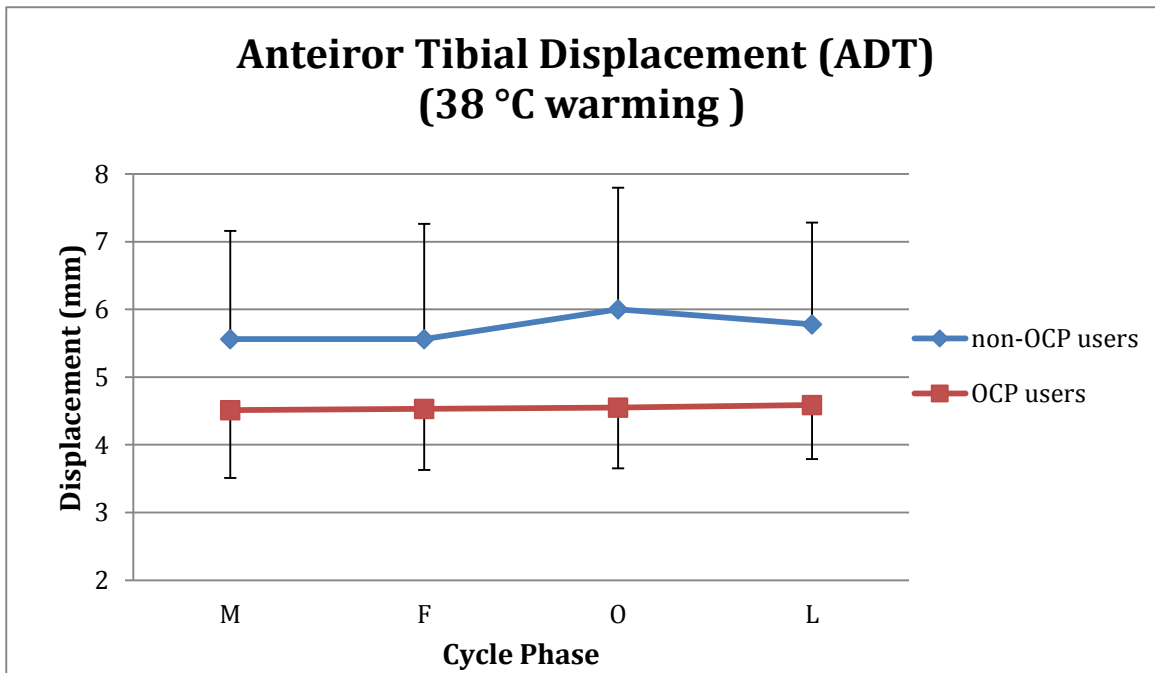


Figure12. Mean \pm SD of the ATD measured in 4 different phases of the menstrual cycle at ambient temperature and after 38 °C warming in non-OCP users and OCP users.

Abbreviations: M, Menstruation; F, Follicular; O, Ovulation; L, Luteal; OCP, Oral contraceptive pills

Measurement of Muscle and Tendon Flexibility

Force to Flex the Knee (FFK)

The FFK decreased 15% from menstruation to ovulation at ambient temperature in non-OCP users ($p=0.04$, Table 7). However, there were no significant changes in the FFK across the phases at 38 °C warming ($P>0.05$). Also, a significant difference was found in the FFK between the ambient temperature and 38°C warming in all phases except ovulation in which estradiol concentrations were relatively higher than in other phases (Table 7). Both at ambient temperature and 38 °C warming, the FFK of OCP users were greater than non-OCP users in all phases (Table 7, Figure 13). There was no significant difference in the FFK between pre and post heat in OCP users. On average, 9.5% decrease was shown in OCP users versus 5.4% decrease in non-OCP users (Table 7).

Table 7. Mean (SD) of the FFK measured in 4 different phases of the menstrual cycle at ambient temperature and after 38°C warming in non-OCP users and OCP users (N=19).

Force to Flex the Knee, N						
Group	Non-OCP users			OCP users		
Phase	Pre Heat Mean (SD)	Post Heat Mean (SD)	p-value ^a	Pre Heat Mean (SD)	Post Heat Mean (SD)	p-value ^a
M	126.0(35.4)	113.2(23.8)	0.04	132.5(26.1)	118.0(25.6)	<0.01
F	116.6(38.0)	110.9(34.6)	0.01	128.2(28.1)	117.1(23.5)	0.04
O	111.2(24.6)*	109.0(25.9)	0.36	130.9(30.0)	117.6(24.5)	<0.01
L	115.2(18.4)	108.9(30.2)	0.21	129.8(32.3)	119.2(24.9)	0.02
p-value ^b	0.24	0.89		0.92	0.99	

