Knee ligament mechanical properties are not influenced by estrogen or its receptors

Stuart J. Warden,1,2 Leanne K. Saxon,3 Alesha B. Castillo4 and Charles H. Turner4

1Department of Physical Therapy, School of Health and Rehabilitation Sciences, Indiana University, Indianapolis, IN, USA
2Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, USA
3Department of Orthopaedic Surgery, Indiana University School of Medicine, Indianapolis, IN, USA
4Department of Biomedical Engineering, Purdue School of Engineering and Technology, Indiana University-Purdue University Indianapolis, Indianapolis, IN, USA

Abbreviated title: Direct effects of estrogen on knee ligament mechanics

Address for reprint requests and other correspondence: Stuart J. Warden, Department of Physical Therapy, Indiana University, 1140 W. Michigan Street, CF-326, Indianapolis, IN 46202, USA.
Phone: +1-317-278-8401; Fax: +1-317-278-1876; Email: stwarden@iupui.edu.
ABSTRACT

Females are at greater risk of tearing their knee anterior cruciate ligament (ACL) than males participating in similar athletic activities. There is currently no conclusive explanation for this disparity; however, as ACL injuries in females have been linked with estrogen fluctuations during the menstrual cycle, one hypothesis is that estrogen has a direct detrimental effect on knee ligament mechanical properties. This study investigated the influence of estrogen and its receptors (ERα and ERβ) on knee ligament mechanical properties. This was achieved by testing the viscoelastic and tensile mechanical properties of knee medial collateral ligaments (MCL) and ACLs from: 1) male Sprague-Dawley rats treated with either estrogen (17α-ethynylestradiol; 0.03 mg/kg) or an ERα-specific agonist (propyl pyrazole triol; 2 mg/kg), and 2) female mice with a null mutation of the gene encoding for ERβ. Estrogen treatment had no significant effects on the viscoelastic or tensile mechanical properties of the rat MCL or ACL. Similarly, pharmacological stimulation of ERα using a selective agonist in rats and genetic modulation of ERβ by null mutation of its gene in mice did not influence MCL or ACL properties. These data indicate that estrogen does not have a major direct effect on ligament mechanical properties. Energies for the prevention of the disproportionately high rate of knee ligament injuries in females may be better spent focusing on more established and modifiable risk factors, such as abnormalities in neuromuscular control about the knee.

Keywords: anterior cruciate ligament (ACL); biomechanics; gender differences; medial collateral ligament (MCL); neuromuscular control
INTRODUCTION

Females are at two to eight times greater risk of tearing their knee anterior cruciate ligament (ACL) than males participating in similar athletic activities (1, 2, 17). There is currently no conclusive explanation for this disparity; however, differences in sex-specific hormones have been shown to play a role (3, 37, 49, 55, 56). In particular, estrogen fluctuations during the menstrual cycle have been linked to ACL injuries in females (3, 37, 49, 55, 56), with preliminary data reporting that significantly more ACL injuries than expected occur during the midcycle surge of estrogen (ovulatory phase) than during phases where estrogen concentrations are low (follicular and luteal phases) (55). Although other investigators have found ACL injury risk to be heightened during alternative phases of the menstrual cycle (3, 37, 49), the link between the menstrual cycle and injury has raised questions regarding direct detrimental effects of estrogen on knee ligament mechanical properties/strength. These questions have been further stimulated by recent findings of the presence of estrogen receptors in ligaments (30, 43-45), and the response of fibroblasts (28, 29, 31, 59, 60) and ligaments (48) to estrogen.

Estrogen effects are mediated by two ligand-activated transcription factors called estrogen receptors (ERs)—ERα (14, 15) and ERβ (26). Both ER subtypes are widely expressed, where they have distinct biological functions. The actions of estrogen on cellular proliferation seem to require ERα, whereas the effect of estrogen on cellular differentiation may be mediated principally by ERβ (7). Given these differing roles, the function of each ER subtype is important to determine when investigating the biological effect of estrogen on a particular tissue. In terms of ligaments, transcripts for ERs have been identified in mRNA isolated from rabbit and human knee medial collateral ligaments (MCLs) and ACLs (44), and ERs have been identified via immunohistochemistry in human ACL fibroblasts (30), and ovine (45) and rodent (43) ACLs. Albeit, the relative expression each ER subtype has not been confirmed in knee ligaments.
The identification of ERs in ligament fibroblasts suggests that estrogen may directly modulate fibroblast functioning. Indeed, studies have shown that fibroblasts derived from knee ligaments are responsive to estrogen (28, 29, 31, 59, 60), indicating that their ERs are functional. Although findings have been contradictory with reports of both increased (28, 29) and decreased (31, 59, 60) fibroblastic collagen production in response to physiologic levels of estrogen, the data indicate that estrogen can directly influence knee ligament fibroblast activity. As fibroblasts maintain ligaments, and are responsible for both collagen production and degradation, estrogen may directly influence knee ligament mechanical properties.

