ESTROGEN EFFECTS ON

ACL LAXITY AND

NEUROCOGNITIVE FUNCTION

IN YOUNG WOMEN

by

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ABSTRACT

Objective: To examine the effect of 17β estradiol (E2) on anterior cruciate ligament (ACL) laxity and neurocognitive function in healthy, young women.

Subjects: Six healthy, young women between ages 18-35 years old were recruited for this pilot study.

Design and Setting: Women underwent a controlled hormone intervention to prevent endogenous production of estrogens and progesterone using a gonadotropin releasing hormone antagonist (GnRHant, Ganirelix) for 10 days; E2 was selectively added back (0.1mg/day patch, Vivelle Dot) over the last 7 days.

Measurements: ACL laxity was measured in millimeters during hormone suppression (day 3 of GnRHant) and again after 7 days of E2 administration using the KT 2000 knee arthrometer with customized computer software. Neurocognitive function was assessed via computerized test (Immediate Post-Concussion Assessment and Cognitive Test, ImPACT) on the same days. Composite scores from the ImPACT test were used to evaluate neurocognitive function. A two-tailed, paired samples t-test was used to compare differences between GnRHant and E2 conditions.

Results: Six women enrolled in the study, and 2 dropped out; pilot data presented is on four women (25±6 yrs, 169.2±6.7cm, 69.6±10.4 kg). ACL laxity during GnRHant (4.43 ± 2.15 mm) and E2 conditions (5.44 ± 1.08 mm) were not different ($p = 0.437$). Verbal memory (91.87 ± 2.29, 98.00 ± 2.65, $p=0.424$), visual memory (85.00 ± 11.27, 74.67 ± 21.73, $p=0.247$), motor speed (34.90 ± 8.15, 38.52 ± 5.74, $p=0.135$) and reaction time (0.703 ± 0.10 s, $0.610 \pm .061$ s, $p=0.327$) were also not different between GnRHant and E2 conditions..

Conclusion: These preliminary results suggest minimal changes in ACL laxity or neurocognitive function during short duration E2 administration. Additional research is needed to fully characterize the interactions among ACL laxity, estrogen, and neurocognitive function in women.

Key Words: estrogen, hormones, anterior cruciate ligament, neurocognitive function, KT-2000, knee laxity, female

Chapter 1

INTRODUCTION

Due to the multi-factorial nature and frequency within athletic events, anterior cruciate ligament (ACL) tears have become a concern for healthcare professionals. Each year it is predicted that over 100,000 ACL tears will occur in the United States.[1] These injuries are most common in younger adults, between ages 15-45 years old,[2] and according to the CDC, it is estimated that ACL injuries in the United States result in costs that surpass $2 billion dollars annually.[3] Not only are ACL tears and their outcomes common and expensive, but also they disproportionately affect women. Women are up to nine times more likely than men to suffer from an ACL injury each year, even when considering football to be a factor in the epidemiology.[1] Furthermore, in commonly played sports such as basketball and soccer, women show at least a 3-fold greater risk in ACL injuries compared to men.

There are a number of factors that may contribute to ACL injuries, as 70% of ACL injuries are caused by a non-contact mechanism.[2] Some suggested factors include joint laxity, influx of sex hormones (estrogen, progesterone, relaxin), biomechanics (lower extremity alignment, increased femoral anteversion or Q angle, foot pronation), playing surface, muscle development, body composition, and neuromuscular control.[2,4] There has also been speculation that a neurocognitive insufficiency may be present in the appropriate judgment and coordination for movement planning that results in injury.[4,5]

Although many of these factors appear to suggest a sex-specific vulnerability for a non-contact ACL injury mechanism, emerging literature reinforces that the laxity of the ACL may be a risk factor, especially in relation with new research that finds if neurocognitive function of the brain sending signals to the musculoskeletal system to respond to a certain situation that will later be discussed. Furthermore, a study reported that there were core proprioception deficits and excessive lateral trunk displacement were risk factors for female athletes.[6] Due to lack of neuromuscular control of the core and the proprioceptive deficits, women tend to land with a quadriceps-dominant force, not otherwise seen in men.[6] The quadriceps activation is of importance because it is a primary agonist of the knee for extension, especially at end range, and contributes to high compressive loads on the tibiofemoral joint.[6] Men also manifest more knee stiffness than women, which may aid in the protection and transmission of force throughout the knee,[6] although this has been argued. When compared with controls, studies have shown that women also have more anterior and internal-rotation knee laxity.[4] Therefore, changes in ACL laxity is an important influence specific to women that may contribute to the increased prevalence of injury. Although biomechanical components are often well-recognized as a main contributing factor for ACL injury, it is not the sole contributor. In a review article by Hewett et al.[26], 7 studies supported a greater prevalence of ACL injuries sustained during the first half of the menstrual cycle or in relation to the preovulatory phase. They also demonstrated an effect for oral contraception and non-oral contraception both having an influence on ACL injury during the first half of the menstrual cycle. As a result, the authors concluded that preventions utilized during these phases would help to reduce injuries.

Although neuromuscular control is a common suspect contributing to injury, neurocognitive function is just as important to consider due to the high rate of noncontact mechanisms because of its influence on motor behavior and its response. Coordination may be a significant factor in ACL injuries because if there are errors, then an athlete may be further predisposed to that injury due to lack of attention to the task, which is manifested through visual and verbal memory scores, which contribute to situational awareness, in testing.[7] It is interesting to note that neurocognitive tasks may influence injury because studies have also shown concussed athletes with similarly poor scores have correlations with lower-extremity musculoskeletal injuries as well.[5] Sex differences can be seen when examining neurocognitive tasks in visual-motor-related function that have been suggested to interfere with knee neuromuscular control.[8] Women tended to show greater alterations in neuromuscular control at the knee during anticipatory-response movements integrating reaction time or visual processing.[8] The literature suggests that there is evidence that estrogen has an advantageous effect on tasks in which females typically perform well at, such as verbal memory, learning, and fine-motor skills.[21] Research looking at estrogen replacement therapy for post-menopausal women had evidence from RCTs that demonstrated this intervention has protective effects these skills during this point in aging. [21] These studies also claim that tasks requiring visual and spatial ability had worse performance scores during the high estrogen, mid-luteal phase.

Due to the aforementioned sex differences in ACL injury occurrence, fluctuations in reproductive hormones have become a center of interest regarding ACL injury. The idea that joint laxity is a risk factor for ACL injury has been brought about frequently in research. In a study done by Rozzi et al., females were found to have almost 2mm of laxity more than men.[9] As such, ACL injury rates and laxity have been assessed at different time points of the menstrual cycle. Women were tested during the early follicular phase of the menstrual cycle (at low estrogen and progesterone), ovulatory phase (high estrogen), or luteal phase (high estrogen and progesterone). Increases in knee laxity were apparent when estrogen and progesterone levels were high compared to baseline.[10] However, increases in knee laxity were associated with increased estradiol[11] and the ACL Research Retreat declared that risk for ACL injury seems to be higher at the pre-ovulatory, or late follicular, phase during the menstrual cycle.[4] The mechanism underlying this process is still not completely understood and continues to be difficult because of individual fluctuations across the menstrual cycle. In animal models, ligament laxity is increased where estrogen receptors are found.[12] Furthermore, high doses of estrogen, complemented with progesterone, manifested greatest ranges of knee laxity in rats.[12] These results lend support to the idea that women may be at higher risk of non-contact knee injury because of fluctuations in sex hormone. [12] In contrast, there has been a study using animal models also that did not find any significant effects of estrogen treatment on the viscoelasticity or tensile mechanical properties on male rat ACLs.[13] Due to these opposing findings, more research is needed to determine whether the receptors in the ACL are affected by estrogen in humans. Because fluctuations in hormone levels across the menstrual cycle differ between women and menstrual disturbances often going undetected, it can be challenging to determine the individual contribution of ovarian hormones on physiological variables.[14] Within the menstrual phase, estrogen rises in the follicular phase and slightly at the end of the luteal phase and as such, amounts of estrogen and other ovarian hormones reach varying levels between individuals.

With this background in mind, the purpose of this study was to examine the effects of estrogen on ACL laxity in the knee and neurocognitive function in young women. In order to control endogenous hormone production, women used a gonadotropin-releasing hormone antagonist (GnRHant) for 10 days; 17β estradiol (E2; Vivelle Dot, 0.1mg/day, patch) was administered during the last 7 days. ACL laxity was measured utilizing a knee arthrometer accompanied by a computerized test to assess neurocognitive function during GnRHant and E2 conditions. We hypothesized that E2 administration will increase knee laxity, but will decrease neurocognitive function in young women.

Chapter 2

METHODS

Participants

We recruited 6 healthy, young women (aged 18-35 years) with normal menstrual cycles from the local community and college campus. This study was conducted as a part of a larger protocol, which examined the estrogen effects on vascular function in women. The inclusion criteria for this study were any young, healthy females. Exclusion criteria include: women who were pregnant or were planning on becoming pregnant, were breast feeding, had a latex allergy, had a history of blood clots, pulmonary embolism, deep vein thrombosis, stroke, breast cancer, liver disease, abnormal vaginal bleeding not related to their period, have had an abnormal gynecological exam within the last year, were obese (body mass index > 30 m/kg^2) or smoked cigarettes, did not participate in this study. In addition, women who had a history of high blood pressure, fibroids in their uterus, diabetes, epilepsy, gallbladder disease, or have had a hysterectomy, did not participate in this study. Women using Depo-Provera or IUDs or extended birth control pill cycle like Seasonale or Seasonique were excluded. Furthermore, participants that suffered an ACL tear within one year or concussion within six months were excluded. All women signed the university IRB-approved consent form. A healthy control group of 4 young women and 8 men were also recruited separately to be tested only for ACL laxity without the hormone intervention to demonstrate intra-tester reliability. These participants were tested about one week apart with the same methods as the current study in order to provide reliability to support the parent protocol. The goal was to obtain measurements that were consistent and prove intra-tester reproducibility. These participants were not required to go through any additional screenings. The study was approved by the Institutional Review Board (IRB) of the University of Delaware and conformed to the Declaration of Helsinki.

