

Sex hormones attenuate pro-inflammatory pathways in cells of the intervertebral disc in a cell-type specific manner.

Hutchinson, Jeffrey¹, Leung, Andrew^{1,2}, Séguin, Cheryle¹.

¹University of Western Ontario, ON, Canada, ²London Health Sciences Center, London, ON, Canada
Jhutch48@uwo.ca

AUTHOR DISCLOSURES

None

INTRODUCTION: The *Global Burden of Disease* study reported that low back pain is the leading cause of years lived with disability worldwide, with a lifetime prevalence of up to 84%.^{1,2} Back pain affects roughly 632 million people globally³ and is associated with an enormous socioeconomic impact. The occurrence of back pain increases with age; and while men are at higher risk for back pain early in life, women are more likely to experience back pain after the depletion of endogenous estrogen due to menopause.⁵ Though multifactorial, intervertebral disc (IVD) degeneration is associated with low back pain, in ~ 40% of cases.⁶ Despite its prevalence, current treatments are limited to physiotherapy and pain management. The current research is based on clinical observations of increased IVD volume in competitive athletes and suspected anabolic steroid users. These findings are unexpected as increased IVD volume is not normally seen and may provide a therapeutic target for IVD degeneration. Moreover, anabolic steroid and growth hormone injections are being used clinically based on a reported decrease in incidence and severity of patient self-reported back pain despite no investigations on their effects on the IVD. Given this, we hypothesize that exposure to steroid hormones will alter cell signaling within the IVD, resulting in increased cell proliferation and matrix synthesis, and decreased matrix degradation.

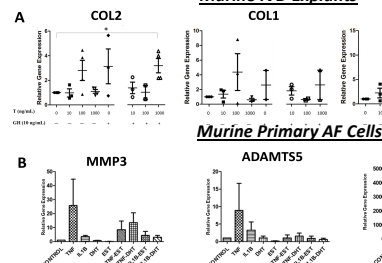
METHODS: Intact IVDs (NP, AF, and CEP) or primary AF cells were isolated from 2-month-old CD-1 mice and cultured in hypoxic (2% O₂), low glucose (1 g/L) conditions. Explants were cultured with testosterone (10, 100, 1000 ng/mL) with or without human growth hormone (10 ng/mL) for up to 7 days. Tissues were harvested for RNA isolation and RT-PCR or histological analysis using Safranin-O-Fast/Green staining (N=4). AF cells were cultured in pro-inflammatory conditions (TNF α ; 25 ng/mL, or IL-1 β ; 10 ng/mL) prior to hormone treatment (dihydrotestosterone; 100 nM, 17 β -estradiol; 100 nM) to assess the effects of hormone treatment in a model of IVD inflammation (N=3). To characterize cell type-specific effects, primary nucleus pulposus (NP) and annulus fibrosus (AF) cells were isolated from bovine caudal IVDs and cultured in micromass in hypoxic, low glucose, and low serum (2% O₂, 1 g/L glucose, 1% FBS). Cells were treated with 5 α -dihydrotestosterone or 17 β -estradiol (0 to 125 nM) for 72 h alone or following pre-treatment with TNF α (25 ng/mL) or IL-1 β (10 ng/mL) to stimulate proinflammatory pathways (N=5). Cells were harvested for RT-PCR analysis of extracellular matrix gene and pro-inflammatory gene expression. Data presented as mean \pm SEM. Analyzed using One-way ANOVA and Tukey's Multiple Comparisons test. * p-value <0.05.