Few studies have specifically studied the direct influence of estrogen on knee ligament mechanical properties with those that have providing conflicting results. Slauterbeck et al. (48) showed post-ovariectomy estrogen treatment for 30 days reduced ACL ultimate force in rabbits. In contrast, Räsänen and Messner (42) found five months of estrogen treatment in normal growing rabbits to have no effect on MCL ultimate force or elastic modulus; however, estrogen treatment did significantly reduce MCL cross-sectional area. Most recently, Strickland et al. (52) found estrogen to have no influence on knee MCL or ACL mechanical properties in sheep. Given these contrasting results, a need persists to establish the direct effect of estrogen on knee ligament mechanical properties.

The aim of this study was to further investigate the influence of estrogen on knee ligament mechanical properties and investigate the contribution of each ER. This was achieved using a multidirectional approach by: 1) assessing ligaments from male rats treated with either estrogen (non-specific ER agonist), an ERα-specific agonist or a vehicle solution, and 2) assessing ligaments from female mice with and without a null mutation of the gene encoding for ERβ.
MATERIALS AND METHODS

Animals. Estrogen and ERα effects were investigated in 24 male Sprague-Dawley rats purchased at six weeks of age from Harlan Sprague-Dawley, Inc. (Indianapolis, IN). Male rats were selected to minimize the effects of endogenous estrogen production. ERβ effects were investigated in female ERβ homozygous mutant (ERβ⁻⁻) mice (BERKO) with a C57BL/6 background (N=10). These animals were generated by inbreeding of heterozygotes for ERβ disruption (ERβ⁺⁻), as described by Dupont et al. (11). Control mice consisted of female wild-type (ERβ⁺⁺) littermates (WT; N=10). Progeny genotype was determined from tail-derived DNA by polymerase chain reaction amplification (PCR) using established primers (11). Animals in both studies were maintained under standardized environmental conditions with ad libitum access to standard mouse or rat chow and water. All procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Indiana University.

Interventions. To investigate estrogen and ERα effects, rats were acclimatized until eight weeks of age and randomly divided into three intervention groups (N=8/gp): 1) estrogen treated (EE₂); 2) propyl pyrazole triol treated (PPT); and 3) vehicle treated controls (VEH). The EE₂ group was treated via oral gavage with 17α-ethynylestradiol (0.03 mg/kg; Sigma-Aldrich, St Louis, MO), which is a synthetic estrogen that is used in oral contraceptive formulations. The dose was chosen as it has been shown to correct the body weight and skeletal effects of estrogen deficiency in rats (12), whereas higher doses have been found to be detrimental to development in young male rats (53). The PPT group was treated via subcutaneous injection with propyl pyrazole triol (4-propyl-1, 3, 5-Tris (4-hydroxy-phenyl) pyrazole—2 mg/kg in 50% DMSO/50% Dulbecco’s phosphate-buffered saline; Sigma-Aldrich, St Louis, MO). PPT is an ERα-specific
agonist that has a binding affinity that is 410-fold greater for ERα than ERβ (51), and the chosen
dose of PPT corrects the bone loss caused by estrogen deficiency in rats (19). The VEH group
received via oral gavage a vehicle solution (0.25ml of 20% hydroxypropyl-β-cyclodextrin; Sigma-Aldrich, St Louis, MO). All rats were treated seven days per week for five consecutive
weeks and killed at 13 weeks of age. To investigate ERβ effects, mice were aged until 20 weeks
before being killed. There was no specific intervention in these animals.