Screening

All women underwent rigorous screening. A general physical at the Nurse Managed Health Center and screening at Reproductive Associates of DE prior to the hormone intervention were performed, where each participant completed a medical history and menstrual history questionnaire. At that time their height and weight was measured. Resting blood pressure and a resting electrocardiogram (ECG: a test to record electrical activity of the heart) was measured. Another measurement was a blood sample, which was collected by inserting a needle into an arm vein (approximately 3 tablespoons of blood was removed). The blood sample was used to make an assessment of liver and kidney function, electrolytes such as sodium, a cholesterol profile, red blood cells, glucose (blood sugar).

The results from the screening, as well as the medical history form and physical activity questionnaire, were reviewed by a nurse practitioner to determine if the participant qualified for the study. A vaginal ultrasound was also performed at Reproductive Associates of DE to confirm that the participant was not pregnant. A small blood sample was taken for the analysis of hCG, progesterone, estradiol, FSH, and LH.

At that time, a generalized ligament laxity assessment was done using the Beighton Scale. The Beighton scoring was used in order to determine if the participants demonstrate joint hypermobility. This scale has been validated and used in studies that examine factors that influence laxity in various joints, and have particularly examined looking at anterior cruciate ligament injury in females, ages 16 and older.[19]

Hormonal Intervention

Ganirelix acetate (Rosemont Pharmacy, Bryn Mawr, PA, USA) is an antagonist derived from gonadotropin-releasing hormone (GnRHant) and when given in doses for therapy, acts as a blocker to the gonadotropin-releasing hormone receptors that exist on the pituitary gonadotroph and its transduction pathway. The GnRHant has been shown to be effective in young women as a way to control hormone levels during a study done by Wenner et al. that examined mechanisms contributing to low orthostatic tolerance influenced by estradiol.[15] When administered to young women, ganirelix acetate suppresses endogenous estrogen and progesterone. These changes occurred between 36 to 48 hours within taking the dose.[15, 25] Women began the gonadotropin-releasing hormone antagonist between days 25 and 28 of the menstrual cycle so that the corpus luteum has decreased and the endometrium has begun to shed. Through subcutaneous injection, the women self-administered the dosage every day for ten days to suppress production of the reproductive hormones. An estrogen patch containing 0.1 mg of 17β-Oestradiol (Vivelle Dot) was applied on day four through day ten to have a constant concentration. Women who used oral contraception ceased taking their pills before the injections, which was the final day of their pill cycle. The time points of hormone administration days of measurement were demonstrated in Figure 1.1. Testing did not occur then until three full days after cessation of their contraceptive pills.

Laxity Measurement

The KT-2000 arthrometer was utilized for measuring total joint displacement, as well as anterior and posterior knee displacement individually. It provides objective measurements of the sagittal plane of motion of the tibia in relation to the femur. The participant lay supine on a table with a 6.5 cm thigh support platform under both legs proximal to the region behind the knee and a foot support platform under both feet aligned with the lateral malleoli. The thigh support increased the angle of knee flexion necessary to obtain a good measurement, which should be between 20 to 35 degrees. This flexion angle to align the patella in the trochlea is important because patellar mobility may produce testing error. There was also a Velcro thigh strap to prevent excess hip rotation. The arthrometer was then placed on the anterior aspect of the tibia where the line arrow aligns with the knee joint line using the Velcro strap. The arthrometer should have pressure on the patella pad to stabilize the patella in place. The arthrometer was adjusted so that the patella sensor adjustment knob and position of the tibia and patella shows almost parallel and then the knob can be tightened. The displacement dial on the patella reference pad so that the needled aligns with zero. The maneuver to obtain the measurements was to pull and then push on the force handle. A consistent force application that did not exceed 30 lbs (134 N) anteriorly and 20 lbs (89N) was applied. There were three trials done at each measurement session and then averaged. The knee chosen was from the dominant leg. The changes in millimeters were recorded on a customized software program shown in Figure 3.1.Multiple studies measure knee ligament laxity in regard to the fluctuations over the menstrual cycle, the device used in a majority of them was the KT-1000 or KT-2000 knee arthrometer and provided significant results shown in Figure 2.1. Therefore, this device should be considered to continue to be used for future studies, as it demonstrates to have the most reliability and validity.[16]

Neurocognitive Function Assessment

The Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT) test is widely used and has been scientifically validated computerized concussion evaluation exam.[17] It has been found to be particularly helpful in acquiring a quick and applicable way to detect changes in neurocognitive function after concussion. It compares reaction time, verbal memory, visual memory, and processing speed. Examples of the ImPACT testing battery sections are shown in Figure 4.1 and 5.1. Although it has some limitations with a learning effect[24], this may not be a conflict, as each of our subjects only took it twice. The participant completed the test on day three and day ten in conjunction with the ACL laxity measurements. The participant was in a quiet environment and the test was taken on a desktop kept in the investigator's laboratory. The assessment testing approximately took around ten minutes. At the end of the exam, the ImPACT test divided the scores into separate sections for each task that was completed. The scores were broken down into four composite sections. These scores determined how performance was affected by the hormonal intervention. The use of the computerized neurocognitive test battery that is the ImPACT was done in a previous study done by Swanik to detect a relationship between neurocognitive function and noncontact anterior cruciate ligament injuries that indicated significance, unrelated to concussion.[7]

For the majority of results in a systematic review done by Alsalaheen et al., the ImPACT convergent validity was supported, and though some other factors were inconclusive, this continues to be a standard for testing neurocognitive performance and one of the best tools currently.[17]

Data Analysis

The independent variables in this study were two time points of testing. The dependent variables were the ACL laxity and the scores of the ImPACT test reflecting performance. ACL laxity was measured in millimeters from the average of 3 trials performed on each testing day. The ImPACT generated composite scores were taken from 4 sections of the assessment battery at completion, which were visual motor (motor speed), reaction time, visual memory, and verbal memory. The software provided the composite scores for these variables on each testing day. As previously mentioned, the composite scores were reviewed and compared. The baseline was established by testing the participant on day 3 of hormone suppression.

Statistical Analysis

The independent variable (GnRHant and E2 conditions) and dependent variables (ACL laxity and neurocognitive performance scores) was used in the statistical analysis. ACL laxity was analyzed using a paired samples t-test. Neurocognitive performance from the ImPACT assessment was examined from the scores of each task item and then analyzed using paired samples t-test. All data was analyzed using SPSS version 20.0 (SPSS Inc., Chicago, IL.). An *a priori* alpha level was set at 0.05. Effect size was calculated using the SPSS program. Data measurements for ACL laxity are presented individually as well as mean+/- SD. ImPACT composite scores are presented as mean +/- SD.

Reliability

The reliability of the primary investigator's measurements was established using a paired samples t-test using Excel® 2008. All measurements displayed reliability between men and women manifesting that there was no difference in both groups. This demonstrates the investigator's ability to be consistent with the technique. The male participant measurements were taken in two trials a week apart (3.913 mm, 3.389 mm, $p = 0.22$). The female participant measurements were taken in two trials within a week during the placebo phase of oral contraceptive use (3.735 mm, 2.915 mm, $p = 0.11$). All female participants used oral contraceptives.

Chapter 3

RESULTS

There were 6 participants that were initially involved in this study (healthy females, ages 18-35), but 2 dropped out from the study. Demographic information for the four complete pilot participants is presented in Table 1.1.

ACL LAXITY COMPARISON

Results between GnRHant and E2 conditions for anterior displacement are presented in Figure 1.1. There were no significant statistical differences in anterior cruciate ligament laxity when examining anterior displacement in millimeters between GnRHant (4.43 ± 2.15 mm) and E2 condition (5.44 ± 1.08 mm; $p = 0.437$). The effect size was calculated (0.540) and was indicative of having a medium effect. None of the participants met the criteria to be considered to have hypermobility according to the Beighton Score scale standards in which state that 5+/9 points would be hypermobile.

NEUROCOGNITIVE PERFORMANCE COMPARISON

Results for the composite scores for three participants from the neurocognitive performance test are shown in Table 2.1. There were no significant statistical differences found between GnRHant or E2 conditions for composite scores in verbal memory (91.87 ± 2.29, 98.00 ± 2.65, $p=0.424$) in

Figure 7.1, visual memory (85.00 ± 11.27, 74.67 ± 21.73, p=0.247) in Figure 8.1, motor speed (34.90 ± 8.15, 38.52 ± 5.74, p=0.135) in Figure 9.1 or reaction time (0.703 ± 0.10 s, 0.610 ± .061 s, p=0.327) in Figure 10.1. The effect size was calculated for verbal memory (0.763, large), visual memory (0.549, medium), motor speed (0.499, medium), and reaction time (1.05, large).

Chapter 4

DISCUSSION

To our knowledge, this pilot study was the first to use a controlled hormone intervention to explore the relationship among estrogen, ACL laxity, and neurocognitive function in young women. Our preliminary results indicate that ACL laxity did not statistically change with short term E2 administration, as well as verbal memory, visual memory, motor speed, and reaction time composite scores. There were no significant statistical differences in ACL laxity or neurocognitive performance. As a result, the effect sizes were calculated and demonstrated that there may be reason to believe that hormones, especially estrogen, could have an effect on ACL laxity and neurocognitive performance if there were larger sample sizes applied including 10 participants for reaction time, approximately 22 participants for motor speed and visual memory, 13 participants for verbal memory, and 29 participants for ACL laxity.

ACL LAXITY

In this pilot study, we found no changes in anterior displacement (mm) between GnRHant and E2 conditions. One possible reason as to why there was no significant effect from this investigation may be a result from a delay in the onset of hormones within the body. One study that took ACL laxity measurements daily within the menstrual cycle demonstrated that there was a delay by one day from the effects of fluctuating hormone levels when estrogen peaks.[11] Another reason may be due to the dose-response relationship in which previous studies have used 0.2 mg of E2, while this investigation used 0.1 mg.[15] Similar to this investigation, there was a study done that also reported that estrogen fluctuations had no significant effect on anterior knee laxity during a normal menstrual cycle, but a decrease in stiffness.[22]

Therefore, a future study could examine the effect on stiffness as well. As a result, this could suggest that the role of other hormones helps balance out the effects of estrogen on the anterior cruciate ligament during the normal menstrual cycle, whereas this investigation examined estrogen at isolated levels and should include a larger sample to see if there is an effect.

