

Estrogen, But Not Progesterone, Reduces TNF-Induced Inflammation in Chondrocytes Via ER Alpha.

Emma Nunez¹, Meena Meiyappan¹, Elmira Mikaeiliagah, and Paula A Hernandez¹

¹University of Texas Southwestern Medical Center, Dallas, TX.

Emma.Nunez@UTSouthwestern.edu

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INTRODUCTION: Knee osteoarthritis (OA) affects 27.2 million US adults and is more prevalent in post-menopausal women, associated with the decrease in systemic estrogen levels [1,2]. Despite the well-documented differences in knee OA incidence and severity between males and females, a deeper understanding of whether there are sex-specific chondrocyte responses to inflammation and how sex hormones affect this response in a sex-specific manner is essential. Recent findings indicate that progesterone (P4) and 17-β Estradiol (E2) reduce the expression of catabolic and pro-inflammatory markers in postmenopausal chondrocytes [3], and E2 has been shown to reduce the nitric oxide (NO) production induced by Interleukin-1 beta (IL-1β) in female chondrocytes [4]. As circulating levels of tumor necrosis factor (TNF) and Interleukin-6 (IL-6) have been associated with knee radiographic OA [5], here we studied whether the expression of IL-6 triggered by TNF in chondrocytes depends on the biological sex and whether sex hormones (E2 and P4) are able to reduce this pro-inflammatory response. We hypothesize that E2 and P4 reduce the inflammatory response induced by TNF in both male and female cells.

METHODS: Chondrocytes were sourced from bovine knees (14♂, 14♀, 24-30 months old, equivalent to 18-24 years in humans) and seeded in DMEM with 10% FBS. **Treatment:** Media was replaced by estrogen-free media (phenol red-free DMEM, 10% charcoal-stripped FBS, 1x antibiotics-antimycotics, 1x GlutaMAX, 1mM sodium pyruvate) 24h prior to treatment. Cells were treated with or without TNF (10 ng/μl) for another 24h in the presence or absence of premenopausal concentrations of E2 (3.7 nM, 1 ng/ml) or P4 (20 nM). To evaluate estrogen receptor (ER) involvement, we included the specific antagonists MPP (ER-α antagonist, 100 nM) and PHTPP (ER-β antagonist, 100 nM), or the specific agonists PPT (ER-α agonist, 1 nM) or DPN (ER-β agonist, 5 nM). **NO production:** was determined from conditioned media using Griess Reagent (Promega). **qPCR:** mRNA expression of IL-6 was assessed by real-time PCR using TaqMan and a commercial probe (Bio-Rad). Relative expression was calculated using the 2^{-ΔCt} method, with YWHAZ as a normalizer. **Statistical analysis:** two-way ANOVA was used to evaluate the effect of treatment and sex, with Dunnett's correction for multiple comparisons and significance as p<0.05. Analysis was done in Prism GraphPad v.10.

RESULTS: The production of NO increased significantly in both male and female chondrocytes after TNF treatment, but it was significantly higher in female chondrocytes (Fig. 1A, n=14 per sex). The effect of sex and the interaction between sex and treatment trended to significance (p= 0.053 and 0.058, respectively). The mRNA expression of IL-6 was upregulated by TNF in both sexes, and resembling NO data, levels were significantly higher in female chondrocytes (Fig. 1B, n=14 per sex). P4 was unable to reduce both the NO release (Fig. 2A, n=4♂-5♀) and the upregulation of IL6 induced by TNF in both sexes (Fig. 2B, n=4♂-5♀). On the other hand, E2 decreased NO release in response to TNF treatment in both male and female cells (males p<0.0001, females p<0.05; Fig. 2C, n=8♂-9♀). Similarly, the TNF-induced IL6 upregulation was significantly reduced by E2 in both sexes (males p<0.05, females p<0.05; Fig. 2D, n=8♂-9♀). To further analyze the mechanism behind E2 effect, we inhibited the estrogen receptors α (ERα) and β (ERβ) using the specific antagonists MPP and PHTPP, respectively. Inhibition of ERα, but not ERβ, was able to block the E2 protection to TNF-induced inflammation in both sexes, for NO production (Fig. 3A, n=6♂-7♀) and for IL-6 upregulation (Fig. 3B, n=6♂-7♀). Likewise, preliminary data shows that specific activation of ERα, but not ERβ, was able to mimic the effect of E2 (Fig. 3C, 2♂-2♀), indicating that ERα, but not ERβ, is responsible for the protection to TNF-induced inflammation in both male and female chondrocytes.

DISCUSSION: We reveal sex differences in chondrocytes response to TNF-induced inflammation. Both NO production and IL-6 upregulation were elevated in female chondrocytes compared to male chondrocytes, indicating an exacerbated response to TNF in females. Interestingly, P4 did not reduce NO production nor IL-6 expression in both sexes despite prior research indicating anti-inflammatory effects in postmenopausal chondrocytes [3]. Here we used young chondrocytes exposed to TNF, therefore, it is possible that the P4 effect is context dependent (age, inflammatory status, cytokine involved, etc.). Opposite to P4, E2 effectively reduced NO production and IL-6 expression in both sexes. Contrary to our hypothesis, this indicates that only E2 is protective against pro-inflammatory conditions in male and female cells. Mechanistically, we observed that the effect of E2 reducing TNF-induced inflammation was triggered via ERα and not ERβ. These results indicate that ER-α signaling mechanisms have a chondroprotective effect under pro-inflammatory conditions. We are currently evaluating whether this mechanism also reduces the expression of ECM catabolic markers.

SIGNIFICANCE/CLINICAL RELEVANCE: Exploring how sex hormones influence the inflammatory response in chondrocytes based on biological sex may reveal critical factors driving sex-specific differences in the prevalence of knee OA. Furthermore, this can be informative for individuals undergoing hormonal treatments such as the transgender population, and females using contraceptives or hormone replacement therapy.

REFERENCES: [1] Ouyang & Dai, 2025; [2] Patel et al., 2024; [3] Gilmer et al., 2025; [4] Richette et al., 2007; [5] Stannus et al., 2010.

