THE EFFECT OF ESTROGEN ON THE RABBIT ANTERIOR CRUCIATE LIGAMENT. A BIOMECHANICAL STUDY.

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INTRODUCTION
Recent epidemiological studies have recognized a disproportionately higher anterior cruciate ligament (ACL) injury rate in female athletes as compared to male athletes. Although the etiology of this phenomena remains unclear, possible explanations include gender differences in ligament or muscle strength, conditioning, endurance, and anatomy. Unique to the female athlete is her exposure to a constantly changing hormonal milieu throughout her reproductive years. The identification of the estrogen receptor in the fibroblasts of the human and rabbit ACL suggests that female sex hormones may have an effect on the structure and composition of this ligament. Furthermore, on the molecular level, estrogen has been shown to dramatically decrease collagen and procollagen synthesis by ACL fibroblasts, in as little as 7 days. The purpose of this study is to assess how these estrogen-induced metabolic changes affect the biomechanical properties of the rabbit ACL.

METHODS AND MATERIALS
After obtaining approval from the Institutional Animal Care and Use Committee, 40 age- and weight- matched New Zealand White rabbits (Lepus brachyrus), were obtained and subsequently equally divided into 5 groups (16 knees per group). The males served as the control group (Group I). The 32 females were randomly assigned to: oophorectomized group (Group II), unoperated, normal group (Group III), and two separate groups, each receiving twice logarithmic concentrations of 17β-estradiol (Sigma Chemical Co., St. Louis, MO) at 6 ng/ml (Group IV) and 600 ng/ml (Group V), respectively via an osmotic pump (Alza Corp, Palo Alto, CA)). Operated groups were subjected to their outlined treatments for a total of 7 days based on previous studies in primary tissue cultures which demonstrated that estradiol had significant effects on both collagen and procollagen synthesis within 7 days of treatment. With the exception of the ACL and its attachments to the femur and tibia, all remaining soft tissues about the knee were sharply dissected free. Throughout the course of dissection and preparation, care was taken to avoid damage to the attachment sites and to keep the specimen moist with 0.9% saline solution at all times. All specimens appeared grossly normal, as there was no evidence of joint disease or degeneration. The tibia and the femur were osteotomized approximately 6 cm from the joint and the respective ends were potted in polyurathane (BJB Enterprises, Tustin, CA). Thereafter, the length and width of the ACL was measured at three separate points (proximal and distal attachment, and mid-substance) with the use of a Digidigic digital caliper (Mitatoyo, Japan). For mechanical testing of the ACL, the potted specimen were mounted on the 858 biaxial materials testing machine (MTS Systems Corporation, Eden Prairie, MN) at full extension while the femur was externally rotated 90 degrees on the tibia. This allowed the fibers of the ACL to be oriented in line with the direction of tension. A small pre-load of 0.5 N was applied to the specimen mounted in the MTS. The specimen were then preconditioned with 10 cycles of lengthening, from 0.0 to 0.5 mm, at a rate of 0.2 mm/sec (5% strain/sec). Subsequently, the specimens were loaded to failure at 10 mm/sec (100% strain/sec). The calculated data is presented as the average with its standard error mean (± SEM). Statistical analysis was performed with an analysis of variance to detect differences between the mechanical properties of the rabbit ACL as a function of relative concentrations of estradiol. A 95% confidence level was chosen to determine significance of all statistical analyses. Statmost (DataMost, Salt Lake City, UT) was used to make the statistical calculations.  

RESULTS

FORCE AT FAILURE
With increasing estradiol exposure, less force was required to tear the ACL. The force at failure for Group I was highest at 224.43±37.28 N, followed by Group II at 218.35±24.76 N. There was no statistical difference between these two groups (P=0.68). Group III demonstrated a load to failure of 190.10±29.97 N, which was significantly weaker than Group I (P=0.02) and Group II (P=0.03). Group IV had a peak force at failure at 147.98±23.26 N, while Group V had a peak force of failure at 151.56±51.55 N. Although there was no statistical difference between these two groups (P=0.89), there was a statistical significant decrease in force of failure when comparing Groups IV and V to Groups I, II and III (P=0.02, P=0.003, and P=0.002, respectively).

ULTIMATE TENSILE STRESS
A decrease in the ultimate tensile strength is observed with increasing estradiol exposure. There was no significant difference (P=0.37) in ultimate tensile stress between Group I (36.05±10.69 N) and Group II (32.4±10.11 N). Compared to Group I and Group III, Group II (25.47±4.87) was significantly weaker (P=0.0004 and P=0.008, respectively). There was no statistical difference (P=0.50) between Group IV (29.61±10.58) and Group V (26.06±10.57). There was no significant difference between Group IV and Group III and Group V and Group III (P=0.08 and P=0.42, respectively).

MODULUS OF ELASTICITY
With increasing estradiol exposure, the modulus of elasticity is noted to increase. Specifically, the modulus of elasticity of Group IV was 308.08±69.84 and Group V was 307.03±83.54. There was no statistical difference between the two groups (P=0.97). Meanwhile, the modulus of Group I was 184.51±37.42, Group II was 199.05±58.50, and Group III was 186.53±48.89. There were no statistically significant differences amongst these three groups. In comparison to Group III, Groups IV and V demonstrated significant increases in the modulus of elasticity (P=0.0001 and P=0.0005, respectively).

STIFFNESS
With increasing estradiol exposure, an increase in stiffness is observed. There was no statistical difference (P=0.08) in stiffness between Group I (121.31±19.63 N) and Group II (128.35±14.20 N). There was no difference between Group I and Group III (107.90±26.05 N) and Group II and Group III (P=0.123 and P=0.08, respectively). There was no statistical difference (P=0.74) in stiffness between Group IV (183.00±47.76 N) and Group V (175.72±64.27 N). There was a significant difference between Group IV and Group III and Group V and Group III (P=0.00001 and P=0.00008, respectively).

MAXIMUM DISPLACEMENT PRIOR TO FAILURE
With increasing estradiol exposure, a decrease in maximum displacement prior to failure is noted. There was no statistical difference (P=0.08) in maximum displacement prior to failure between Group I (2.44±0.62 mm) and Group II (2.10±0.24 mm). Furthermore, there was no significant difference between Group I and Group III (2.19±0.23 mm) and Group II and Group III (P=0.19 and P=0.37, respectively). There was no statistical difference (P=0.70) between Group IV (1.64±0.39 mm) and Group V (1.71±0.55 mm). However, there was a significant decrease in maximum displacement prior to failure when comparing Groups IV and IV and Groups III and V (P=0.0002 and P=0.0008, respectively).

ULTIMATE TENSILE STRAIN
With increasing exposure to estradiol, the ultimate tensile strain decreased. There was no significant difference (P=0.11) in ultimate tensile strain between Group I (0.23±0.07) and Group II (0.19±0.02). Nor was there a significant difference between Group I and Group III (0.21±0.03) and Group II and III (P=0.35 and P=0.16, respectively). There was no statistical difference (P=0.72) between Group IV (0.15±0.03) and Group V (0.17±0.06). However, Group IV and V were significantly weaker than Group III (P=0.003 and P=0.04, respectively).

DISCUSSION
With increasing participation of female athletes in sports, and the relative increased incidence of ACL injuries in women, a clear association between ligament strength and estrogen exposure has been demonstrated. Although further studies are needed, we believe that preventive measures should be directed to protect the female athlete from either high estrogen exposure (via oral contraceptives) or from high-demand sports during the follicular phase of the menstrual cycle, when estrogen levels are highest.