Ages of the participants has also been a consideration as to whether there is more of a response to estrogen due to the repeated long-term exposure of cycles with older ages (16.3 ± 0.65 years) compared to ages to right after puberty, affecting the connective tissue.[22] While two of our other participants showed a less than 1 mm increase in laxity, one participant had a 4 mm increase. Nonetheless, though the changes were small and not significant, the effect size for anterior displacement may help to support the cause to develop more research regarding ACL laxity increase at peak-estrogen levels. Something to note is that one participant had a minimal response to the hormone suppressant (she demonstrated almost no changes in hormone levels) and was the only participant to manifest a decrease in laxity, although not significant, at her body's natural estrogen levels. Hewett et al, found that recreational female athletes that used oral contraception showed better dynamic stability and passive knee stability in comparison to females that did not use oral contraceptives.[27] This could also explain that besides ACL laxity and hormones, there may be other factors contributing to injury. The clinical relevance when examining this data could be something to be considered, as a change in ligament laxity could possibly influence stiffness within the knee, thereby altering the demand of neuromuscular or neuropsychological responses to allow protection of the ligaments.[5] Typically stiffer muscles help to counteract excessive forces, and if there is a decrease in stiffness as mentioned before, the only protection left is the passive restraint of anterior displacement from ACL laxity.[22] Therefore, the possible combination of both occurring, increased laxity with decreased stiffness at high estrogen levels could be a risk factor for ACL tears.

Another factor to consider is the interference of the potential effects of progesterone on ACL laxity in the menstrual cycle. One investigation evaluated the relationship of knee laxity in sex hormone changes and their interactions and whether there was a time delay.[11] It was found that all hormones contributed to a variance in laxity changing around 3 days after estradiol and 4 days for progesterone. When estrogen and progesterone were combined, this specifically explained laxity variance.

NEUROCOGNITIVE PERFORMANCE

Regarding the neurocognitive performance test, the participants showed no statistical significance in composite scores between GnRHant or E2 conditions. Though the study showed no significance, there is no reason to believe that E2 does not have an effect on neurocognitive performance. Estrogen is crucial in creating synaptic plasticity[23], an important process for memory and learning, throughout human development.

Looking at our limited data regarding effect size only, it is consistent with the literature, which specifically reports that there is evidence that estrogen has an advantageous effect on tasks that females usually do well in, such as verbal memory, learning, and fine-motor skills.[21] These same studies also report that tasks that require visual and spatial ability had worse performance scores during the high estrogen, mid-luteal phase. This was not found in this study, but could apply to a larger population. It also suggests that though the p-values in the scores were not significant, the importance of such findings are based on the manifestation that changes in hormone levels in a typical menstrual cycle that can have an influence on differences that are measurable in healthy young women.[21] Another analysis to consider is to examine reliable change indices using an 80% confidence interval. When looking at each composite score's average separately, reaction time was shown to have a reliable change, while the others neared it. Verbal memory at both conditions (91.7±9.3, 98±2.6, p=0.42) as shown (Figure

7.1) manifested a 6.3-point difference for a 9-point reliable change. Visual memory at both conditions (85±11.3, 74.7±21.7, $p=0.25$) as shown (Figure 8.1) manifested a 10.3-point difference for a 14-point reliable change. Motor speed at both conditions (34.9±8.2, 38.5±5.7, $p=0.14$) as shown (Figure 9.1) manifested a 3.6-point difference for a 7-point reliable change. Reaction time at both conditions (0.70±0.10, 0.61±0.06, $p=0.33$) as shown (Figure 10.1) manifested a .09-second difference for a .06-second reliable change.

For post-menopausal women, numerous randomized control trials have shown that estrogen-replacement therapy also enhanced verbal memory and learning, without any changes in visual memory or spatial abilities. This is not consistent with the outcome of this study, but may be relevant because due to the GnRHant conditions during our study, the participant is in a "menopause-like state" for the duration of the injections. Visual memory has been found to enhance during the mid-luteal phase, but was correlated to the progesterone levels, not estrogen.[23] Therefore, this study did not support these results from previous studies perhaps due to the isolation of estrogen, where progesterone (and other hormones) levels were suppressed. The effect sizes show that the verbal memory and reaction time would have a large effect if more participants could be recruited for a future, bigger study. Along with that, the visual memory and motor speed had a medium effect size, therefore still giving us reason to believe that estrogen could show some influence in a bigger sample size as well. Numerous earlier investigations have shown that estrogen introduced after menopausal surgery improved reaction time, versus those who only received a placebo.[23] This is not consistent with our study when looking at our results, but the state of hormone levels is similar and can be looked at further for future studies. Another factor to consider when discussing estrogen influence on these neurocognitive components is to determine whether there is a dose-response relationship in these situations.

There are also individuals with genetics that predisposed them to have unusual levels of sex hormones during prenatal life, who have been investigated and which led to the belief that females have improved cognition as a result of estrogen.[21] Again, there may be some clinical relevance from our results because any disorientation in visual-spatial tasks could lead to uncertainty in completing it and cause injury. The reaction time may be of clinical relevance as well because it is a key component of injury avoidance. If the reaction time is slowed, then the biomechanical chain could be disrupted and also result in errors that cause some type of injury consequence.[5]

CLINICAL RELEVANCE

There is enough evidence in the literature to support that knee joint laxity in females is more than males and as a result, the changes throughout the menstrual cycle could influence changes in ACL laxity. It has been found that even when there are 1.3 mm differences between dominant and non-dominant knees, there is an increased four-fold risk of ACL injury.[28] If this investigation found significance through a confidence interval within a small change such as this, then it could be possible that a small, but non-significant change in this investigation may be clinically relevant. Another study also looking at temperature found that there were significant changes at the ovulatory phase by only .8 mm in anterior displacement in female participants that did not use oral contraceptives (but no significance in females that did).[29] Therefore, this refers back to the point that perhaps as a clinical prevention strategy may be to suggest to young women wanting to participate in athletics could consider using oral contraception. Overall, due to the inconsistency in the literature, the influence of estrogen on ACL laxity and its risk for injury is still inconclusive.

When looking at the ImPACT scores, although the difference in conditions did not reach significance, there were reliable changes found in reaction times at an 80% confidence interval, therefore leading to the belief that estrogen may help improve reaction time. If this is the case, a drop in estrogen within the cycle could predispose the female athlete to making an error in judgment landing in response to a quick change in external stimuli. One of the participants was flagged during the ImPACT score, due to decrease in performance, as being seen as neurocognitively affected (concussed) at the E2 condition for visual memory. Visual memory is important in spatial awareness and if that is decreased at high estrogen levels, then the female athlete may be at risk for ACL injury because her planning for what her brain perceives her environment is may not be remembered as well and therefore she may not adjust accordingly.

LIMITATIONS

We recognize the low sample size, but this investigation was a pilot study. As a result, future directions that can be taken based on this investigation is the to increase the duration of the study in order to collect more samples to see if there can be a significant effect. Since the effect sizes were medium to large effects, the next investigation should calculate these numbers and recruit enough participants, ranging from 10 (for reaction time) to 29 (ACL laxity), to accommodate for power. Knee stiffness could also be taken accounted for as another variable to examine risks for ACL injury.

According to the literature, the p-value will not necessarily confirm the outcome of a quantitative study, so the effect size was used to help avoid a Type II error, in which the probability of concluding there is no effect, when one actually may exist.[20] Effect size is not dependent on the sample size. It also helps the investigator to determine the magnitude of any possible differences, whereas significance only considers whether the findings are a result of chance.

CONCLUSION

The results of the present study indicate that the hormone intervention had no significant effects on ACL laxity or neurocognitive performance. However, looking at the effect sizes, there is no reason to believe that estrogen does not have an effect if the conditions were perhaps applied to a larger sample size. The complex interaction of estrogen with connective tissue and neurocognitive function may produce subtle, but clinical changes in the processing that could apply to clinical situations. Future studies should include a larger sample size and possibly incorporate other neurocognitive tasks to find if significant results exist.

TABLES

Table 1.1 – Participant Demographics

Subject	Age (years)	Height (cm)	Weight (kg)
1	23	175.26	78.29
2	20	172.72	77.66
3	33	168.91	65.77
4	24	160	56.70
Average ± SD	25 ± 5.60	169.22 ± 6.68	69.61 ± 10.35

Demographic information for the four complete pilot participants.

Table 2.1 – Neurocognitive Measurements

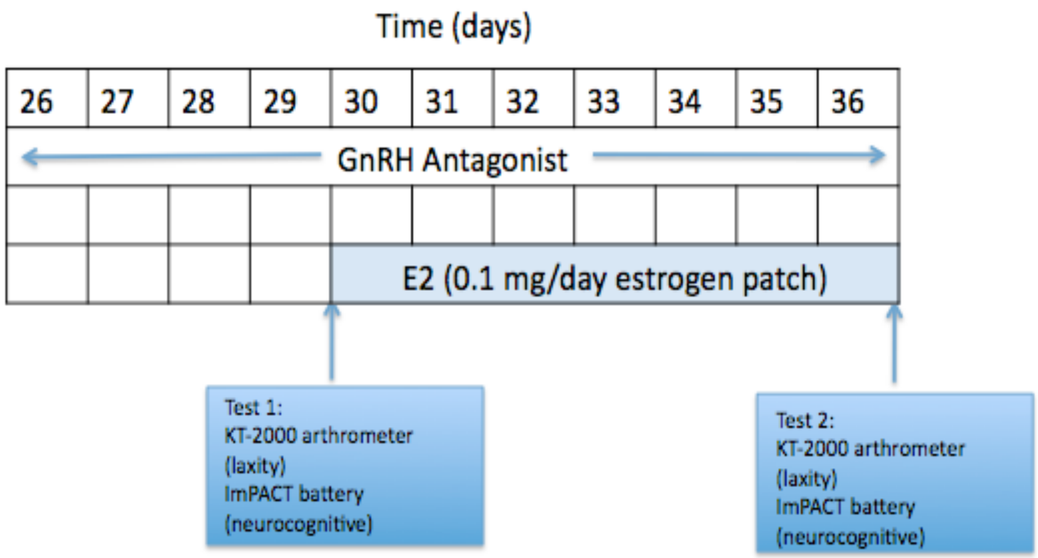
Composite Scores	Pre	Post	P-value	Effect Size
Verbal Memory	91.7 ± 9.3	98 ±2.6	0.42	0.76
Visual Memory	85 ±11.3	74.7 ±21.7	0.25	0.55
Motor Speed	34.9 ±8.2	38.5 ±5.7	0.14	0.50
Reaction Time	0.70 ±0.10	0.61 ±0.06	0.33	1.05

(n = 3)

Results for the composite scores for three participants from the neurocognitive performance test.