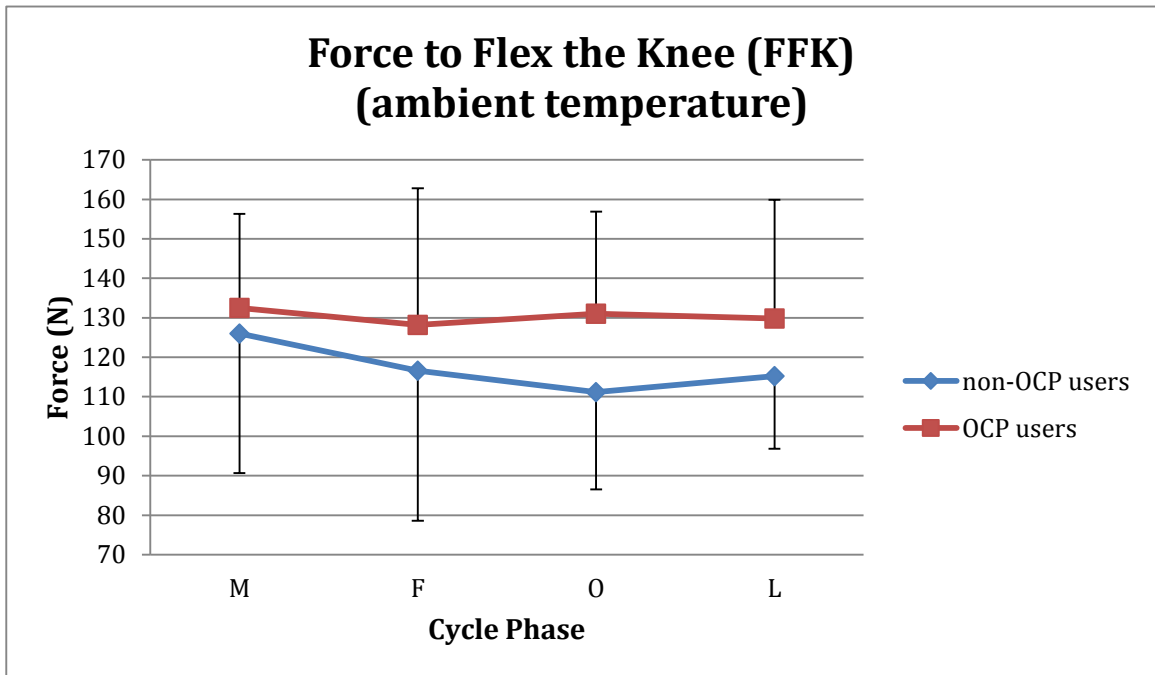
Abbreviations: SD, Standard deviation; M, Menstruation; F, Follicular; O, Ovulation; L, Luteal; OCP, Oral contraceptive pills

* Significant changes from menstruation

^a p- values for the null hypothesis that there is a no difference between pre and post heat

^b p- values for the null hypothesis that there is a no difference across 4 phases of the menstrual cycle

(A)



(B)

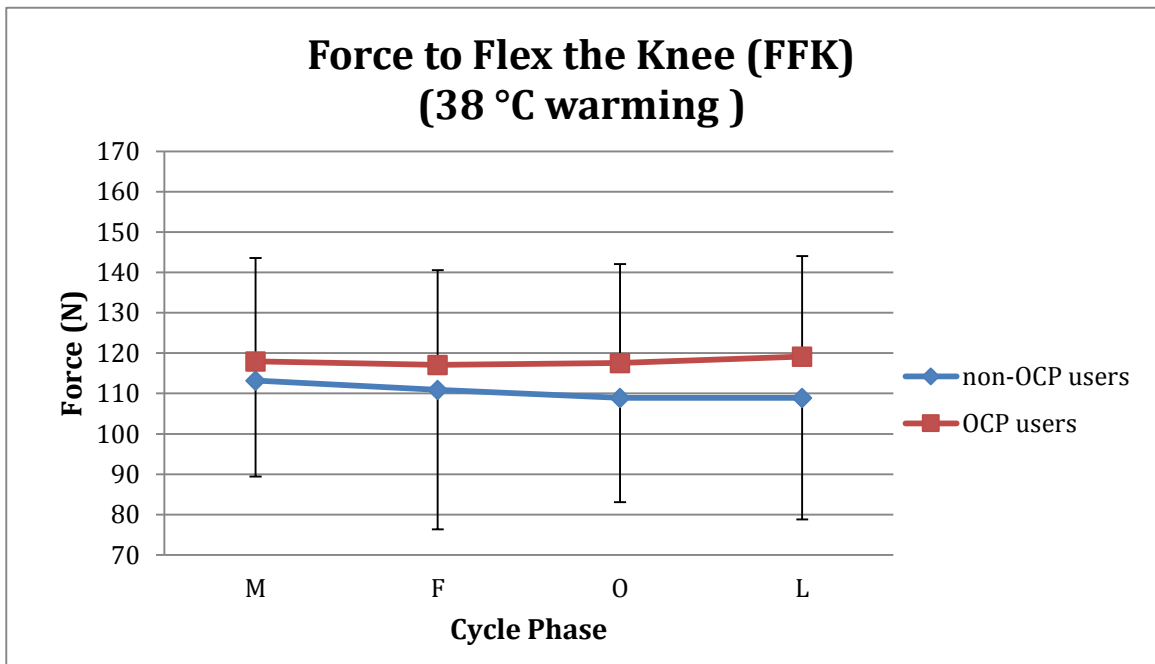


Figure13. Mean \pm SD of the FFK measured in 4 different phases of the menstrual cycle at ambient temperature and after 38 °C warming in non-OCP users and OCP users. Abbreviations: M, Menstruation; F, Follicular; O, Ovulation; L, Luteal; OCP, Oral contraceptive pills

