Relaxin-2 Disrupts DNA Damage Repair and Induces Apoptosis in Female Rat Femur-ACL-Tibia Complexes

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INTRODUCTION: Relaxin-2 weakens ligament structural integrity by upregulating matrix-degrading enzymes and suppressing collagen synthesis. These effects are associated with higher rates of anterior cruciate ligament (ACL) tears in female athletes. Injury to the femur-ACL-tibia complex triggers a cellular response involving DNA damage and subsequent activation of DNA repair or apoptotic pathways. Whether relaxin-2 impairs DNA damage repair and promotes apoptosis in this complex remains unknown. This study investigated the effects of relaxin-2 on ring finger protein 168 (RNF168), an E3 ubiquitin ligase essential for DNA double-strand break repair, apoptosis and biomechanical properties in the femur-ACL-tibia complex of female rats.

METHODS: Female Sprague-Dawley rats (10-12 weeks old, 200-250 g) were randomly assigned to control (saline vehicle) or relaxin-2-treated groups (n = 12 per group). Relaxin-2 was administered daily by oral gavage at 20 μg/kg for 2 weeks. After treatment, blood samples were collected from each animal to evaluate circulating relaxin levels by ELISA. Biomechanical testing of the ACLs from both hind limbs was conducted to measure maximum load, stiffness, and ultimate tensile strength (UTS) using an ElectroPuls E3000 Linear-Torsion All-Electric Dynamic universal material testing machine (Instron). Immunohistochemistry (IHC) was performed in femur-ACL-tibia complexes to detect the relaxin-2 receptor and RNF168, and apoptotic cells were identified using TUNEL staining. Stained sections were imaged using a Nikon Eclipse Ni-E microscope. The number of positive stained cells was counted using ImageJ and normalized to the control group as a ratio. Animal experiments were approved by Rhode Island Hospital IACUC.

RESULTS: Relaxin-2 treatment significantly increased relaxin concentrations in blood and relaxin-2 receptor expression in the femur-ACL-tibia complexes. The number of cells expressing RNF168 was decreased in the ACL of relaxin-2-treated rats compared with controls. Apoptotic cell counts showed a trend toward higher apoptosis in the ACL of relaxin-2-treated group relative to controls. Maximum load, stiffness and UTS of the ACL were not significantly altered by relaxin-2 treatment.

CONCLUSION: Relaxin-2 increased receptor expression while reducing RNF168 levels, suggesting impaired DNA damage repair and enhanced apoptosis in the femur-ACL-tibia complexes of female rats. The biomechanical properties of the ACL were not impacted by relaxin-2 treatment.

DISCUSSION: RNF168 is a key E3 ubiquitin ligase that ubiquitylates histone H2A, facilitating the recruitment of DNA repair proteins such as BRCA1 and 53BP1. Reduced RNF168 expression may hinder DNA repair and shift the cellular response toward apoptosis when damage is extensive. Ongoing experiments aim to assess γH2AX, BRCA1, 53BP1, and XRCC1 for DNA damage and repair, and cleaved caspase-3 and annexin V for apoptosis, to further delineate relaxin-2's effects. Further studies are warranted to investigate how relaxin-2 decreases DNA damage repair leading to apoptosis. Although relaxin-2 has no effects on biomechanical properties, whether this increases susceptibility of the ACL to injury is unknown. Understanding these mechanisms could inform hormonal screening, training strategies, and therapeutic interventions for populations at high risk of ligament injury, such as female athletes.

SIGNIFICANCE/CLINICAL RELEVANCE: Our findings suggest a potential strategy to improve ACL repair outcomes by blocking relaxin-2 and promoting DNA damage repair pathway.

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Figure 1

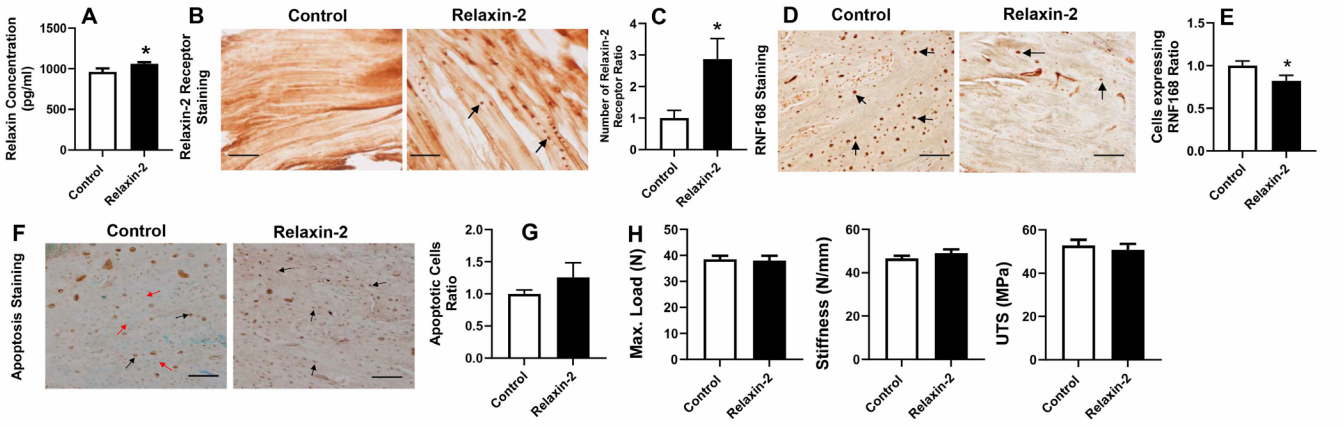

Figure 1. Relaxin-2 increased its receptor expression and reduced RNF168 expression in the femur-ACL-tibia complex of female rats. Female SD rats were administered with relaxin-2 daily by oral gavage at 20 μg/kg for 2 weeks. (A) Blood relaxin levels were detected by ELISA. N=12. (B,C) Relaxin-2 receptor expression by IHC and its quantification. Arrows denote positive cells. N=4. (D, E) RNF168 expression by IHC and its quantification. Arrows denote positive cells. N=11. (F, G) TUNEL staining and its quantification. N=12. Red arrows denote negative, while black arrows indicate positive cells.(H) Biomechanical properties of the ACL were tested. N=9-11. Mean ± SEM. *P<0.05 using an unpaired t test.