FIGURES

Figure 1.1 – Hormone intervention time points

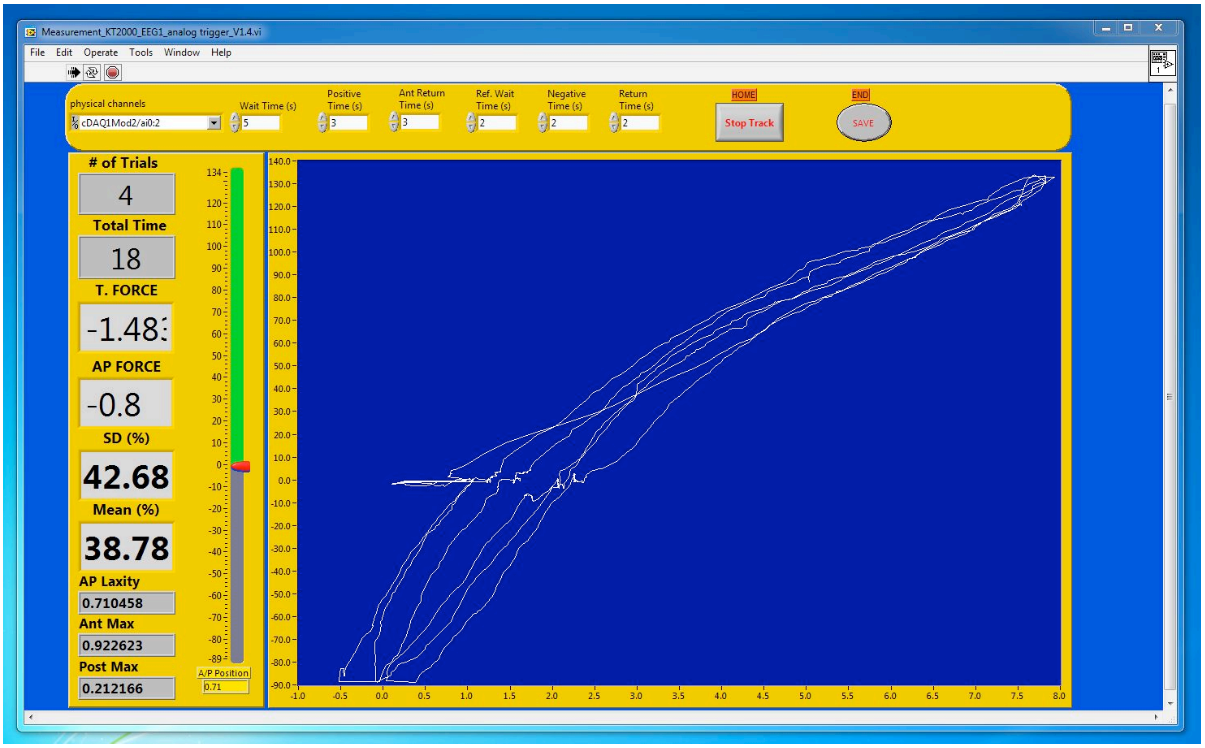


The time points of hormone administration days of measurement were demonstrated.

Figure 2.1 – KT-2000 knee arthrometer

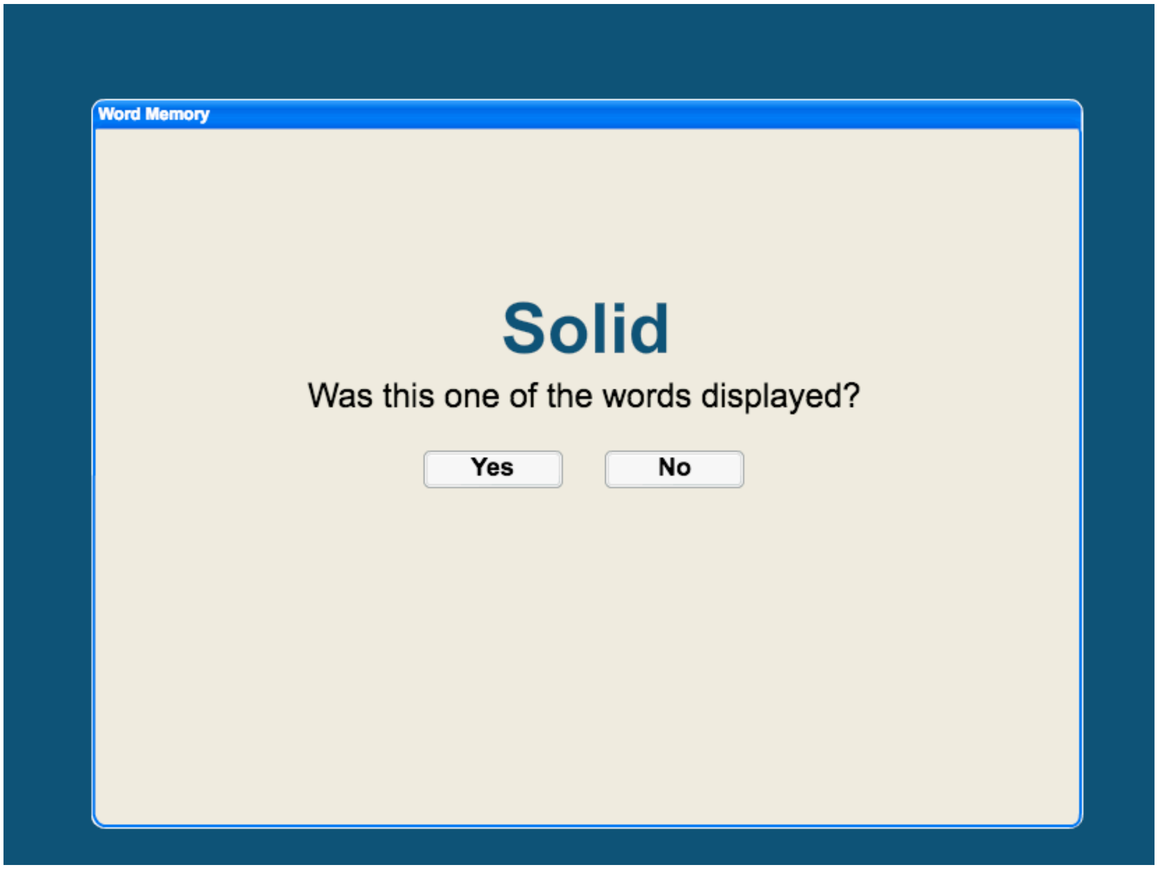
Image of KT-2000 knee arthrometer with participant at rest.

Figure 3.1 - KT-2000 Custom Data Analysis Software Program



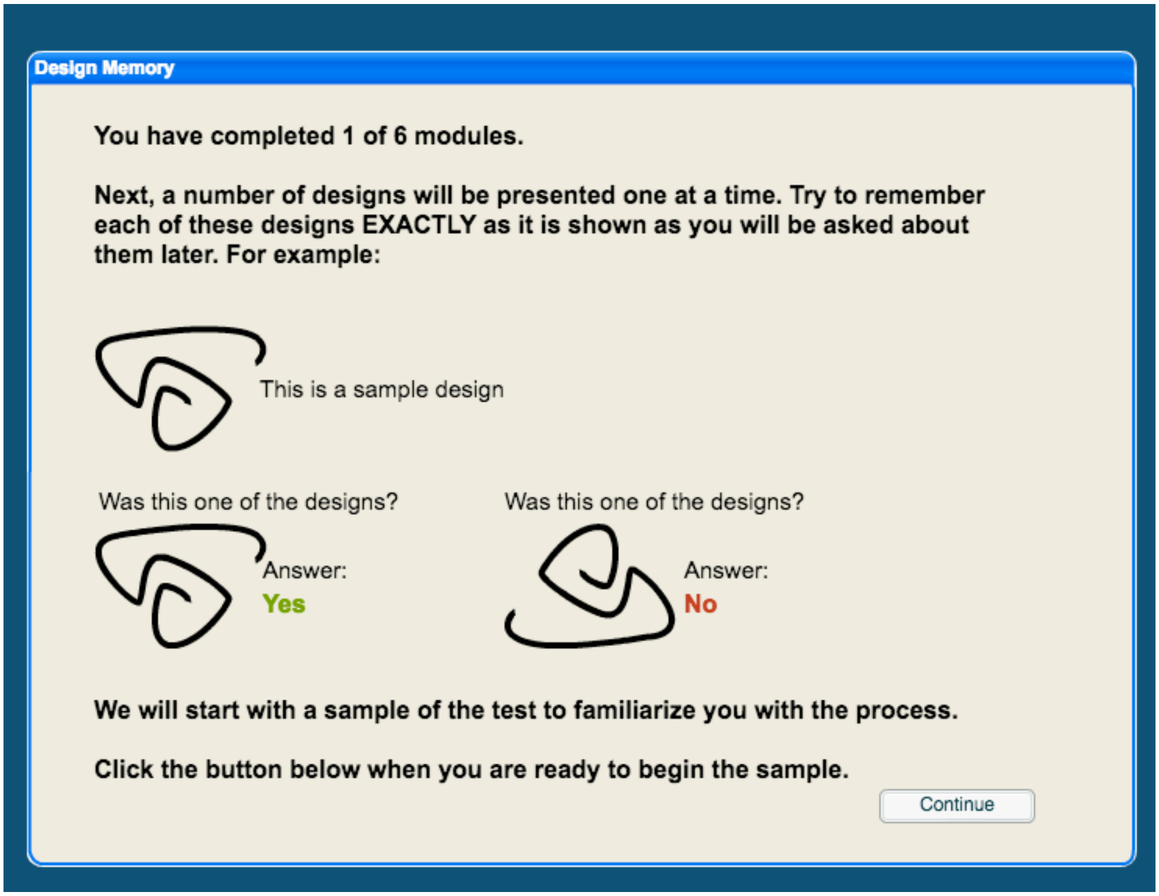
The changes in millimeters were recorded on a customized software program.