Knee Flexion-Extension Hysteresis (KFEH)

At ambient temperature, the KFEH was significantly lower in OCP users than non-OCP users. During ovulation, the KFEH was significantly lower in non-OCP users than OCP users (27.0 ± 6.0 N vs. 34.9 ± 11.2 N, $p < 0.01$, Table 8). Across phases, there were no significant changes in the KFEH in non-OCP users ($p = 0.01$) but not in OCP users ($p = 0.72$). The KFEH increased 24% from ovulation to menstruation at ambient temperature in non-OCP users. In addition, there were no significant changes in the KFEH across the menstrual cycle at 38 °C warming in OCP users ($p > 0.05$). In both groups, the KFEH was significantly lower at 38 °C warming when compared to ambient temperature in all phases except ovulation. After 38 °C warming The KFEH decreased 21.1% in OCP users and 14.0% in non-OCP users (Figure 14, Table 8).

Table 8. Mean (SD) of the KFEH measured in 4 different phases of the menstrual cycle at ambient temperature and after 38°C warming in non-OCP users and OCP users (N=19).

Knee Flexion-Extension Hysteresis, N						
Group	Non-OCP users			OCP users		
Phase	Pre Heat Mean (SD)	Post Heat Mean (SD)	p-value ^a	Pre Heat Mean (SD)	Post Heat Mean (SD)	p- value ^a
M	34.4(9.1)	28.4(8.0)	<0.01	38.0(14.9)	29.3(8.0)	0.02
F	28.9 (7.4)*	25.0 (9.2)	0.03	35.3(14.0)	28.2(11.0)	0.04
O	27.0(6.0)*	24.2(8.7)	0.44	34.9(11.2)	28.1(11.2)	0.07
L	30.0(7.0)#	25.6(5.3)	0.02	36.0(11.1)	28.1(6.9)	0.02
p-value ^b	0.01	0.45		0.72	0.95	

Abbreviations: SD, Standard deviation; M, Menstruation; F, Follicular; O, Ovulation; L, Luteal; OCP, Oral contraceptive pills

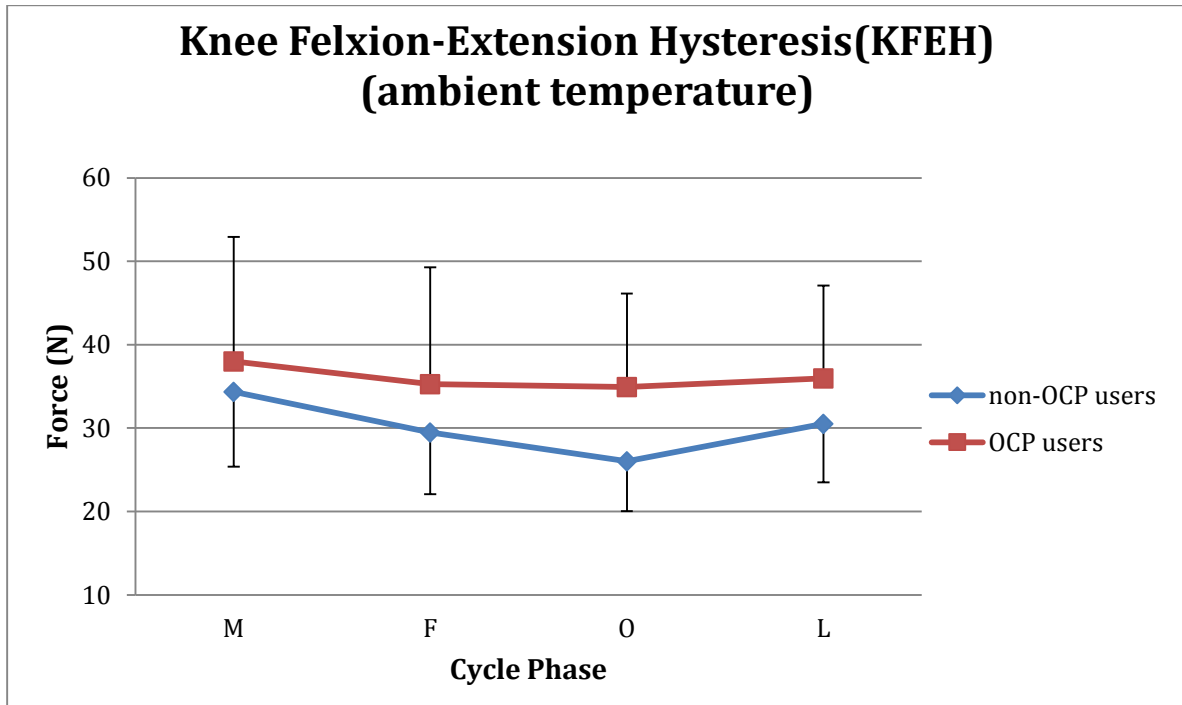
* Significant changes from menstruation

Significant changes from ovulation

^a p- values for the null hypothesis that there is a no difference between pre and post heat

^b p- values for the null hypothesis that there is a no difference across 4 phases of the menstrual cycle

(A)



(B)

