***Testosterone* Therapy, Friend or Foe for the Aging Spine**

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Introduction: The most recent *Global Burden of Disease* study identified back pain as the single most common cause of disability worldwide, with a socioeconomic impact estimated at $100 billion annually. Chronic back pain has a lifetime prevalence of over 80% in Canada. Though complex, back pain is associated with intervertebral disc (IVD) degeneration in 40% of cases. Despite its prevalence and tremendous socioeconomic impact, the etiology of IVD degeneration remains largely unknown, and there are no disease-modifying treatments.

The current research is motivated by observations of increased IVD volume in competitive athletes and suspected anabolic steroid users, made by our clinical collaborator. This phenomenon is similar to that characterized as hyperconcavity of the vertebral endplates (HEPS) reported in professional football players and suspected anabolic steroid users, associated with decreased incidences of back pain compared to age-matched athletes within the same discipline. These findings are unexpected as increased IVD volume is not typically observed and may provide a therapeutic target for IVD degeneration. These findings have led to the hypothesis that HEPS may represent a beneficial adaptation and prompted the exploration of testosterone as a potential therapeutic target for IVD degeneration. However, this interpretation is complicated by the fact that endplate concavity is a traditional hallmark of aging and disc degeneration. Despite this uncertainty, select clinics are administering localized injections of testosterone for chronic low back pain, based on a single case study of reduced patient-reported pain, without understanding the short- or long-term consequences for spine health. To date, there are no studies investigating how anabolic steroids influence IVD biology, biomechanics, or how their effects change with age. We conducted a longitudinal study to assess the effects of testosterone on age-associated degeneration and IVD tissue repair following injury, delivering supraphysiological doses of testosterone to mice (modeling anabolic steroid use) through weekly injections following a caudal IVD injury. *We hypothesized that exposure to supraphysiological levels of testosterone would promote IVD tissue building, resulting in increased disc height and delaying or preventing injury-associated IVD degeneration.*

Methods: At 16.5 months of age, caudal IVD injury was induced through x-ray-guided needle puncture using a 30-gauge needle. Following injury, mice were treated weekly with testosterone enanthate (15 mg/kg) (N=8 mice/group) or vehicle control by subcutaneous injection. Mice were euthanized after 6 weeks (at 18 months of age), and intact spines were isolated for µCT analysis to assess disc height, subchondral bone properties, and vertebral bone parameters. Histopathological analysis was subsequently used to assess degeneration of the lumbar and caudal spine, as well as osteoarthritis of the knee using both midsagittal and coronal sections. Whole body composition was assessed through quantitative magnetic resonance imaging (qMRI) at the endpoint. µCT data were assessed using a parametric Student's T test, or one-way ANOVA, and histopathology was assessed using Friedman's two-way analysis of variance by ranks with Bonferroni correction, or the Mann-Whitney U test. All animal procedures were conducted in accordance with policies and guidelines set forth by the Canadian Council on Animal Care and approved by the Animal Use Subcommittee of the University of Western Ontario (AUP 2022-179).

Results: Following prolonged *in vivo* administration, systemic testosterone injections significantly increased body weight compared to vehicle controls. Surprisingly, there were no differences in overall body composition (lean, adipose, and bone) induced by testosterone. µCT analysis showed that testosterone induced hyperconcavity of the vertebral endplate in the lumbar spine (**Figure 1B, red box**), as well as an increase in disc height in the lower lumbar spine compared to vehicle-treated controls (**Figure 1A**). Increased IVD height and vertebral endplate hyperconcavity in the lumbar spine was associated with loss of subchondral and vertebral bone volume, trabecular number and trabecular thickness. Histopathological analysis in the lumbar spine showed no differences in degenerative features in the NP, AF, or cartilage endplates in testosterone-treated mice compared to controls. However, we noted differences in NP and endplate morphology in IVDs, which were not captured in the IVD degeneration scoring system--, specifically increased height and altered matrix organization (**Figure 1B**). The loss of disc height following caudal IVD puncture (**Figure 2**) was partially restored in testosterone treated mice (**Figure 2D)** although this was not associated with changes in IVD histopathology (**Figure 2E**). Knee histopathology revealed severe osteoarthritis in all groups by 18 months of age, which was unaffected by testosterone treatment.