Figure 4.1 – ImPACT word memory



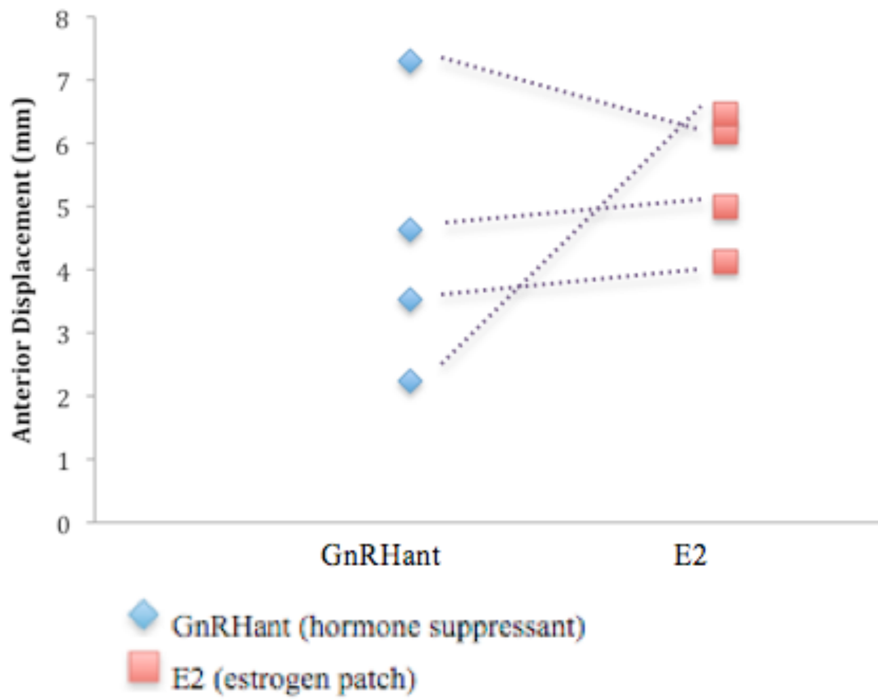
Example of the ImPACT testing battery word memory section.

Figure 5.1 – ImPACT design memory



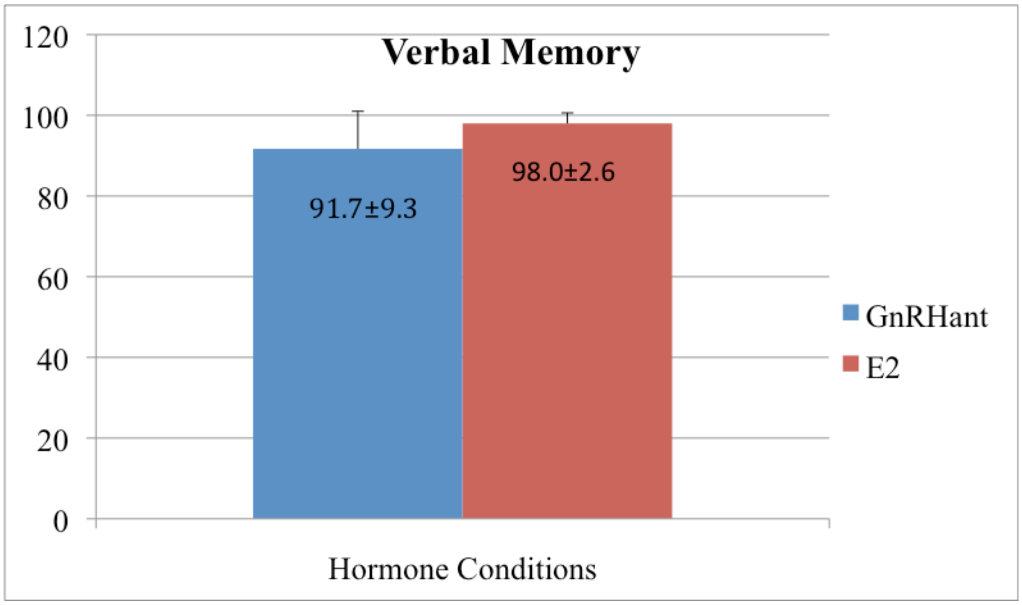
Example of the ImPACT testing battery design memory section.

Figure 6.1 –ACL Laxity Differences Between Hormone Conditions



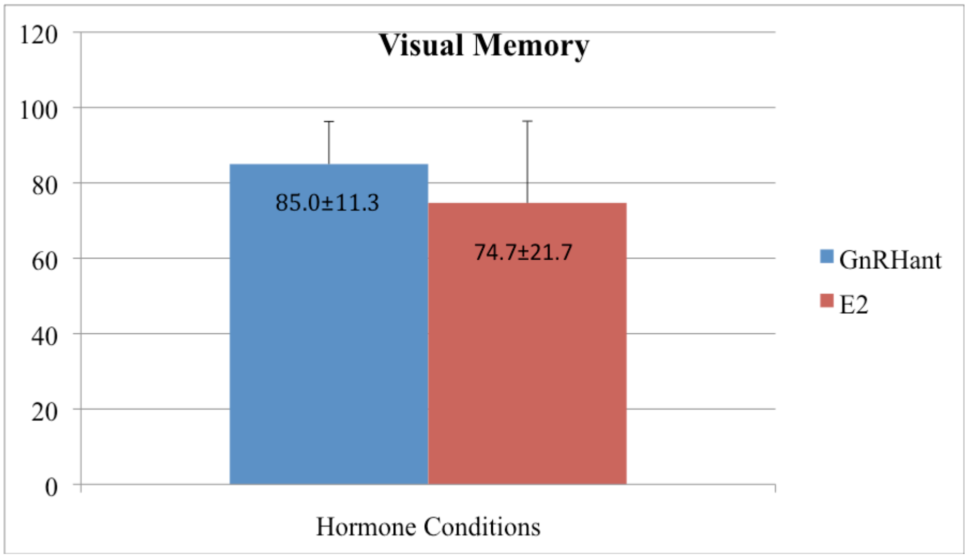
Results between GnRHant and E2 conditions for anterior displacement.

Figure 7.1 - ImPACT Verbal Memory Scores



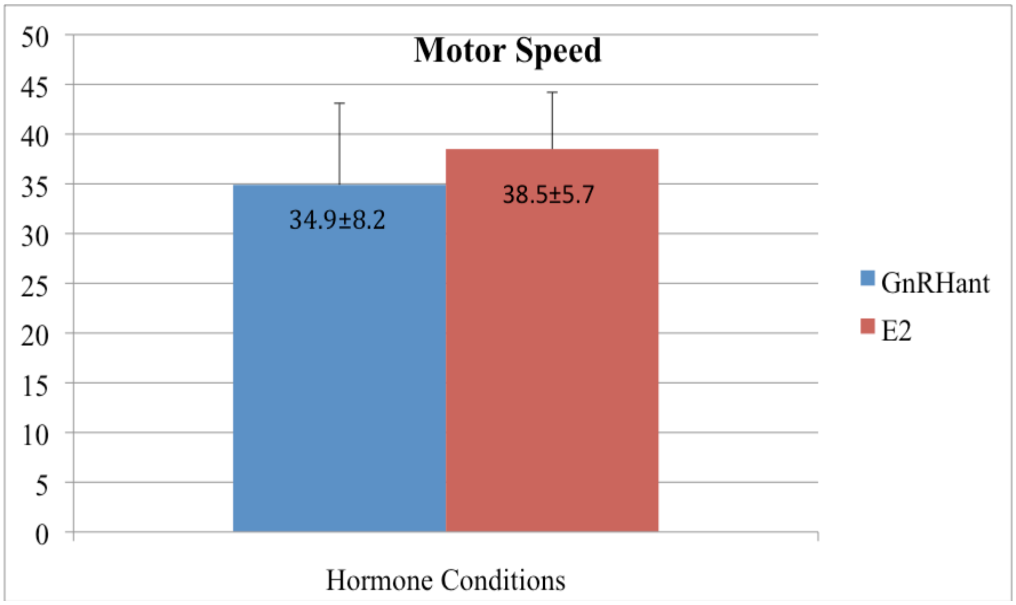
Verbal memory at both conditions (91.7±9.3, 98±2.6, $p=0.42$).

Figure 8.1 - ImPACT Visual Memory Scores



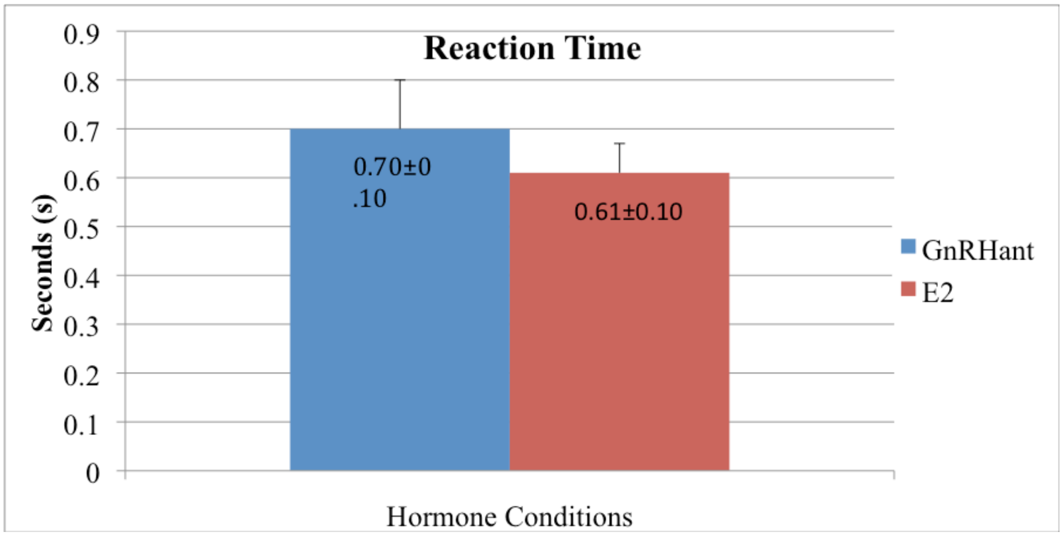
Visual memory at both conditions (85±11.3, 74.7±21.7, p=0.25).

Figure 9.1 - ImPACT Motor Speed Scores



Motor speed at both conditions (34.9±8.2, 38.5±5.7, p=0.14).

Figure 10.1 - ImPACT Reaction Time Scores



Reaction time at both conditions (0.70±0.10, 0.61±0.06, p=0.33).

