INTRODUCTION:
Torsion is an important loading mode of the intervertebral disc (IVD) and increased torsional range of motion has been associated with clinical symptoms from IVD disruption [1]. IVDs have been studied extensively in compression but there are few studies investigating torsion loading in vivo. In general, torsion results in interlaminar shear and tension of annulus fibrosus (AF) fibers while axial compression leads to substantially greater nucleus pulposus (NP) pressurization and tensile hoop stresses in the AF. The purpose of this study is to investigate effects of torsion on promoting biosynthesis and producing injury in rat caudal IVDs through measurements of mRNA expression in a short-term in vivo study. We hypothesize that: 1) cyclic torsion will up-regulate collagen and elastin mRNA expression in the AF with minimal changes in NP mRNA expression; 2) large amplitude torsion will induce injury detectable with increased proinflammatory cytokines IL-1β and IL-6 expression; 3) cyclic torsion will promote greater mRNA expression than static torsion.

METHODS:
As approved by the university IACUC, 50 skeletally mature Sprague–Dawley rats were instrumented with an Ilizarov-type device across caudal disc c-2-9. After a four-day surgical recovery, all animals were anesthetized for 90 minutes. Rats were divided into five groups: cyclic torsion to ±5°, ±15° and ±30°, static torsion to +30° (static), and sham (device was implanted but no loading applied). Cyclic torsion in rotation control was applied with a newly designed loading device at 1 Hz sinusoidally for 90 minutes (Fig. 1). The static group consisted of a ramp to +30° that was held for 90 minutes. Torque and angle measurements were taken during the loading period. At 24hrs following loading, animals were euthanized and the instrumented disc (c-8-9) and internal controls (c-6-7 and c-10-11) were harvested. AF and NP tissue were separately analyzed for gene expression using real-time RT-PCR with 18S RNA as endogenous control (ΔCt) and relative levels of genes in instrumented discs were normalized to internal controls (ΔΔCt) as described previously [2]. ANOVA with Fisher’s PLSD (p<0.05) was performed to determine if loaded groups differed from sham; paired Student’s t-test was used to determine effects of sham on mRNA expression.

RESULTS:
Elastin mRNA expression was significantly (3-fold) up-regulated in the AF (Fig. 2) in response to cyclic torsion at all amplitudes. Aggrecan and collagen II in AF and NP showed a trend of up-regulation with increased cyclic torsion amplitude that was significant at ±30° for aggrecan in the NP (2 fold, p<0.05) and marginally significant for the AF (2 fold, p<0.1). ADAMTS4 was the only catabolic gene significantly up-regulated in the AF (±30°) and NP (±15°) groups. A significant increase of TIMP3 in the nucleus pulposus was measured for ±30°. The static condition showed some trends of upregulation for similar genes as cyclic ±30° but this was not significant. No significant changes in expression of proinflammatory cytokines IL-1β and IL-6 in AF or NP regions were detected.

DISCUSSION:
The most important finding of this study was that cyclic torsion up-regulated elastin gene expression in the AF with minimal alterations in NP mRNA expression. The strong elastin up-regulation in the AF in response to torsion combined with a lack of significant upregulation in collagen I & II gene expression indicated elastin production is mechanically sensitive to tissue shearing. With increasing torsion amplitude no sustained changes were measured in proinflammatory cytokines suggesting that damage was not induced by static or cyclic torsion at 30°. Together, results supports the prior suggestion that elastin promotes AF tissue resilience while resisting damage under shearing [3]. Cyclic torsion stimulated mRNA expression more than static torsion consistent with hypothesis 3 and literature on stimulatory effects of cyclic loads and inhibitory effects of immobilization and static loads.

When comparing mRNA expression in the IVD with prior cyclic compression results [2], cyclic torsion resulted in elastin production while cyclic compression resulted in significant and greater levels of aggrecan, and collagen2 production particularly in the NP. Some aggrecan and ADAMTS4 mRNA stimulation in NP and AF under torsion suggests small amounts of pressurization in the IVD due to volumes of high torsion loading amplitudes.

We conclude that IVD cells in vivo have distinct responses to torsion and compression. Results highlight the importance of elastin in torsional dynamic loading with mRNA findings suggesting the concept that elastin remodels more than collagen in response to shearing.

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