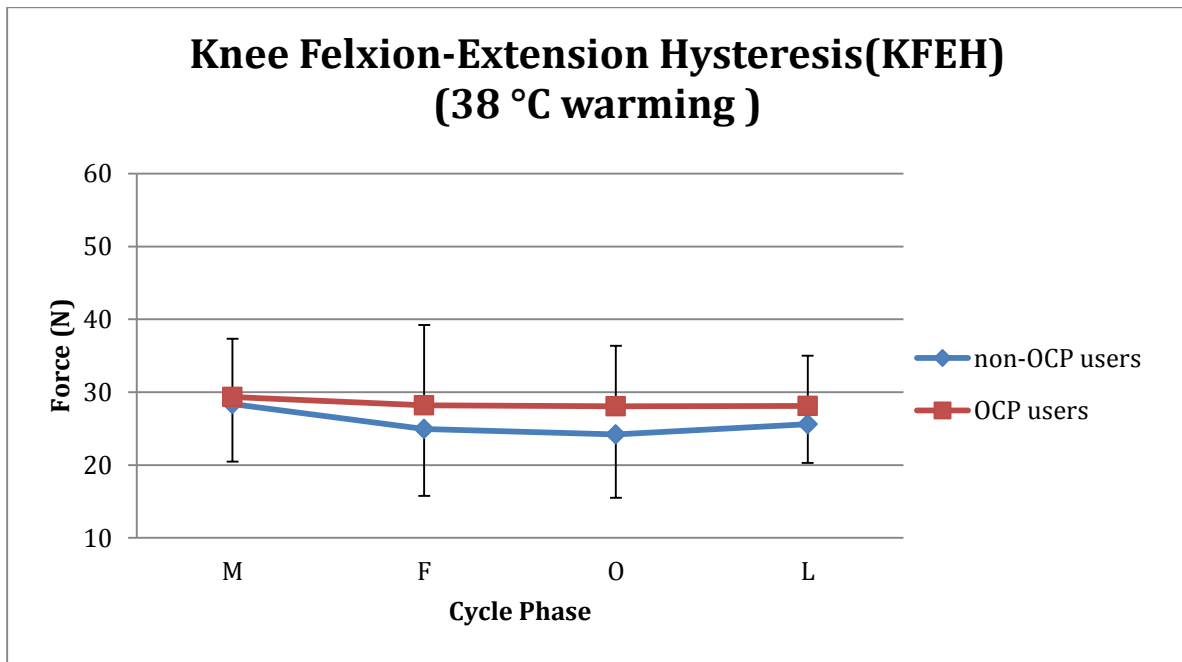


Figure 14. Mean \pm SD of the KFEH measured in 4 different phases of the menstrual cycle at ambient temperature and after 38 °C warming in non-OCP users and OCP users. Abbreviations: M, Menstruation; F, Follicular; O, Ovulation; L, Luteal; OCP, Oral contraceptive pills

Discussion

ACL elasticity and muscle and tendon flexibility are major risk factors for knee injuries during sports^{23,24}. Women have fluctuating estrogen during the menstrual cycle, and are more susceptible to have injuries²⁵. In previous studies, women had greater knee laxity during ovulation at a time when estrogen level peaks²⁶⁻²⁸. The finding supports these results that estrogen receptors are found in connective tissue and thus, it changes its mechanical properties of tendons and ligaments^{7,28}. However, OCP makes women's hormone level more consistent during the menstrual cycle. If it is true that female reproductive hormones cause connective tissue laxness, female OCP users should show more stable tissue extensibility. Therefore, it is of no surprise that the few studies addressing the effects of OCP on knee injury prevention show little change in ligament elasticity during the menstrual cycle^{29,30}. Researchers, however, have reached different conclusions on the relationship between OCP and knee laxity^{13,30}.

In the present investigation, ACL elasticity, FFK, and KFEH were measured during four different phases of the menstrual cycle with subjects exposed to ambient temperatures. In addition, to stabilize temperature fluctuation from the day, the experiments were repeated by warming the leg to 38°C in both young healthy OCP and non-OCP users. We have found that non-OCP users have a greater ACL elasticity, less FFK, and KFEH in all phases compared to OCP users at ambient temperature. We further found that non-OCP users' biomechanical outcome variables were altered throughout the menstrual cycle, but not in OCP users during the menstrual cycle. When comparing estradiol serum concentration between the two groups, OCP users estradiol levels were lower in all phases and did not alter during the menstrual cycle. These results imply that

estradiol serum concentration has an impact on human ligament, muscle, and tendon. In other words, more knee stiffness was found in women on OCP. Here estradiol was lower in blood.

Tissue temperature is one of the factors that alters knee elasticity and heat is commonly used to reduce soft tissue stiffness³¹. Since basal body temperature fluctuates during the menstrual cycle, it is used to accurately detect if women are ovulating. This change is also influenced by progesterone and estrogen during the menstrual cycle but not in OCP users^{32,33}. Previous research found that muscle temperature increased on ovulation and it continuously remained elevated during the luteal phase^{15,17}. To increase central core temperature, the skin removes less heat to reduce body temperature than skin loses more heat. In non-OCP users, muscle and skin tissue temperatures fluctuate during the menstrual cycle; thus playing an important role in knee elasticity.