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Appendix A

LITERATURE REVIEW

INTRODUCTION

Anterior cruciate ligament (ACL) injuries are a growing problem in the United States and worldwide.[1,2,3] Each year in the US alone, it is predicted that over 100,000 ACL ruptures will occur over each year.[1] These injuries most often occur within persons between 15 to 45 years of age, over a 30-year period, which results in an ACL injury once in every 1,750 individuals.[4] Considering such a high incidence, this can contribute to significant healthcare costs being spent. According to the CDC, reported ACL injuries in the United States result in costs that surpass $2 billion dollars annually.[5] This is without considering the management and rehabilitation follow-ups that are also acquired for initial care. Furthermore, this does not account for any complications that may arise in the future, such as infections or graft rejections. These statistics are not restricted to the US alone. In New Zealand, ACL surgeries were found to contribute to the highest cost of treatment when compared to non-surgical injuries and other knee ligament surgeries within sport.[2] Thus, not only are ACL tears and their outcomes common and expensive, but also they disproportionately affect women.[1,4] The purpose of this literature review is to describe the underlying factors that are known to contribute to this higher incidence in women, and also propose a novel way to explore the role sex hormones (particularly estrogen) may play in this higher incidence.

SEX DIFFERENCES IN ACL INJURY

Epidemiology

As previously stated, there is a higher incidence of ACL injuries in women compared to men. Annually, women have found to be up to nine times more likely than men to suffer from an ACL injury, even when considering male-only sports such as football, to be a factor in the epidemiology. [1] The number of males that have ACL injuries is higher than females due to the higher number of males participating in athletics. When the unequal populations are accounted for, females proportionally injure the ACL more often. [4] Particularly, in basketball and soccer, female to male ratios showed at least a 3-fold risk. [1] The epidemiological research that has been done clearly shows that the injury rate for females is significant enough to warrant further investigation. Delving deeper into these factors, examining soccer and basketball at the college level, females had a 30% risk of ACL tears after 1,000 exposures, which is interesting to consider since men are at a third of this, and therefore means females have a 5% chance of ACL tear as opposed to males with a 1.7% chance per year. [1] One study found that 4.4% incidence rate for female athletes with ACL tears over one year. [1] Since athlete exposures are directly proportional to number of ACL tear incidence rates, it is important to understand that when looking at a population, the idea that the higher prevalence of female ACL tears is correct, but relative to the amount of female athletes participating, since it less than males overall. [1]

It is important to note that these sports require fixed-foot or pivoting motions often in participation. There are a number of factors that contribute to ACL injuries, where 70% of ACL injuries are caused by a non-contact mechanism, leaving only 30% from direct contact.[4] Knowing this, numerous studies have been done analyzing all the factors that contribute increased ACL injury. Commonly, some of those suggested are caused by playing surface, lower extremity alignment, muscle development, increased femoral anteversion or Q angle, foot pronation, joint laxity, stiffness, influx of hormones, biomechanics, neuromuscular control, body composition, and so on and so forth. [4,5] Many of these factors appear to suggest a sex-specific vulnerability and within the non-contact ACL injury mechanism. As a result, there has been an increase in research regarding the effect of sex hormones, such as estrogen, on the structural integrity of the ACL and processes in the human body. There has also been speculation that a neurocognitive insufficiency may be present in the appropriate judgment and coordination for movement planning that result in injury due to hormone fluctuations.[5,7] According to the National Athletic Training Association's ACL research consensus statement, clinicians and researchers agreed that risk factors might not be the same for women versus men, although whether the mechanism is female-specific or same external-loading injury is undetermined.[5] Lastly, male and female athletes are at the same low risk for ACL injury before puberty; after onset, Hewett et al. proposes that the general maturation process includes multiple risk factors contributing towards the increased risk of ACL injuries.[8] Thus, this change in injury risk after puberty between men and women suggests that the rise in sex hormones may play an essential role in these injury rates. A detailed discussion of the sex differences in these factors is presented below.

Anatomical and Biomechanical Differences

Examining knee-joint geometry, females have a smaller length, cross-sectional area, and volume of their ACL, even with adjustments.[5] Not only that, but females have less collagen fiber density with a lesser strain and stress at failure and elasticity modulus.[5] It may also be worth considering that imaging has shown females having a taller, but smaller femoral notch width and greater tibial slopes. The role of the femoral notch is still in discussion as it is difficult to obtain valid and reliable measurements, due to there being no standard, to support whether the size is related to injury risk.[34] The tibial slope has been looked at in ACL-deficient individuals, where the lateral tibial plateau slope was much greater than the medial tibial plateau slope.[35] There is the exception of a reduced coronal tibial slope, due to the fact that when landing, these factors influence hip adduction and knee valgus.[5] It is commonly known in the sports medicine world that excessive knee valgus in the athlete population is highly undesirable due to the interference of good mechanics. The valgus angles have been found in numerous studies to be related with ACL injury risks experimentally and through computational models. A study found that athletes screened after sustaining an ACL injury showed knee abduction angles that were eight degrees more than controls.[8]

Furthermore, a study was reported that there were core proprioception deficits and excessive lateral trunk displacement were risk factors for female athletes.[9] Since landing mechanisms require certain positioning and control, the knee valgus and any influence of an abducted hip and internally rotated femur changes the stability of the proximal limbs and can result in injuring an aspect of the lower extremity, since instability requires correction by control.[34] Due to lack of neuromuscular control of the core and the proprioceptive deficits, females tend to land with a quadriceps-dominant force, not otherwise seen in males.[9] The lack of ability to control trunk displacement after being affected by pertubation ahs been found to be a strong predictor for ACL and other knee ligament injuries.[8] Ahmad et al. found that after menarche, athletes utilize their quadriceps strength more than hamstring strength during activity.[10] The quadriceps activation is of importance because it is a primary agonist of the knee for extension, especially at end range, and contributes to high compressive loads on the tibiofemoral joint.[9] If an athlete is quadriceps-dominant, this can contribute to high strains in anterior forces, which may predispose that athlete to injuring their ACL due to a shearing motion in dynamic movements. So when the knee is close to being fully extended, the quadriceps will contract to create a shearing force of the tibia that affects the ACL. This is why it is important that the simultaneous contraction of the hamstring muscles work in harmony with the quadriceps to reduce strain.[35] Males also manifest more knee stiffness than females, which may aid in the protection and appropriate transmission of force throughout the knee.[9] When compared with controls, studies have shown that females also have more anterior and internal-rotation knee laxity.[5] It will be discussed later that sex hormones, especially estrogen, have an influence on the integrity of the ligament, which is predominantly found in women.

ACL Laxity

Commonly, laxity appears to be considered a risk factor for ACL injuries. Ligamentous laxity is the amount of "looseness" that a joint demonstrates, whether in passive or active movements. The amount of displacement of the ligament in a certain direction determines whether a certain ligament is towards high or normal laxity. Several researchers have examined some possible correlations between ligament laxity and ruptures. When looking at general laxity of ligaments and the amount of knee ligament tears, one study found that 72% of thirty-nine professional football players with three or more indicators of laxity had ligament-related knee injuries. [11] Detecting generalized laxity seems to be a characteristic that needs consideration. Hyperextension has actually been named a culprit in the odds of injuring the ACL by 5-fold.[12] Many studies examine laxity regarding exercise and one study found that females showed higher knee laxity than their male counterparts, even across the entire menstrual cycle.[13] On the other hand, the menstrual cycle has been reported not to influence knee laxity, at least during exercise such as bicycling.[11] It is of interest to mention that this study only had 18 participants, to which there has been a pattern that studies with lower numbers tended to show no significance. There are external factors that can influence such a small cohort and therefore needs further consideration before ruling out that the phases of the menstrual cycle do not affect passive knee laxity. Laxity has shown to be equal in increasing for both males and females as well. Knowing that laxity is still greater in females, prevention programs may have to be customized separately for each sex in order to accommodate for the compensations of the athlete.

Laxity is measured with instruments that compare side-to-side differences of anterior-posterior tibiofemoral translation. Studies have shown that examining these measurements in young female athletes are significant because for every increase by 1.3 millimeter side-to-side difference, there is an odds ratio of four times more likely for ACL injury.[12] Therefore at the very least, if passive laxity is greater, there may be an increased risk. Although ACL injury is multi-factorial and complex, there are variables that are examined individually to detect whether one contributes more towards the risk than others.

Common practice typically involving stretching before activity is encouraged because it is believed to help with preventing injury. In contrast, research has shown that this may not actually be the case, and at times may interfere with the body's signals. For example, more recently there was a study that examined the effects of static stretching on knee laxity in soccer players, which showed that musculoskeletal stretching and playing soccer increased anterior tibial translation up to 45.6% in the participants.[14] This is concerning due to the fact that stretching is integral in everyday warm-ups before activity and if joint laxity is a predictor of injury, then individuals may actually be exposing themselves to a higher risk. A possible reason why increased laxity may be a risk is that there is an association with delayed muscle reflexes and joint-loading sensitivity.[14] This means there is a change with the neuromuscular connection from the brain to the muscles and therefore the brain doesn't necessarily interpret the information coming in accurately to prepare correctly for shift movements or landing. As a result of changes in the passive stability of the knee, the sensorimotor system may compensate[14] for the increased laxity and neuromuscular, or even neurocognitive disruption. The neurocognitive disruption that may occur could be a result from a decrease in reaction time. Externally, stretching may be a factor, but regarding intrinsic function, greater knee laxity may also be a result from collagen turnover being greater, causing the tissue to be weaker, and therefore cannot tolerate higher failure loads,[6] as well as various other causes.

In contrast, research regarded laxity is commonly revisited, but there is less known about how ligamentous stiffness can influence injury rates. Most cases reveal that females demonstrate a reduced stiffness in their knees than males, especially in transverse and frontal planes.[38,39] Typically, the research that has been done further investigates on the negative effects of reduced stiffness and increased joint laxity. There is some research that argues that in conditions of high impact loading, joint structures, such as a ligament, with increased stiffness may be more sensitive to joint position errors.[40] Therefore, more quality information is needed on the physiological effects on high impact loads and how higher knee stiffness may or may not contribute to injury.