RESULTS: Culture of murine IVD explants with testosterone and growth hormone showed synergistic effects on anabolic gene expression, inducing a significant increase in Type 2 collagen expression (Fig 1A). Histologic evaluation demonstrated the accumulation of hypertrophic cells in the AF of testosterone treated IVDs. In primary murine AF cells, extracellular matrix gene expression and cell proliferation were unaffected, but both testosterone and estrogen attenuated cytokine-induced inflammatory gene expression (Fig 1B). In bovine cell cultures, acute exposure to testosterone or estradiol did not significantly alter the expression of extracellular matrix genes in NP or AF cells. However, when we recapitulated the pro-inflammatory microenvironment of IVD degeneration by pre-treating cells with pro-inflammatory cytokines prior to steroid hormone treatment, significant changes were observed in a cell-type and cytokine specific manner. IL-1 β stimulation induced the expression of matrix degrading enzymes (MMP3, ADAMTS5) and IL-6 in NP cells (Fig 2B), while TNF α treatment induced a similar response in AF cells (Fig 2C). In NP cells, exposure to either testosterone or estradiol attenuated the pro-inflammatory response induced by IL-1 β . However, in AF cells only testosterone attenuated the pro-inflammatory response induced by TNF α , not IL-1 β .

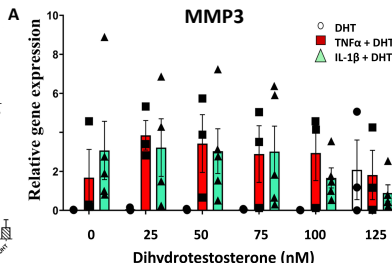
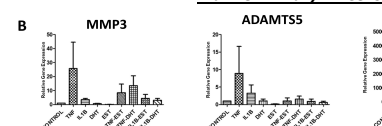
DISCUSSION: Given that anabolic steroids are a controlled substance in many countries, including Canada and the US, doses for anabolic effects are widely variable depending on frequency, route of admission, and formulation of steroid⁷⁻⁹. Our study reports dose response curves for 5 α -dihydrotestosterone and 17 β -estradiol for NP and AF cells in healthy and pro-inflammatory environments to establish biologically relevant doses. Although it has been suggested that sex hormones regulate IVD health and degeneration^{7,10,11}, their mechanisms and potential as a therapeutic remains unclear. Our study demonstrates that in the IVD, steroid hormones can both regulate anabolic pathways and attenuate pro-inflammatory gene expression in a cell type-specific manner. Future work will assess how signaling pathways such as NF-kB or MAPK, are regulated by sex hormone exposure and explore the effects of steroid hormones in-vivo, where crosstalk between IVD tissues may regulate the biological response.

SIGNIFICANCE: While current treatments for disc degeneration are limited, our findings suggest that steroid hormone treatment may modulate inflammatory pathways in the IVD, providing a potential novel therapeutic intervention.

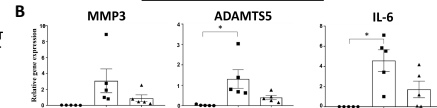
Murine IVD Explants



Murine Primary AF Cells



Bovine Nucleus Pulposus



Bovine Annulus Fibrosus

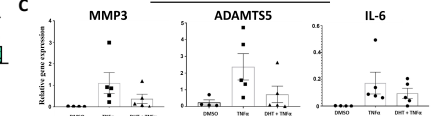


Figure 1. Gene expression analysis of murine (A) IVD explants following 7 days of testosterone +/- growth hormone treatment, or (B) primary AF cells following 72 hours TNF α or IL-1 β treatment, with or without dihydrotestosterone (DHT) or 17 β -estradiol (EST). Relative gene expression was assessed using $\Delta\Delta C_t$, normalized using the housekeeping genes HPRT and RPS29 and expressed relative to vehicle control.

Figure 2. Gene expression analysis of bovine NP and AF cells pre-treated with IL-1 β (10 ng/mL) or TNF α (25 ng/mL), followed by 5 α -dihydrotestosterone or 17 β -estradiol for 72 hours. A. Representative dose response curve for MMP3 gene expression in NP cells treated for 72 hr with dihydrotestosterone (DHT) following 24 hr pre-treatment with TNF α (25 ng/mL, red) or IL-1 β (10 ng/mL, green). Robust doses for NP (B) and AF (C) cells display attenuation of inflammatory gene expression. Transcript levels were calculated relative to a 5-point standard curve made.

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