In addition, there were heat effects on muscle and tendon flexibility both in non-OCP and OCP users. The FFK and FFEH significantly decreased after 38 °C warming of the leg in all phases in OCP users. In a similar manner, knee flexibility was significantly reduced after using heat in all phases of the menstrual cycle except ovulation, where estradiol serum concentration was relatively higher in non-OCP users. These results imply that muscle and tendon flexibility is more affected by temperature than estrogen. Interestingly, we found that FFK and FFEH decreased more after 38 °C warming in OCP users compared to non-OCP users during the menstrual cycle. This indicated more effect of heat on FFK and FFEH in OCP users. Thus, using heat increases knee flexibility in OCP users and is still beneficial to non-OCP users during menstruation where estradiol levels are relatively low. However, for the ACL, the effects of estradiol

were different. The ADT did not significantly decrease after 38 °C warming in both groups of subjects. Also, it was significantly higher during ovulation than menstruation after heat.

ACL elasticity , muscle and tendon flexibility is related to knee injuries ³⁴. Women who are taking OCP have less knee laxity due to estradiol effects during menstruation. Previous studies have examined the association between ACL injuries and use of oral contraceptives ^{29,35}. However, results from these studies were perhaps inconclusive because these studies did not examine the effect of both knee elasticity and tissue temperature. It is not easy to examine the effects of OCP on ACL injuries only with ACL elasticity. Other factors should be considered such as tissue temperature. Thus, in further investigation, both estrogen and tissue temperature should be considered as key risk factors that can cause ACL injuries during menstrual cycle in both OCP and non- OCP users.

In addition, interestingly, it is common to do a warm up prior to exercise in order to reduce injuries since less elasticity is related to tissue tears. However, in women, the reverse seems to be true . Perhaps, the structure strength (e.g. collagen cross bridges) is also altered by estrogen. This may explain differences in the results of some studies on women versus men. These knee injuries may be caused by changes in collagen strength not just elasticity. Estrogen may not only alter ACL elasticity, but may cause a change in the breaking strength of the ACL. Just as bone can fracture due to glycogen cross linking even when it is dense, there may be a similar relationship with collagen in ligaments whereby they may tear even if elastic. This would involve a shift in the force stress curve for the ACL making the elastic region shorter and the plastic region larger due to

estrogen. There are no studies on structural strength of the ACL in relation to estrogen, only elasticity. It will be interesting to see if there are collagen structure strength changes during the menstrual cycle and its relationship to incidence of knee injuries in OCP users and non-OCP users.

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CHAPTER FOUR

DISCUSSION

Women have a higher risk factor for ACL injuries compared to men who participate in the same sports activities³⁻⁶. ACL elasticity and muscle and tendon flexibility are major risk factors for knee injuries during sports^{42,43}. Numerous studies have studied changes in knee ligament elasticity during the menstrual cycle to examine the relationship between sex hormones and ACL laxity^{44,45}. In several previous studies, women had greater knee laxity during ovulation at a time when estrogen level peaks^{23,35,46}. The finding supports these results that estrogen receptors are found in connective tissue and thus, estrogen changes the mechanical properties of tendons and ligaments^{12,23}. In spite of that, researchers are still debating on the results of these studies because the study methods and procedures were vastly different²². However, female reproductive hormones are more consistent during the menstrual cycle in women on the OCP. If knee ligament laxity was affected by hormone fluctuation, female OCP users should show more stable tissue extensibility.

Increased tissue temperature could be one factor that causes ligament and tendon laxity and heat is commonly used to reduce soft tissue stiffness^{25,47}. Basal body temperature fluctuates during the menstrual cycle in women, and this change is also influenced by progesterone and estrogen^{48,49}. Previous research found that muscle temperature continuously increased throughout the menstrual cycle and it remained elevated during the luteal phase^{29,31}. Muscle temperature changed not just by

one but by four to five degree in Celcius which is fairly significant changes temperature in shell tissues during the menstrual cycle ³¹. Muscle and shell tissue temperature fluctuates during the menstraual cycle, thus temperature is also playing an important role in knee elasticity. Therefore, in the present investigation, two series of experiments were conducted to examine the relationship ACL elasticity, FFK, and KFEH during the menstrual cycle. In series 1 (Chapter 2), we examined the relationship between estradiol serum concentration and ACL elasticity, FFK, and KFEH and how this relates to tissue temperature in young healthy women with a regular menstrual cycle. In series 2 (Chapter 3), we investigated the differences in ACL elasticity, FFK and KFEH between OCP users and non-OCP users and its relationship to tissue temperature during the menstrual cycle.

The most important finding from series 1 was a significantly greater ACL elasticity, less FFK, and KFEH during ovulation where estradiol serum concentration peaks compared to the menstruation where estradiol level is lowest during the menstrual cycle. Greater ACL elasticity was found to occur during ovulation. There are two factors that may cause this: 1) estrogen and 2) tissue temperature. Ligaments must have more elasticity with relatively higher temperature since these are elastic stuctures ⁴². In this investigation, skin temperature was comparably lower during ovulation than menstruation and the early follicular phase. But the only improtant temperature is the temperature of deep tissue. There are very few papers on deep shell tissue temperature in deep tissues during the menstrual cycle ³¹. These investigations found that muscle temperature was significantly elevated during ovulation and the well documented increase in core temperature. This is because heat has to be conserved by skin to get increased body temperature. Heat goes through the muscle and leaves the skin by radiation, convection,

conduction, and evaporation. Skin needs to be cooler than the core temperature to aid in heat loss or conserve heat³⁴. Therefore, to keep the body cool at ovulation, the shell needs to remove more heat; hence muscle temperature increases at ovulation.

