Orthopaedic Surgery
School of Medicine
University of Missouri Health

Thompson Laboratory for Regenerative Orthopaedics

Metabolic Responses of ACL Explants to Estradiol and Pro-inflammatory Cytokine Stimulation

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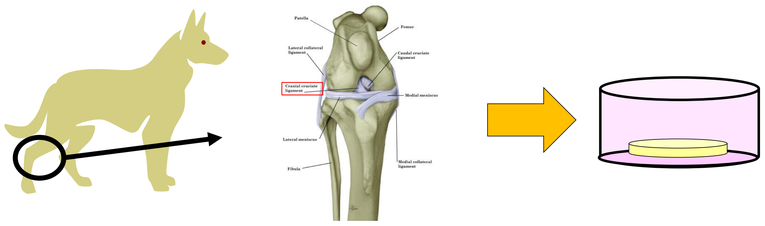
Introduction and Purpose

- Anterior cruciate ligament (ACL) injury in females are almost ten times more common than in males.
- Estrogen levels present during the menstrual cycle have been associated with increased ACL injury rates.
- The mechanistic effects of estrogen on the ACL that may contribute to the increased risk for rupture have not been fully elucidated
- Inflammatory changes in the joint may contribute to risk for ACL rupture and/or ACL reconstruction failure
- This study was designed to assess the responses of ACL explants to estrogen and pro-inflammatory cytokine stimulation.

Hypothesis

17-β estradiol and IL-1β stimulation, individually or in combination, will significantly increase production of degradative enzymes and pro-inflammatory cytokines in ACL explants when compared to unstimulated controls

Methods



1) With ACUC approval, ACL tissues were harvested from female dogs (n=12) euthanized for reasons unrelated to this study
2) Four 6mm explants were created using a dermal biopsy punch. Explants (n=12/group) were randomly assigned to one of four culture groups: (1) Control (ACL-N), (2) IL-1β (1ng/ml) stimulated (ACL-I), (3) Estrogen (300ng/mL) stimulated (ACL-E), or (4) Cytokine and estrogen stimulated (ACL-IE).

3) The explants were cultured for 12 days in supplemented DMEM at 37˚C and 5% CO_2, and media was changed and collected every 3 days for biomarker analysis.
4) Media were assessed for IL-6, IL-8, IL-10, IL-18, KC, MCP-1, TNF-α, nitric oxide, PGE-2, MMP-1, MMP-2, MMP-3, MMP-13, and MMP activity using commercially available assays according to manufacturer's instructions.
5) Significant differences between tissue types were determined by ANOVA with significance set at $p<0.05$.

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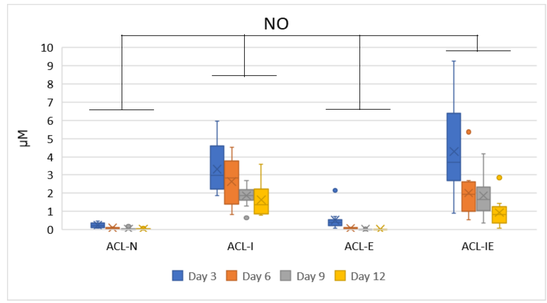
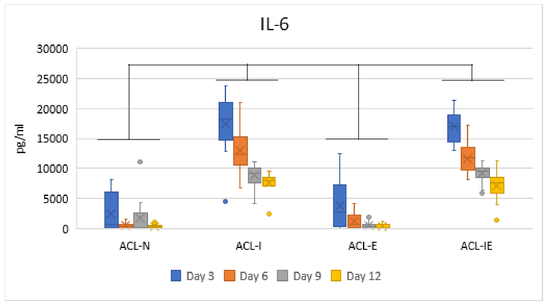
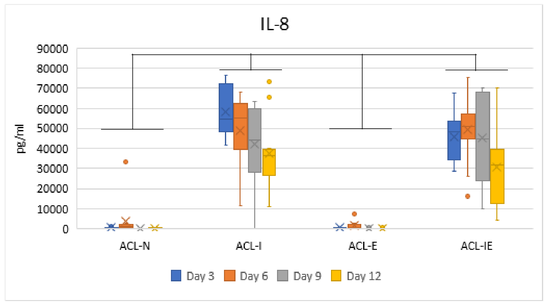
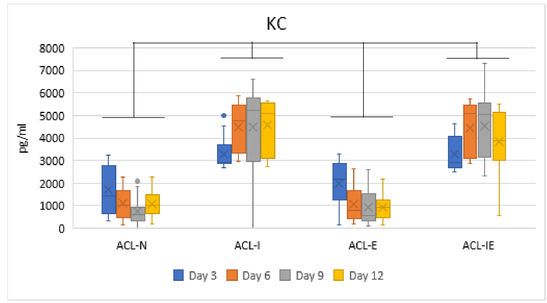
Results