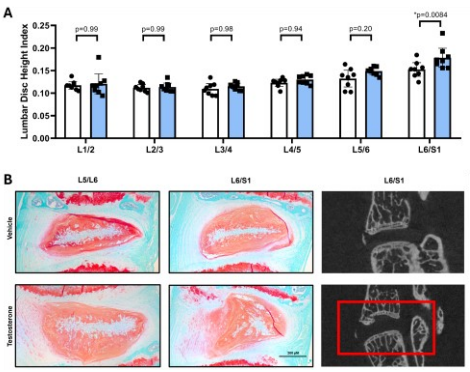

Figure 1. Testosterone increases disc height in the lumbar spine, and induced HEPS at the L6/S1 IVD. (**A**) Disc height was significantly increased at the L6/S1 motion segment. (**B**) Midsagittal sections of lumbar IVDs stained with Safranin-O Fast Green. Organization of NP matrix and morphology were altered in discs displaying HEPS, confirmed by µCT (red box). N=8 mice per group.

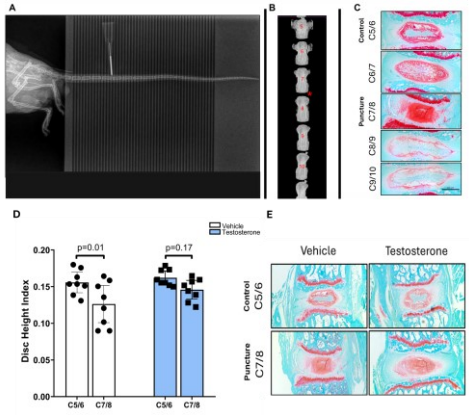

Figure 2. Loss of caudal IVD height following injury was rescued by anabolic steroid use. (A) Caudal IVD needle puncture **(B)** Location of injury **(C)** representative histopathology 1-week following injury **(D)** Disc height was reduced by anabolic steroid exposure after expected loss by needle-puncture injury at the C7/8 IVD compared to an uninjured internal control (C5/6). (**E**) Midsagittal sections of caudal IVDs stained with Safranin-O Fast Green. N=8 mice per

Discussion: While the use of testosterone for musculoskeletal conditions is becoming increasingly common, our study using aged male CD-1 mice demonstrates that supraphysiological testosterone had context-dependent effects. Supraphysiological doses of testosterone induced endplate hyperconcavity and increased disc height in the lumbar spine, resembling clinical observations in athletes and anabolic steroid users. However, the increased disc volume was associated with a loss of subchondral and vertebral bone volume, and trabecular number and thickness. Moreover, although testosterone partially restored disc height following caudal IVD puncture, histopathological analysis revealed no improvement in degeneration. Here, we did not assess changes in biomechanical properties after anabolic steroid use, though these properties are likely impacted given the observed changes in disc height and matrix organization in discs displaying HEPS. Since alterations in joint biomechanics caused by altered tissue composition can increase the onset and severity of disc degeneration, we postulate the observed changes may be detrimental to tissue health in the long term. We acknowledge our findings are limited by the use of only male mice, and will address this limitation in future studies using female mice, with alterations to the study design to prevent heightened estrogenic effects by the active conversion of testosterone to estrogen by using a non-aromatizable testosterone such as dihydrotestosterone, as used by female athletes.

Significance: This study demonstrates that while testosterone improved disc height index in the lumbar spine and promoted repair following caudal IVD injury, there were minimal differences at the level of histopathology. Instead, we observed differences in vertebral and subchondral bone parameters, likely impacting tissue biomechanics. Future work on anabolic steroid use should address changes in IVD and vertebral bone biomechanics, as well as the complex interactions of testosterone with the various tissues of the spinal motion segments to clarify the short- and long-term impacts on spine health.

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