When the knee was heated to 38 °C, there was still difference in ACL elasticity at ovulation when compared to other phases of the menstrual cycle. Therefore, while the temperature change in deep tissues causes increased laxness, so must estradiol. Both are synergistic at ovulation in increasing ACL elasticity. However, previous studies examining ACL elasticity did not control tissue temperature. Even regarding the menstrual cycle, we go through large changes in shell temperature according to various phases of the year. If studies were conducted on skiers, the results would have been different from tennis players. Clothing and ambient temperature become important to consider in also since this alters arm and leg temperature. This may be the reason the results were so varied in different studies. No previous study has even had subjects acclimatized to room temperature before measuring measurements.

This study also measured the FFK and KFEH in knee flexion and extension. These measures examined muscle and tendon flexibility. Human muscles and tendons are soft tissues composed of collagen fibers, which may be also affected by estradiol concentration and temperature as explained above. Results of this study showed a significantly lower FFK and KFEH at ovulation compared to menstruation. This finding indicates that less muscle and tendon stiffness are shown at ovulation than the menstruation stage. In addition, this result may infer that less muscle flexibility occurs with increased estradiol concentration and lower skin temperature which may lead to higher muscle tissue temperature in young healthy women. However, for the knee, the

probable effect of estradiol is different. There was more energy storage at ovulation showing increased laxity. When the leg was warmed to the core temperature, this effect disappeared. This result implies that the flexibility is more affected by temperature than estrogen; although estrogen is responsible for the temperature fluctuation. Thus, using heat would be beneficial on increasing knee flexibility; the optimal time would be in the menstruation and early follicular phase, where the less flexibility occurs.

Results from the series 2 study show that non-OCP users have a greater ACL elasticity, less FFK, and KFEH in all phases compared to OCP users at ambient temperature. We further found that for non-OCP users' biomechanical outcome variables were altered throughout the menstrual cycle, but not in OCP users during the menstrual cycle. OCP users estradiol levels were much lower in all phases of the menstrual cycle and did not alter during the menstrual cycle. These results imply that estradiol serum concentration has an impact on human ligament, muscle, and tendon. In other words, more knee stiffness was found in women on OCP. Here estradiol was lower in blood.

In addition, there were heat effects on muscle and tendon flexibility both in non-OCP and OCP users. The FFK and FFEH significantly decreased after 38 °C warming of the leg in all phases in OCP users. In a similar manner, knee flexibility was significantly reduced after using heat in all phases of the menstrual cycle except ovulation, where estradiol serum concentration was relatively higher in non-OCP users. These results imply that muscle and tendon flexibility is more affected by temperature than estrogen. Interestingly, we found that FFK and FFEH decreased more after 38 °C warming in OCP users compared to non-OCP users during the menstrual cycle. This

indicated more effect of heat on FFK and KFEH in OCP users. Thus, using heat increases knee flexibility in OCP users and is still beneficial to non-OCP users during menstruation where estradiol levels are relatively low. However, for the ACL, the effects of estradiol were different. The ADT did not significantly decrease after 38 °C warming in both groups of subjects. Also, it was significantly higher during ovulation than menstruation after heat.

However, as seen in this investigation, we did not examine how knee elasticity is related to injuries. ACL elasticity and knee muscle and tendon flexibility may cause injuries or may not. Temperature may be another factor that affects ACL injuries but previous studies did not examine ACL elasticity under the same condition such as controlling for the effects of clothing or environment temperature. Also, previous studies have examined the association between ACL injuries and use of oral contraceptives^{53,54}. However, results from these studies were perhaps inconclusive because these studies did not examine the effect of both knee elasticity and tissue temperature. It is not easy to examine the effects of OCP on ACL injuries only with ACL elasticity. Other factors should be considered such as tissue temperature. Thus, in further investigation, both estrogen and tissue temperature should be considered as key risk factors that can cause ACL injuries during menstrual cycle in both OCP and non- OCP users.

In addition, interestingly, it is common to do a warm up prior to exercise to reduce injuries since less elasticity is related to tissue tears. However, in women, the reverse seems to be true. Perhaps, the structure strength (e.g. collagen cross bridges) is also altered by estrogen. This may explain differences in the results of some studies on women versus men. These knee injuries may be caused by changes in collagen strength

not just elasticity. Estrogen may not only alter ACL elasticity, but may cause a change in the breaking strength of the ACL. Just as bone can fracture due to glycogen crosslinking even when it is dense, there may be a similar relationship with collagen in ligaments whereby they may tear even if elastic. This would involve a shift in the force stress curve for the ACL making the elastic region shorter and the plastic region larger due to estrogen. There are no studies on structural strength of the ACL in relation to estrogen, only elasticity. It will be interesting to see if there are collagen structure strength changes during the menstrual cycle and its relationship to incidence of knee injuries in OCP users and non-OCP users.