**Specimen preparation.** Immediately after death, the right hindlimb was removed from
each animal at the hip joint, placed intact in a plastic bag and stored at -80°C. Postmortem
storage by freezing does not change ligament properties during destructive mechanical testing
(57). On the day of mechanical testing, the hindlimbs were allowed to thaw to room temperature
in phosphate-buffered saline (PBS). Dissection of extraneous tissue exposed the knee joint and,
with further careful dissection, femur-ACL-tibia (F-A-T) and femur-MCL-tibia (F-M-T)
complexes were created. As rats were concurrently being used to investigate estrogen effects on
the skeleton, only one knee joint was available from these animals for ligament testing. To
permit testing of both the MCL and ACL in the same knee in these animals, a Dremel drill
(Robert Bosch Tool Corporation, Mount Prospect, IL) with attached diamond-embedded wafer
blade (Super Flex Diamond Disc; Miltex Inc., York, PA) was used to cut the femur and tibia
sagittally. This was not performed in the mouse knees as there were sufficient numbers to test
the MCL and ACL in different animals. At all times the ligament and insertion sites were kept
hydrated with PBS.

MCL thickness and width were measured optically, and the MCL area estimated using an
elliptical geometry (40). Geometry of the ACL was not determined due to its dual bundles and
intimate position within the knee joint. The femoral and tibial portions of the F-A-T and F-M-T
complexes were placed in a custom-built testing jig and the knee positioned at 90° and 0° flexion
for ACL and MCL testing, respectively (Figure 1A, B). The femoral and tibial portions were
embedded in Wood’s low melting-point metal (Bismuth alloy LMA-117; Small Parts, Inc., Miami Lakes, FL) for fixation, and the jig coupled to an electromagnetic material testing device (TestBench 200 N ELF LM-1; EnduraTEC Systems Group, Bose Corp., Minnetonka, MN). A 50 N load cell was used to measure forces during testing of rat ligaments, which resulted in a force and displacement resolution of 0.01 N and 0.001 mm, respectively. A 5 lb (22 N) load cell was used to measure forces during testing of mouse ligaments, which resulted in a force and displacement resolution of 0.001 N and 0.001 mm, respectively. A preload of 0.05 N was applied to each ligament, and the ligaments were preconditioned in displacement control by cyclically loading at 1 Hz for 10 cycles to ~1% strain to rectify any alteration in low-load properties of the ligament due to deep freezing (57). The ligaments were subsequently unloaded and allowed to recover for ten minutes prior to testing of viscoelastic properties.

**Ligament viscoelastic properties.** Viscoelastic properties were determined via stress relaxation tests. Following re-establishment of preload (0.05 N), ligaments were stretched to ~5% strain and held for 100 sec. To achieve this strain, rat MCLs and ACLs were lengthened 0.32 mm and 0.12 mm, respectively, while mouse MCLs and ACLs were lengthened 0.05 mm and 0.03 mm, respectively. A strain magnitude of 5% is below the threshold for structural damage in ligaments (39), and testing for 100 sec enables accurate estimation of the viscoelastic behavior of ligaments (34). Loading ramped at a strain rate of ~10%/sec and data was collected at 20 Hz during testing. Force-time data were plotted on both linear-linear and log-log scales. The percent force reduction from the peak force was calculated from linear-linear plots (peak force–final force)/peak force x 100) (Figure 2A). Log-log scales were curve fitted with a power law, \( t^n \) (Figure 2B). The dimensionless parameter \( n \), which is the slope on a log-log plot, was considered as the ‘rate’ of relaxation (34, 38).

**Ligament tensile mechanical properties.** Following viscoelastic testing, ligaments were unloaded and allowed to rest for 1000 sec (16-17 min) while being kept hydrated with PBS.
Experiments subjecting ligaments to displacements below 5% tissue strain have shown that a recovery time equal to 10-times the test time allows the return to the initial preload gauge length (38). Following tissue recovery, preload (0.05 N) was re-established and the ligaments were pulled to tensile failure in displacement control at a strain rate of ~10%/sec (Figure 1C, D). To achieve this strain rate, rat MCLs and ACLs were lengthened at 0.8 mm/s and 0.5 mm/s, respectively, while mouse MCLs and ACLs were lengthened at 0.5 mm/s and 0.3 mm/s, respectively. During testing, force and displacement data were collected at 100 Hz. From the force-displacement curves the mechanical properties of ultimate force and stiffness were obtained (Figure 2C). Ultimate force data from the MCLs were divided by MCL area to obtain ultimate stress.