Another important factor to consider when examining laxity is to determine which instrument is the most valid and reliable for measuring. The most common devices that have been used are KT-1000 (or updated KT-2000) arthrometer, Genucom, and Telos radiography. [15] This systematic review reported that predominantly, the KT-1000 was the most recommended device for measuring anterior knee laxity with an interexaminer reliability of 0.85, ICC of 0.92, and intraexaminer reliability of 0.83, ICC of 0.84, generally. [15] Myrer et al. noted that anterior tibial displacement between different examiners supported these findings, but had higher reliability for the same examiner.[16] When testing side-to-side difference for ACL or anterior laxity is measured, it has been recommended that less than 3mm is the appropriate range to determine stable knees.[16] The mean measurements in an uninjured knee at 134N were found to be between 6 mm to 7 mm[16] Disrupted knees, or knees with ligamentous tears were found to have up to 13 mm of displacement at 89N.[41] Furthermore, in previous studies that have been done that measure knee ligament laxity in conjunction with studies that observe effects over the menstrual cycle, the device used in a majority of them was the KT-1000 or KT-2000 knee arthrometer. Therefore, this device should be considered to continue to be used for future studies, as it appears to have the most reliability and validity.[17] Consistency of measurements is ideal when comparing data over long periods of time and numerous amounts of studies. The KT-2000 arthrometer measures the ligaments in passive knee stability, but it has been shown that there is an association that has been identified between changes in hormones and ACL injury risk. The interaction between knee laxity and the menstrual cycle may not be a cause of injury, but it is of importance to determine this in order to guide research in the appropriate direction.

Neurocognitive Insufficiency

Although very little research has been done, few studies have shown an association with non-contact ACL injuries and slower reaction times, processing speeds, as well as worse visual and verbal memory scores.[18] Although neuromuscular control is a common suspect contributing to injury, neurocognitive function is important to consider because of its influence on motor behavior and its response. Coordination is a significant factor in ACL injuries because if there are errors, then an athlete may be further predisposed to that injury due to lack of attention to the task, which are manifested through visual and verbal memory scores in testing.[18] It is interesting to note how neurocognitive tasks may influence injury because studies have also shown that concussed athletes with decreased visual-spatial scores have shown to have correlations with lower-extremity musculoskeletal injuries as well.[7] There have been shown to be sex differences when examining neurocognitive tasks in visual-motor-related function that have been suggested to interfere with knee neuromuscular control.[19] Since there has been a sex disparity present in neurocognitive tasks, then further research should be done to address this, as was done with hormones and ACL injury. This can further contribute to assisting in the creation of ACL injury prevention programs.

Typically, when looking at neurocognitive performance, the Immediate Post Concussion Assessment and Cognitive Testing (ImPACT) battery has been the golden standard for evaluating brain function after a concussion, although not necessarily always reliable. The ImPACT is a computerized battery assessment divided into four scored sections to examine neurocognitive function that is used widespread. These sections are verbal memory, visual memory, processing speed, and reaction time. Each of these sections attempts to evaluate memory, attentiveness, or the speed that the brain processes. While participants are watching a computer monitor, there is test modules that contain memory recall of words or letters, designs, x's and o's tracking, matching symbols or colors, as well as the reaction for a trigger to appear on the screen that the test-taker must click in a timeframe to measure the quickness of a response.[20] Swanik et al. found that the ImPACT test was not only useful for such an injury, but was able to find statistical differences in scores of athletes who had suffered noncontact ACL injuries and poor performance.[18] The highest numbers of athletes that injured the ACL were women soccer players, which follow the trend of female increased risks and why studies looking at hormones may be vital.

SEX HORMONE EFFECTS ON ACL INJURY

Hormonal Fluctuations during the Menstrual Cycle

When recruiting participants for research relating to hormones, there are several challenges in a way that the studies must be done to observe the physiology in young women. A significant factor being that the menstrual cycle for each woman is different. Therefore, the tracking of certain phases during menstruation are not consistent between participants. The menstrual cycle is a complex physiological process that encompasses whole body fluctuations. Primarily, the first day of the menstrual cycle begins with the onset of menses, or blood flow, which is the endometrium lining in the uterus that is shed. At this time and leading into the early follicular phase, estrogen and progesterone are at their lowest levels. This phase of the cycle will last around 3 to 6 days until the next part of the phase.[21] This next week, 7 to 14 days of the cycle, is considered to be the follicular, or proliferative, phase, which leads up to ovulation, or the ovulatory phase, where estrogen is highest. After ovulation, the corpus luteum develops and creates more progesterone than the rest of the menstrual cycle, occurring during the luteal phase. Estrogen typically increases the most before ovulation, but does rise slightly again about five days after the ovulatory phase.[21] So to summarize, the three main phases that exist across the menstrual cycle are the follicular phase, ovulatory phase, and luteal phase. Within these phases, there are specifically, luteinizing hormones, follicle-stimulating hormones, estrogen, and progesterone. The typical menstrual cycle has fluctuations in which luteinizing hormones and FSH peak at ovulation, while estradiol peaks right before ovulation and progesterone increases most at mid luteal phase. The follicular phase is within a week of menstrual bleeding, and then ovulatory phase is around thirteen days after the start the cycle, with the luteal phase following after. The follicular phase is the phase before ovulation and is when menstruation occurs. During menstruation, the lining of the endometrium is shed from the uterus.

According to Stachenfield et al., estradiol is the most active form of estrogen in young women, so it is crucial in studies concerning hormones in females.[22] So, in order to combat the confounding variables and differences in types of estrogen between women, the same phase of the menstrual cycle has to match up in the participants.[22] The lowest levels of estrogen are during the follicular phase in its earlier stages and the highest right before ovulation. Therefore, if any future studies need to be done, they should observe the contrast of effects at these two points. Hormonal contraceptives have also found to be useful in maintaining a regular menstrual cycle, but it adds progestin and/or estrogen to the body, and is unable to isolate one sex hormone at a time or have the levels return to as low as the body normally functions. Contraceptives have been allowed in some studies, but the challenge with this is that there are various types ranging from non-hormonal, such as IUDs, or progestin-only pills. This supports the idea the need that research limiting use of contraceptives in order to target one sex hormone specifically would be enlightening since not only are menstrual cycles not consistent among all women, but that contraceptives also vary from each individual. Lee et al. found that young women using oral contraceptive pills, meaning lower estrogen levels, had significantly less ACL laxity and subjectively reported significantly higher pain after exercise (squatting) than regular menstrual cycle participants, especially within the first day or two.[23]

For the most possible controlled study examining sex hormones, the ideal method has been to utilize a hormone-suppressant, called Ganirelix or a GnRH antagonist, to temporarily suppress the menstrual cycle to look at only a specific hormone at a time.[22] The downsides to using this method for studies is that it may have full body side effects for participants, although they return to normal once finishing with the suppressant. Since the body does not naturally have phases within the menstrual cycle where one hormone is isolated at a time, it would be beneficial to gain a better understanding of which hormones are most influential. One hormone may have more of an influence than another, or certain hormones, when together, may be the determining factor for a physiological change.

Sex Hormones and ACL Injury

Additional factors that influence knee laxity also arise intrinsically from genetics, structural integrity, and hormones.[6] Hormones, particularly estrogen, progesterone, and relaxin, have shown to have a greater risk of ACL injury during peak levels and possibly decrease tensile strength and motor skills.[9] Hormones have particularly become a center of interest regarding ACL injury due to the differences between men and women. In order to measure these instances, research has looked at pregnant women, as well as examining young women throughout their menstrual cycles, controlling for regularity.

Due to the hormonal fluctuations during menstruation, it has been detected that at peak levels of estrogen are associated with a change in collagen synthesis, as previously mentioned.[6, 24] These peak levels of estrogen tend to occur at the ovulatory phase, days 10-14 of the cycle.[17] Shultz et al. completed a study that examined non-athlete females with normal menstrual cycles and utilized ovulatory kits to determine the phase of the cycle and confirm no pregnancy.[24] It was found that there was a time delay between hormone levels and knee laxity by a few days, with phase shifts at about 3 days for estrogen, 4 days for progesterone, and 4.5 days for testosterone. [24] Understanding this, further research needs to ensure that measurements taken for knee laxity occur around 3 to 4 days after hormone concentration changes.[24] The same study indicated findings that knee laxity increases were associated with increased estradiol or testosterone, but could go either way with progesterone levels. [24]

In numerous studies,[3, 25] typically case-control studies, those that had a higher number of participants found that the pre-ovulatory phase showed significance between menstrual cycle phases and time of ACL injury. It is important that more data be collected regarding the phase of the menstrual cycle when the ACL tear occurred. A study that reinforces this belief was one done in French female recreational skiers, where out of 172 women, approximately 71% of them tore their ACLs.[3] A systematic review was done for 13 clinical trials, which examined how the menstrual cycle affected ACL laxity and found that there were statistically significant differences for laxity variation, noticeably during the pre-ovulatory phase, yet again. [17]

Sex Hormones and ACL Laxity

Other studies have shown a significant increase of ACL laxity with increased estrogen and progesterone levels compared to baseline. [26] For example, the first study to look at sex hormones in females and ACL laxity performed by Heitz et al. measured healthy knees of seven young females using a KT-2000 knee arthrometer, on the day of menses onset and days 10-13, as well as days 20-23 to focus on peak levels of estrogen/progesterone during follicular or luteal phases.[26] They found the greatest change in ACL laxity during the luteal phase, as well as a significant change at the follicular phase. Since their measurements were examining both estrogen and progesterone, the combined effects could be causing the increase in ACL laxity. The limitations from this study were that the researchers did not take measurements between the follicular and luteal phase, nor was the hormone, relaxin, taken into consideration. Since there are so multiple variations of these sex hormones for each woman, it would be beneficial to discover which of these hormones specifically contribute the most towards the increase in laxity. Further research, perhaps such as isolating estrogen, could be done to understand that perhaps the delayed response of initial estradiol increase may influence knee laxity, as opposed to an interactive effect with progesterone.[24] Dehghan et al., examined the passive range of motion in rat knees by giving them estrogen and progesterone as well and found that the rats that were given progesterone and high estrogen doses correlated with the most range of knee laxity.[27] The hormonal component of ACL tears continues to appear in research and it is important especially to understand that estrogen, in particular, does in fact, have receptors in the ACL of a human ligament.[9] The reason that it is crucial to understand this is that as a result of these receptors, studies have shown that collagen synthesis is reduced as well as fibroblast proliferation.[9] As mentioned before, this changes the integrity of the ligament and therefore the body's adaptation to this process may demonstrate a weak point time of it. Since these two processes occur when estrogen is binding to the receptors on the ACL, then the tensile properties are decreased. Since estrogen is greatest over the other sex hormones during the pre-ovulatory phase, days 1-14 of the menstrual cycle, then perhaps this the period that a female may be most at risk for injury, as research has suggested. It is crucial to consider this because for healthcare professionals that take measures to apply prevention from re-injuring an ACL, programs that utilize neuromuscular mechanisms and balance may not necessarily be applicable in situations regarding hormone fluctuations.