We identified several limitations in the present investigation. Sample size of the present study was small. Moreover, we only examined one full menstrual cycle. Although we tested subjects enough times during the menstrual cycle, results may be normalized if we examined more than one full menstrual cycle. Lastly, leg muscle temperature should be taken in future studies instead of taking leg skin temperature. It could be powerful evidence to support the relationship between estradiol serum concentration and ACL elasticity, FFK, and KFEH and how this relates to tissue temperature in young healthy women with a regular menstrual cycle.

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APPENDIX A
INFORMED CONSENT FORM



LOMA LINDA UNIVERSITY

Informed Consent to Participate in Research

School of Allied Health Professions
Department of Physical Therapy

"Effects of the menstrual cycle on ligament and tendon laxness in women"

Loma Linda, California 92350
(909) 558-4632
(800) 422-4558

PURPOSE

You are invited to participate in a research study to evaluate effect of the menstrual cycle on ligament and tendon laxness in women who are either having a normal menstrual cycle or taking oral contraceptives see if there will be variations in ligament, muscle, and tendon laxness while controlling knee temperature across the menstrual cycle between those two groups. This study is being conducted by a student investigator for academic credit.

PROCEDURES

To be a voluntary participant in this study, you will be asked to be tested twice a week for 1 full menstrual cycle. The test will begin with menstruation. The test time will take an hour each experiment day. All procedures will be performed by a qualified student investigator at room A640, Nichol Hall.

Since the concentrations of estradiol in blood and body temperature fluctuate during the day, the test will be performed at the same time period of each experiment day. Upon arriving in the examination area, the subjects will be rest comfortably in a regulated temperature room at 77 °F for 20 minutes to stabilize their body temperature. First, a blood sample (two tsp) will be taken to assess estradiol levels before measuring the body temperature and knee skin temperature. Next, to assess the baseline, the knee ligament, muscle, and tendon flexibility will be measured. After, the subject's leg will be submerged in a whirlpool bath at 100 °F for 20 minutes to control their leg temperature. Skin temperature, ligament laxity, muscle, and tendon flexibility measurements will be repeated. Wearing shorts will be recommended for comfort.



____ Initial
____ Date

Page 1 of 3

Loma Linda University
Adventist Health Sciences Center
Institutional Review Board
Approved 1/9/13 Void after 1/8/2014
5120352 Chair R. L. Ruffolo

"Effects of the menstrual cycle on ligament and tendon laxness in women"

Measurements of each visit

Enter the room (Regulate body temp.)	Phase I (Pre-measurement)	Regulate knee temperature	Phase II (Post-measurement)
Rest comfortably in a regulated temperature room at 77 °F for 20 minutes	Estradiol serum concentration Body temperature Skin temperature Knee ligament laxity Knee tendon and muscle flexibility	Submerge leg into whirlpool at 100 °F for 20 minutes	Skin temperature Knee ligament laxity Knee tendon and muscle flexibility

RISKS

Participation in this study may cause skin infection or dizziness due to the blood draw. However, this risk will be minimized by having an experienced nurse present at the blood draw.

BENEFITS

There will be no benefits to you personally. However, the study may help improve the knowledge about how the menstrual cycle affects ligament, muscle and tendon laxness.

PARTICIPANTS RIGHTS

Participation is voluntary. You may leave the study at any time. If at any time during a procedure you experience tiredness or discomfort beyond what you are willing to endure, just tell the person conducting the procedure you want to stop. This decision will NOT affect your standing with those conducting the study or loss of benefits that you are entitled to.

CONFIDENTIALITY

All records will be confidential. We will not disclose your participation without your written permission. Any publication resulting from this study will refer to you by ID number and not by your name. See attached Authorization for Use of Protected Health Information (PHI) form regarding your privacy rights.

_____ Initial
_____ Date

*Loma Linda University
Adventist Health Sciences Center
Institutional Review Board
Approved 1/9/13 Void after 1/8/2014
5120352 Chair R. J. Reynolds*

COSTS/COMPENSATION

There is no cost for participating in these studies; you will receive 50\$ when completing the study. Social security number will be requested.

IMPARTIAL THIRD PARTY

If you wish to contact a third party not associated with the study for any questions or a complaint, you may contact the Office of Patient Relations at Loma Linda University, Loma Linda University Medical Center, Loma Linda, California 92354. Phone (909) 558-4647.

INFORMED CONSENT STATEMENT

I have read the contents of the consent form and the California Experimental Subject's Bill of Rights and have listened to the verbal explanation given by the investigator. My questions regarding the study have been answered to my satisfaction. I hereby give voluntary consent to participate in the study described here. Signing this form does not waive my rights nor does it release the responsibilities of the principal investigator, Jerrold Petrofsky Ph. D. or Loma Linda University of their responsibilities. I may call Dr. Jerrold Petrofsky during routine office hours at (909) 558 4300 ex 82186 or leave a voice mail message at this number during non office hours.

Signature of subject

Date

INVESTIGATOR'S STATEMENT

I have reviewed the contents of the consent form with the person signing above. I have explained potential risks and benefits of the study.