Statistical analyses. Statistical comparisons were performed with the Statistical Package for Social Sciences (SPSS 6.1.1; Norusis/SPSS, Chicago, IL), with a level of significance set at 0.05. Differences between VEH, PPT and EE2 rat groups for body weight, viscoelastic properties and ultimate stress were assessed using one-way ANOVAs followed by Fisher’ protected least-significant difference (PLSD) for pairwise comparisons. ANCOVAs were used to assess for rat group differences in ligament ultimate force and stiffness with body weight as the covariate. Differences between WT and BERKO mice were assessed using unpaired t-tests. All data are presented as mean ± SD.
RESULTS

Animal characterization. Body weight did not differ between rat groups at baseline ($P > 0.05$). However, after five weeks of intervention the groups differed significantly ($P = 0.02$), with the PPT group ($304.5 \pm 18.2$ g) being lighter than both the EE$_2$ ($333.4 \pm 33.4$ g; $P = 0.04$) and VEH ($342.3 \pm 23.0$ g; $P = 0.01$) groups. The reduction in weight gain in the PPG group is consistent with feminization of male rats, similar to that produced by high-dose estrogen (53). Genotype in the mice did not influence body weight ($P = 0.92$), with WT and BERKO mice weighing $19.2 \pm 2.6$g and $19.1 \pm 1.7$ g, respectively.

Viscoelastic properties. There were no significant group differences in the peak force obtained or percent force reduction that occurred during the stress relaxation tests in either rat (Table 1) or mouse (Table 2) ligaments. The rate of stress relaxation in rat MCLs and ACLs was not influenced by intervention group ($P = 0.27$ and 0.12, respectively) (Figure 3A). Similarly, mouse genotype did not influence the rate of stress relaxation in MCLs and ACLs ($P = 0.10$ and 0.13, respectively) (Figure 3B).

Tensile mechanical properties. Estrogen and ERα specific agonist treatment did not influence knee ligament strength in rats, with no differences being found between the VEH, EE$_2$ and PPT groups for MCL ($P = 0.62$) or ACL ($P = 0.30$) ultimate force (Figure 4A). Similarly, no differences were found between groups for knee ligament stiffness (all $P = 0.29$-0.41) (Figure 4B) or MCL ultimate stress (all $P = 0.44$) (Figure 4C).

Null mutation of the gene encoding for ERβ did not influence knee ligament strength in mice, with no differences being found between WT and BERKO groups for MCL ($P = 0.49$) or ACL ($P = 0.29$) ultimate force (Figure 5A). Similarly, no genotype differences were for knee ligament stiffness (all $P = 0.29$-0.52) (Figure 5B) or MCL ultimate stress (all $P = 0.39$) (Figure 5C).
A possible cause for the disproportionately high rate of knee ligament injuries in females is a direct detrimental effect of estrogen on knee ligament mechanical properties/strength. This study used controlled animal models to investigate the influence of estrogen and its receptors on knee ligament mechanical properties. Estrogen treatment had no significant effects on the viscoelastic or tensile mechanical properties of the rat MCL or ACL. Similarly, pharmacological stimulation of ERα using a selective agonist in rats and genetic modulation of ERβ by null mutation of its gene in mice did not influence MCL or ACL viscoelastic or tensile mechanical properties. These data indicate that estrogen and its receptors do not directly influence the mechanical properties of these knee ligaments.

The current findings indicate that direct ligament effects of estrogen may not be responsible for the disproportionately high rate of knee ligament injuries in female athletes. However, statistical power and the potential of committing a Type II statistical error needs to be explored in any study that observes a non-significant finding. Power calculations for ultimate force indicated that there was 80% probability of detecting a mouse genotype effect of 10% and 21% for the MCL and ACL, respectively. Similarly, there was 80% probability of detecting a difference of 15% to 24% in ligament ultimate force between VEH, PPT and EE2 rats. Although post-hoc power calculations need to be interpreted cautiously (24), these minimal detectable differences are considered to be clinically relevant and indicate that we addressed our research question with sufficient statistical power.

It is possible that estrogen and its receptors had effects smaller than could be statistically detected. Previous studies have shown estrogen to negatively impact the collagen content of a variety of connective tissues (16, 47). As collagen fulfills the major load-bearing role in ligaments, these studies suggest that estrogen may also influence the mechanical properties of
However, this has only been confirmed by one study which showed estrogen treatment following ovariectomy reduced ACL ultimate force in rabbits by 11.3% (48). This small effect was generated in animals exposed to high levels of estrogen, with the serum estrogen level being two times higher than normal and equivalent to that experienced by rabbits during pregnancy. Such an effect was not observed in the current study using more physiological levels of estrogen, nor has it been confirmed by previous studies (42, 52). Also, the removal of estrogen does not result in the opposite effect of an increase in ligament mechanical strength and subsequent decrease in injury risk (50, 54). These data indicate that any direct detrimental effect of physiological levels of estrogen on knee ligament mechanical properties is at most small.