On the other hand, studies have shown significance in the ovulation phase, when estradiol serum concentrations were high and ACL elasticity was found to be significantly higher and impacted by the estradiol.[28] In this study particularly, temperature was accounted for and makes the argument that the higher temperature may also contribute to laxity. In a laboratory study examining estrogen effects on laxity, they may better be controlled. As studies continue to occur, there is some direction toward neuromuscular firing control that may be influenced by estrogen changes.[29] Estrogen manifests some influence on the central nervous system, impairing motor skills, kinesthesia, and performance.[29]

Sex Hormones and Neurocognitive Performance

Farage et al. specializes in the physiological fluctuations that occur throughout the menstrual cycle and she has done studies that examine the cognitive change which occur. She goes on to explain that research has shown that there are differences between each gender in nervous and reproductive systems. Estrogen's main role is to contribute to function within the reproductive system, but it also has an influence on aspects of the nervous system such as cognitive function, fine motor skills, mechanisms of pain, and have shown even to have protective effects in the brain after strokes or those with Alzheimer's.[36] There are sex differences in certain actions within the brain especially seen in relation to the hippocampus.[36] Females tend to perform best on verbal skills and memory, perception speed, accuracy, and fine motor skills.[37] In contrast, males exceed them in visual memory and spatial tasks.[37] It has even been found that estrogens actually influence synaptic formation and degradation regulation, in female rats.[36] Although estrogen induces new synaptic connections, where estrogen increases number and density of dendritic spines on neurons, progesterone can down-regulate these connections.[36] Making new synapse connections is the basis for building memory. The hippocampus is responsible for memory functions, which is a component that is examined when analyzing neurocognitive performance. The sex differences of changes in the hippocampus include the dentate gyrus size, which is larger in males than females. Cues are picked up faster in males over females concerning spatial learning where the description of the size of the dentate gyrus is considered.[36] Estrogen receptors have been confirmed to reside in the brain and may have various impacts on neurocognition. Studies have shown that estrogen influences cyclic changes in the hippocampus, which enhances short-term memory then leading to the sharpness of working memory generally. [30] Numerous trials show that estrogen therapy given to postmenopausal women show an improvement in memory, reaction times, and abstract reasoning.[31] The neuroprotective effects that are understood to work are that estrogen sustains function of the hippocampus and basal forebrain, which control dopaminergic, serotonergic, and noradrenergic systems.[36] The other effect is that estrogen blocks neurotoxic agents from generating and therefore acting on the brain.[36] Throughout the menstrual cycle, since there are fluctuations, it is beneficial to determine which sex hormones influence brain function significantly. Maki et al., saw that no estrogen-related changes in verbal recall in sixteen, young women may have been due to limited power.[32] In the same study, perceptual memory was found to be inhibited in association with high levels of estrogen; since estradiol correlated both significantly and negatively with the memorizing of fragmented pictures. In contrast, a study looking at verbal and spatial functions across the menstrual cycle in eight young women, saw no difference in spatial scores, but did see an improvement with verbal working memory.[33] These women were selected if they had a normal menstrual cycle and a postovulatory rise in the middle of their cycle. They were each tested four times during a cycle with two tasks. The study also examined a birth control group that had steady sex hormone levels and found that their verbal scores were not affected as much. The sample size were small and there was not actual measurements of hormones, but the improvement then decrease in performance of verbal working memory was parallel with the cycle of the body's estrogen levels.[33] Consistently in the overall literature of menstrual cycle studies, the male-dominant tasks (visual and spatial) show to improve in women during phases of which there are low levels of estrogen, whereas females excel in the midluteal phase, in verbal and fine motor abilities, when estrogen is more prevalent.[37] This idea that working memory is influenced by estrogen is reinforced by the research explained in the systematic review done previously.[30] If there is a trend that estrogen may help with working memory, then research may be needed to isolate this sex hormone to determine that other hormones in particular do not influence it.

CONCLUSION

In summary, there are known sex differences in the anatomical and biomechanical actions between women and men regarding the anterior cruciate ligament. One of these differences between the sexes is that there is the evidence of hormonal influence, particularly the fluctuation of estrogen in the menstrual cycle in women that may contribute to ACL injury. Estrogen has shown to influence laxity in the ACL in some studies and has also been seen to impact the performance of neurocognitive function. Although research is growing with examining factors that contribute to ACL injury, the multi-factorial complexity of this problem raises the need for more research, as the results have been mostly inconclusive so far. The more that can be learned about the effect of sex hormones on the anterior cruciate ligament and neurocognitive performance in young women, the prevention of the injury can be enhanced.

Therefore, the purpose of this study was to examine the effects of a hormone intervention using estrogen on anterior cruciate ligament (ACL) laxity and neurocognitive function.

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Appendix B

APPROVED IRB LETTERS

FFICE

210 Hullihen Hall University of Delaware
Newark, Delaware 19716-1551
Ph: 302/831-2136
Fax: 302/831-2828

DATE: November 3, 2016

TO: Meeja Kinsey, BS
FROM: University of Delaware IRB

STUDY TITLE: [971298-1] Estrogen effects on ACL laxity and neurocognitive function in young women

SUBMISSION TYPE: New Project

ACTION: APPROVED APPROVAL DATE: November 3, 2016
EXPIRATION DATE: October 18, 2017
REVIEW TYPE: Full Committee Review

Thank you for your submission of New Project materials for this research study. The University of Delaware IRB (HUMANS) has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Full Committee Review based on the applicable federal regulation.

Please remember that <u>informed consent</u> is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

Please note that any revision to previously approved materials must be approved by this office prior to initiation. Please use the appropriate revision forms for this procedure.

All SERIOUS and UNEXPECTED adverse events must be reported to this office. Please use the appropriate adverse event forms for this procedure. All sponsor reporting requirements should also be followed.

Please report all NON-COMPLIANCE issues or COMPLAINTS regarding this study to this office. Please note that all research records must be retained for a minimum of three years.

Based on the risks, this project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Nicole Farnese-McFarlane at (302) 831-1119 or nicolefm@udel.edu. Please include your study title and reference number in all correspondence with this office.

UNIVERSITY OF DELAWARE ARCH OFFICE

210 Hullihen Hall
University of Delaware
Newark, Delaware
19716-1551
Ph: 302/831-2136
Fax: 302/831-2828

DATE: March 27, 2017

TO: Meeja Kinsey, BS
FROM: University of Delaware IRB

STUDY TITLE: [1036688-1] Intra-tester Reliability of KT-2000 Knee Arthrometer

SUBMISSION TYPE: New Project

ACTION: APPROVED
APPROVAL DATE: March 27, 2017
EXPIRATION DATE: March 26, 2018
REVIEW TYPE: Expedited Review

REVIEW CATEGORY: Expedited review category #4

Thank you for your submission of New Project materials for this research study. The University of Delaware IRB (HUMANS) has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Full Committee Review based on the applicable federal regulation.

Please remember that informed consent is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

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Appendix C

DATA COLLECTION FORMS AND QUESTIONNAIRES

Questionnaire for ACL Laxity and Neurocognitive Study

1. Do you suffer from or have ever been diagnosed with a neurological disease?

   Yes_____ No_____

2. Have you suffered a concussion or head injury in the past 6 months?

   Yes_____ No_____

3. Have you every injured your knee or torn your ACL?

   Yes_____ No_____

   If yes, when and which knee (right or left):

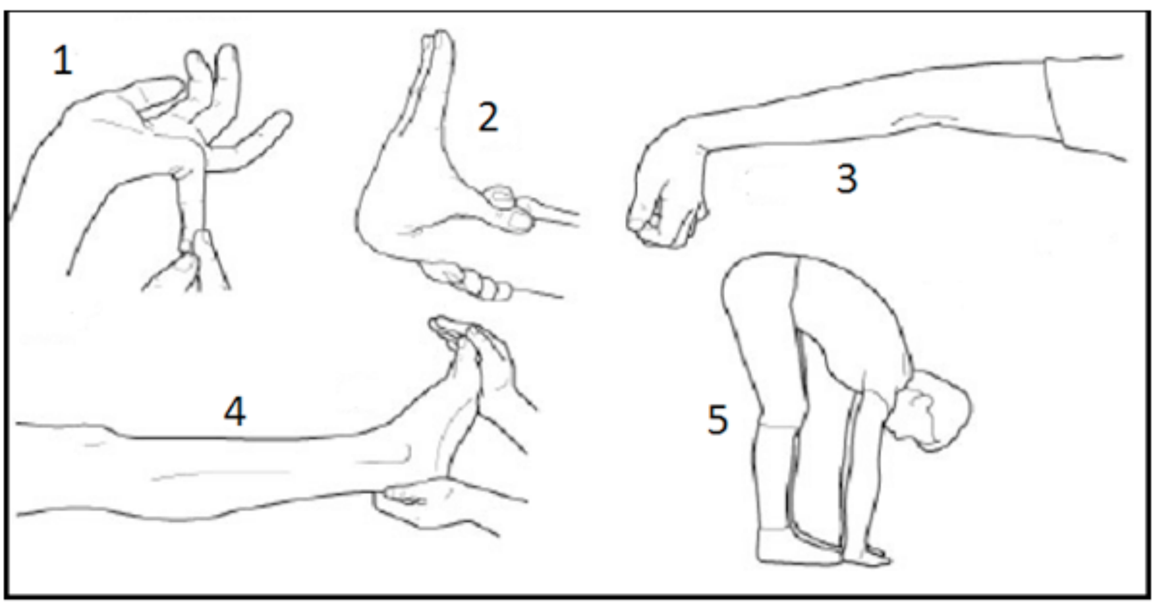
   ____

   ____

Clinical Use of Beighton Scale

Components of the Beighton scoring:

	LEFT	RIGHT
1. Passive dorsiflexion and hyperextension of the fifth MCP joint beyond 90°	1	1
2. Passive apposition of the thumb to the flexor aspect of the forearm	1	1
3. Passive hyperextension of the elbow beyond 10°	1	1
4. Passive hyperextension of the knee beyond 10°	1	1
5. Active forward flexion of the trunk with the knees fully extended so that the palms of the hands rest flat on the floor	1	1
TOTAL	/ 9	



Instructions during the performance of the Beighton scale:
1. I am going to bend your little finger up at 90° to the back of your hand
2. I am going to bend your thumb back on the front of your forearm
3. I am going to bend your elbow backwards

4. I am going to bend your knee backwards
5. Can you put your hands flat on the floor with your knees straight