Signature of investigator _____

Phone Number _____ Date _____

Loma Linda University
Adventist Health Sciences Center
Institutional Review Board
Approved 1/9/13 Void after 1/8/2014
#5120352 Chair R. L. Riquelme

APPENDIX B

AUTHORIZATION FOR USE OF PROTECTED HEALTH INFORMATION FORM



INSTITUTIONAL REVIEW BOARD
Authorization for Use of
Protected Health Information (PHI)

OSR#

Per 45 CFR §164.508(b)

*OFFICE OF SPONSORED RESEARCH
 Loma Linda University • 11188 Anderson Street • Loma Linda, CA 92350
 (909) 558-4531 (voice) / (909) 558-0131 (fax)*

TITLE OF STUDY: Effect of the Menstrual Cycle on Ligament Laxness in Women

PRINCIPAL INVESTIGATOR: Jerrold Petrofsky, Ph.D, JD

Others who will use, collect, or share PHI: Lee Berk, DrPH, MPH, FACSM, CHES
 Haneul Lee, PT, MS

The study named above may be performed only by using personal information relating to your health. National and international data protection regulations give you the right to control the use of your medical information. Therefore, by signing this form, you specifically authorize your medical information to be used or shared as described below.

The following personal information, considered "Protected Health Information" (PHI) is needed to conduct this study and may include, but is not limited to: name, address, telephone number, date of birth, weight, height, BMI, menstrual cycle.

The individual(s) listed above will use or share this PHI in the course of this study with the Institutional Review Board (IRB) and the Office of Research Affairs of Loma Linda University.

The main reason for sharing this information is to be able to conduct the study as described earlier in the consent form. In addition, it is shared to ensure that the study meets legal, institutional, and accreditation standards. Information may also be shared to report adverse events or situations that may help prevent placing other individuals at risk.

All reasonable efforts will be used to protect the confidentiality of your PHI, which may be shared with others to support this study, to carry out their responsibilities, to conduct public health reporting and to comply with the law as applicable. Those who receive the PHI may share with others if they are required by law, and they may share it with others who may not need to follow the federal privacy rule.

Subject to any legal limitations, you have the right to access any protected health information created during this study. You may request this information from the Principal

*Loma Linda University
 Adventist Health Sciences Center
 Institutional Review Board
 Approved 1/9/13 Void after 1/8/2014
 # 420352 Chair D A R. Williams* OSR 12/28/2012

Investigator named above but it will only become available after the study analyses are complete.

- The authorization expires upon the conclusion of this research study.

You may change your mind about this authorization at any time. If this happens, you must withdraw your permission in writing. Beginning on the date you withdraw your permission, no new personal health information will be used for this study. However, study personnel may continue to use the health information that was provided before you withdrew your permission. If you sign this form and enter the study, but later change your mind and withdraw your permission, you will be removed from the study at that time. To withdraw your permission, please contact the Principal Investigator or study personnel at (909) 558 4300 ext. 82186 or e-mail him at jpetrofsky@llu.edu

You may refuse to sign this authorization. Refusing to sign will not affect the present or future care you receive at this institution and will not cause any penalty or loss of benefits to which you are entitled. However, if you do not sign this authorization form, you will not be able to take part in the study for which you are being considered. You will receive a copy of this signed and dated authorization prior to your participation in this study.

I agree that my personal health information may be used for the study purposes described in this form.

_____ Signature of Patient or Patient's Legal Representative	_____ Date
_____ Printed Name of Legal Representative (if any)	_____ Representative's Authority to Act for Patient
_____ Signature of Investigator Obtaining Authorization	_____ Date

Loma Linda University
Adventist Health Sciences Center
Institutional Review Board
Approved 1/9/13 Void after 1/8/2014 OSR 12/28/2012
5/20352 Chair R. L. Ruppel

APPENDIX C

CALIFORNIA EXPERIMENTAL SUBJECT'S BILL OF RIGHTS FORM

CALIFORNIA EXPERIMENTAL SUBJECT'S BILL OF RIGHTS

You have been asked to participate as a subject in an experimental clinical procedure. Before you decide whether you want to participate in the experimental procedure, you have a right to:

1. Be informed of the nature and purpose of the experiment.
2. Be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized.
3. Be given a description of any attendant discomforts and risks reasonably to be expected from the experiment.
4. Be given an explanation of any benefits to the subject reasonably to be expected from the experiment, if applicable.
5. Be given a disclosure of any appropriate alternative procedures, drugs or devices that might be advantageous to the subject, and their relative risks and benefits.
6. Be informed of the avenues of medical treatment, if any available to the subject after the experiment if complications should arise.
7. Be given an opportunity to ask any questions concerning the experiment or the procedure involved.
8. Be instructed that consent to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation in the medical experiment without prejudice.
9. Be given a copy of any signed and dated written consent form used in relation to the experiment.
10. Be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on the subject's decision.

I have carefully read the information contained above in the "California Experimental Subject's Bill of Rights" and I understand fully my rights as a potential subject in a medical experiment involving people as subjects.

Date

Patient

For inpatient studies, add: Time

Parent/Legal Guardian

If signed by other than the patient, indicate relationship:

Relationship

Witness