The current study was unable to find any effect of estrogen or its receptors on knee ligament mechanical properties with continuous exposure to intervention. Rats were treated daily for 5-weeks with either estrogen or an ER$\alpha$ selective agonist, while mice had life-long disruption of ER$\beta$. These exposures are somewhat unphysiological as they deviate from the clinical scenario where ligaments and their ERs are exposed to phasic levels of estrogen during the menstrual cycle. It is possible that estrogen may produce a different effect when introduced intermittently versus continuously (41), and that intermittent doses may significantly alter ligament mechanical properties within a matter of days as collagen turnover can occur rapidly (3-5%/day in adult rat skin) (27). However, normal physiological fluctuations in estrogen during the estrous cycle have not been found to alter the failure properties of the rat ACL (43), and clinically assessed knee ligament mechanical properties (laxity and stiffness) do not differ across the different phases of the menstrual cycle (5, 6, 8, 25). As such, we believe that intermittent intervention would not have altered the findings in the current study and that the absence of an effect despite constant exposure to intervention actually strengthens the conclusions. The latter theory is based on the finding that ligament fibroblasts exhibit a dose-dependent response to estrogen, with increasing estrogen concentrations resulting in progressive declines in fibroblast
proliferation (31, 59, 60). This indicates that continuous intervention may magnify estrogen effects resulting a larger and more detectable effect than when estrogen levels vary phasically with intermittent intervention.

A possible further limitation of our study is the multidirectional approach used to investigate estrogen and ER effects. The effect of combined ER\(^\alpha\) and ER\(^\beta\) stimulation, isolated ER\(^\alpha\) stimulation and no ER stimulation, were investigated by treating rats with, EE\(_2\), PPT and VEH, respectively, while isolated ER\(^\beta\) effects were investigated by comparing mice with and without a null mutation in the gene encoding ER\(^\beta\). We did not investigate isolated ER\(^\beta\) effects in rats as no selective agonist of this receptor was available at the time of investigation. It is possible to use diarylproprionitrile (DPN) for the stimulation of ER\(^\beta\) as this reagent is more potent for ER\(^\beta\) than ER\(^\alpha\); however, it is not selective (18). Consequently, it was elected to investigate isolated ER\(^\beta\) effects in genetically-modified mice. This multidirectional approach to address the research question may be viewed as a limitation; however, it is actually a strength. Irrespective of the model used, no effect of estrogen or the ERs was found. Further investigation of ER\(^\beta\) effects in rats and ER\(^\alpha\) effects in genetically-modified mice may more completely answer the research question; however, we do not believe that data from these studies would alter our conclusions.

As direct ligament effects of estrogen may not explain the disproportionately high rate of knee ligament injuries in female athletes, other mechanisms need to be considered. The risk of knee ligament injury is multifactorial, and results from a combination of extrinsic and intrinsic factors (4). Females have been shown to exhibit a number of intrinsic risk factors that have been linked with ACL injuries. These have been adequately reviewed elsewhere (10), and include anatomical features, such as a narrower intercondylar notch (46), smaller ACL size (9) and altered lower extremity alignment (32), as well as neuromotor features. The latter are of particular interest as they are potentially modifiable and may be influenced by the differing
levels of estrogen during each phase of the menstrual cycle. Females have been found to utilize different muscular activation patterns compared to males during activity (23). This results in significant gender differences in mechanics, with females displaying increased hip internal rotation and a larger valgus at the knee (13, 21, 35, 58). This ‘dynamic valgus’ knee motion has prospectively been shown to be a significant risk factor for ACL in female athletes (22), suggesting that clinical approaches targeting neuromuscular control issues may be useful in preventing ACL injuries in females. Supporting this, training programs for lower extremity neuromuscular control have been found to significantly decrease knee injury rates (20, 33, 36).