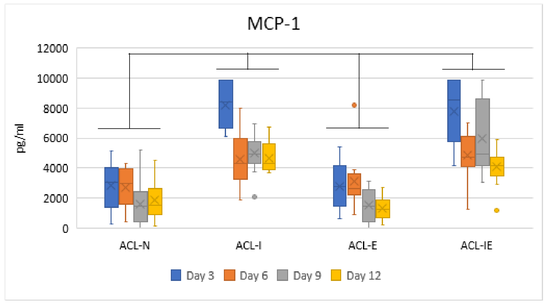
Inflammatory Biomarkers

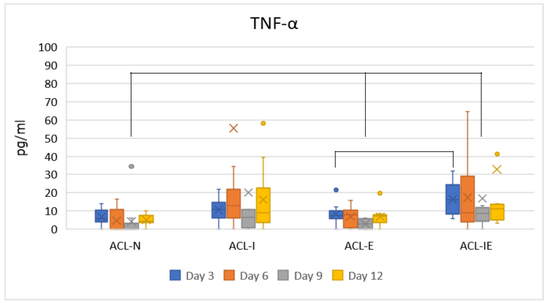


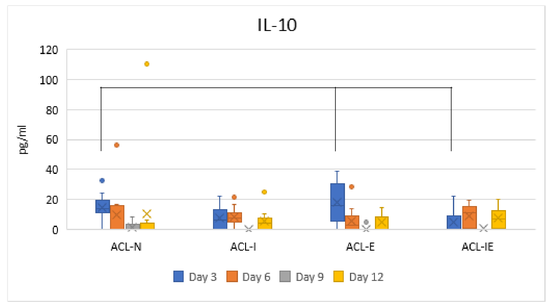


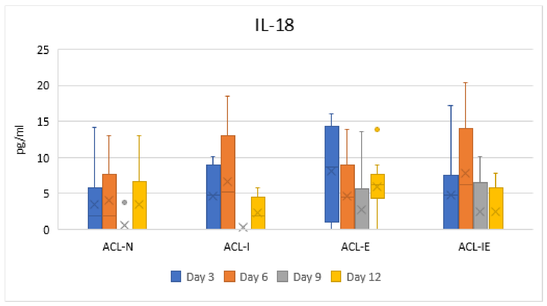








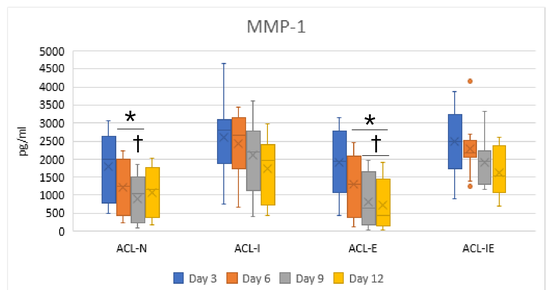


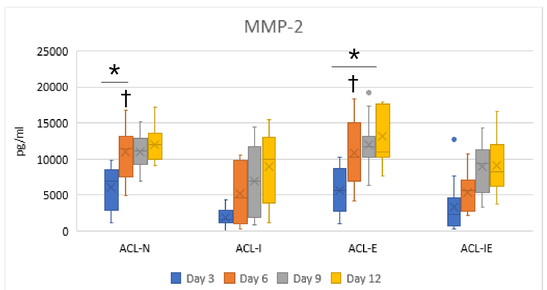


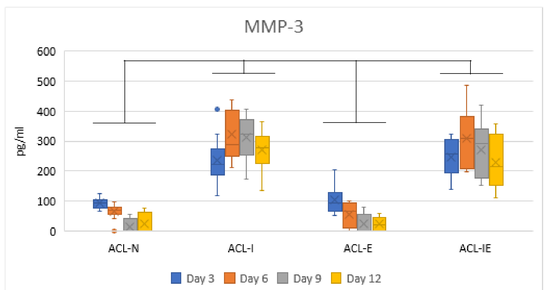
- NO, IL-6, IL-8, KC, and MCP-1 concentrations were significantly higher compared in the ACL-I and ACL-IE groups compared to the ACL-N and ACL-E groups across culture time points
- TNF-α was significantly higher in the ACL-IE group compared to the ACL-E group on day 3 of culture and the ACL-N and ACL-E groups on day 9 of culture
- IL-10 was significantly lower in the ACL-IE group compared to the ACL-N and ACL-E groups on day 3 of culture

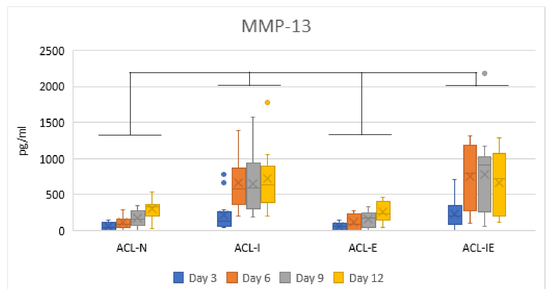
(Brackets indicate significant differences between groups)

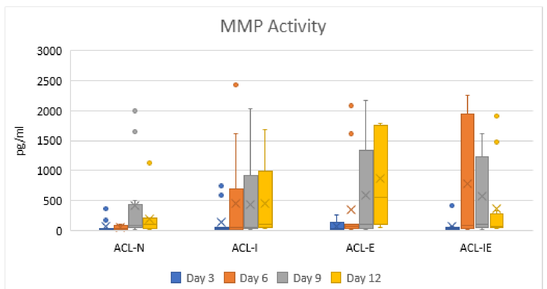
Degradative Biomarkers











- MMP-1 was significantly higher in the ACL-I group compared to the ACL-N (days 6-9) and ACL-E (days 6-12) groups
- MMP-1 was significantly higher in the ACL-IE group compared to the ACL-N (day 9) and ACL-E (days 9-12) groups
- MMP-2 was significantly lower in the ACL-I group compared the ACL-N (days 3-6) and ACL-E (days 3-9) groups
- MMP-3 and MMP-13 were significantly higher in the ACL-I and ACL-IE compared to the ACL-N and ACL-E groups across culture time points

Brackets indicate significant differences between groups
(*) significantly different than the ACL-I group
(†) significantly different than ACL-IE group, culture.

Conclusions

- The addition of estrogen to explant culture did not significantly affect ACL metabolism during culture compared to controls, nor did it consistently enhance or dampen pro-inflammatory cytokine-mediated effects.
- While estrogen was associated with transient increases in TNF-α and MMP-13 production by pro-inflammatory cytokine-stimulated ACL explants, these effects were not consistent, sustained, or statistically significant.
- Taken together, these results suggest that the effects of estrogen on risks for ACL rupture are not primarily mediated through exacerbation of inflammatory or degradative responses by the ACL itself.