In summary, this study found in two separate animal models that estrogen and its receptors did not significantly influence the mechanical properties of the knee ligaments. While an effect smaller than could be statistically detected may exist, the clinical relevance of such an effect is questionable. Combined with previous studies, these data indicate that estrogen does not have a major direct effect on ligament mechanical properties. Energies for the prevention of the disproportionately high rate of knee ligament injuries in females may be better spent focusing on more established and modifiable risk factors, such as abnormalities in neuromuscular control about the knee.
REFERENCES


### Table 1. Peak force and percent force reduction during stress relaxation tests in rat MCLs and ACLs

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>%</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEH</td>
<td>2.37±0.65</td>
<td>66.4±9.0</td>
<td>0.76±0.33</td>
<td>39.5±5.1</td>
</tr>
<tr>
<td>EE2</td>
<td>2.93±0.50</td>
<td>61.4±10.8</td>
<td>0.83±0.35</td>
<td>37.4±9.0</td>
</tr>
<tr>
<td>PPT</td>
<td>2.94±0.72</td>
<td>61.1±7.0</td>
<td>0.99±0.66</td>
<td>42.0±4.4</td>
</tr>
<tr>
<td><em>P</em> values</td>
<td>0.15</td>
<td>0.86</td>
<td>0.65</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Values are means±SD. MCL, medial collateral ligament; ACL, anterior cruciate ligament; VEH, vehicle treated controls; EE2, estrogen treated; PPT, propyl pyrazole triol treated.

### Table 2. Peak force and percent force reduction during stress relaxation tests in mouse MCLs and ACLs

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>%</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0.27±0.08</td>
<td>32.9±9.6</td>
<td>0.31±0.13</td>
<td>32.8±15.5</td>
</tr>
<tr>
<td>BERKO</td>
<td>0.26±0.06</td>
<td>39.6±8.4</td>
<td>0.32±0.10</td>
<td>23.6±8.5</td>
</tr>
<tr>
<td><em>P</em> values</td>
<td>0.86</td>
<td>0.18</td>
<td>0.78</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Values are means±SD. MCL, medial collateral ligament; ACL, anterior cruciate ligament; WT, wild-type littermates; BERKO, estrogen receptor β homozygous mutant mice.
Fig. 1. Representative images of the set-up for rat A) medial collateral ligament (MCL) and B) anterior cruciate ligament (ACL) testing. The knee joint was positioned in 0° and 90° of flexion for MCL and ACL testing, respectively. The femur and tibia were held in place by Wood’s low-melting point metal. Bone slippage and/or growth plate avulsion were avoided by designing fixtures that supported (‘cupped’) the distal femur and proximal tibia. To permit more space within the knee joint during MCL testing, the proximal tibial growth plate was carefully removed prior to mounting. At the completion of testing, the C) MCL and D) ACL were visibly ruptured.
Fig. 2. Representative A) and B) force-time and C) force-displacement curves from rat medial collateral ligament viscoelastic and tensile mechanical tests, respectively. Force-time data were plotted on both A) linear-linear and B) log-log scales to determine ligament viscoelastic properties. Derived properties included the peak force, percent force reduction and ‘rate’ of relaxation during the test. Force-displacement data were plotted on linear-linear scales to determine ligament tensile properties. Derived properties included the ultimate force (peak of the curve on the y-axis) and stiffness (slope of the linear portion of the curve).
Fig. 3. Effect of estrogen and the estrogen receptors on the rate of stress relaxation in (A) rat and (B) mouse knee ligaments. MCL, medial collateral ligament; ACL, anterior cruciate ligament; VEH, vehicle treated control rats; EE₂, estrogen treated rats; PPT, propyl pyrazole triol treated rats; WT, wild-type littermate mice; BERKO, estrogen receptor β homozygous mutant mice. Bars represent mean ± SD.
Fig. 4. Effect of estrogen and an estrogen receptor α specific agonist on (A) ultimate force, (B) stiffness, and (C) ultimate stress in rat ligaments. MCL, medial collateral ligament; ACL, anterior cruciate ligament; VEH, vehicle treated control rats; EE₂, estrogen treated rats; PPT, propyl pyrazole triol treated rats. Bars represent mean ± SD.
Fig. 5. Effect of null mutation of the gene encoding estrogen receptor \( \beta \) on (A) ultimate force, (B) stiffness, and (C) ultimate stress in mouse ligaments. MCL, medial collateral ligament; ACL, anterior cruciate ligament; WT, wild-type littermate mice; BERKO, estrogen receptor \( \beta \) homozygous mutant mice. Bars represent mean \( \pm \) SD.