Hierarchical Evaluation of Mechanically Induced Growth Modulation of the Spine in a Growing Pig Model

Madeline Boyes[1], Axel C. Moore[2], Julie Engiles[1], Benjamin Sinder [3], Klaus Hopster[1], Jason Anari[3], Sriram Balasubramanian[4], Edward Vresilovic[2], Dawn M. Elliott[2], Thomas P. Schaer[1], Brian D. Snyder[5], Patrick J. Cahill[3]

[1]University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA, [2]University of Delaware, Newark, DE, [3]Children's Hospital of Philadelphia, Philadelphia, PA, [4]Drexel University, Philadelphia, PA, [5]Boston Children's Hospital, Boston, MA.

mboyes@vet.upenn.edu

Disclosures: Ed Vresilovic (3A,4-Camber), Dawn Elliott (9-ORS), Thomas Schaer (1-PSI, 1,3B,4,5-ReGelTec, 3B-Peptilogics, 3B,4,5-Acuitive Technologies, 3C-PAX Therapeutics, 3C-OrimTech, 3C,5-SINTX Technologies, 3C-OsteoCentric Technologies, 5-DePuy Synthes, 5-Alcyone Therapeutics, 5-Camber Spine, 6-Heraeus), Brian Snyder (3B-Orthopediatrics)

INTRODUCTION: Intervertebral disc (IVD) distortion contributes initially to scoliotic spine deformity in juvenile and adolescent scoliosis, with subsequent vertebral wedging as deformity progresses. [1]. Ian Stokes [2] hypothesized that spinal deformity progresses as a "vicious cycle" due to asymmetric stresses/strains applied to the growing spine over time and space. Predicated on the Heuter-Volkmann principle (i.e., tension applied to an apophysis/physis stimulates growth, while compression inhibits growth), interventions such as Vertebral Body Tethering (VBT) attempt to correct scoliosis, while preserving spinal motion, by predictably modulating spine growth via mechanical manipulation of affected functional spinal units (FSU) via the application of compression across the convexity of the scoliotic curvature. However, there is evidence that cartilaginous endplate thickening secondary to compression may reduce tissue diffusivity and lead to disc degeneration. We created a reciprocal model of scoliosis by applying a posterolateral tether to a straight spine to induce an asymmetrical lateral bending moment to provoke scoliosis in a growing pig. The objective of this work is to investigate the effect of asymmetric spine loading on tissue remodeling and FSU mechanics at a hierarchical level.

METHODS: Under IACUC approval, to incite a progressive scoliosis, three rapidly growing 12-wk old Yorkshire pigs were instrumented with a subcutaneous CoCr cable tether spanning thoracolumbar (TL) and lumbar (L) vertebrae to create a lateral bending moment. Changes to the vertebral body (VB) and IVD anatomy over time and space were measured by serial CT, plane radiographs and MRI (T1-FLASH, T2-CPMG). CT and radiographic images were used to monitor deformity progression (Cobb angle) and vertebral body growth, while MRI was used to evaluate IVD tissue composition and geometry. MRI post-acquisition analysis included calculating T2* and ΔT1 relaxation times of the nucleus pulposus (NP) outlined manually on a mid-sagittal slice through each IVD. ΔT1 relaxation times were derived from pre and post gadolinium contrast injection images, where: ΔT1=(T1pre-T1post)/T1pre*100. Pigs were sacrificed at 20-wks after deformity generation; functional spine units (FSU) were isolated at the apex of the induced deformity for µCT and histology. Additional FSUs within the instrumented region were mechanically tested under a combined axial load of 0.54 MPa and lateral bending ± 3° for 5 cycles.

RESULTS: Acutely, scoliosis (17°) was mediated by IVD wedging. From 6-12 wks., scoliotic deformity (35-45°) was shared between TL (45-38%) and L (41-47%) regions, with wedging of both the IVD (40-46%) and VB (60-54%) contributing to the overall scoliosis. By 19 wks., deformity (58°) was mainly imparted by VB wedging (**Fig 1A**). MRI and histology (**Fig 1A,B,D**) demonstrate translation of the NP from the concavity to the convexity of the curved spinal segments. MRI ΔT1 relaxation times 10-wks after tethering demonstrated a 5% reduction in T1 relaxation time at all instrumented levels, relative to un-instrumented control segments, which progressively decreased to 45% in ΔT1 @ T13-T14-L1-L2-L3 IVD 19-wks after tethering (**Fig 2**). µCT (**Fig 1C**) and histology (**Fig 1I,J**) suggest that compression inhibits physeal growth manifest by diminished epiphyseal height at the concavity of the scoliosis and sclerotic bone remodeling as evidenced by: ↑6.4% bone volume fraction; ↓17.8% bony endplate (BEP) cortical porosity. Cartilaginous endplate (CEP) thickening may reduce tissue diffusivity, leading to IVD degradation as evidenced by degeneration of the annulus fibrosus (AF), indicated by chondroid metaplasia and fibrillation of the inner AF rings (**Fig 1D-G**); degeneration of the NP, indicated by multifocal loss of notochordal cells (NC) and extracellular matrix with NC necrosis (**Fig 1H**). FSU mechanics (stiffness) remained relatively symmetric, unaffected by the induced anatomic and histologic tissue asymmetry (**Fig 3**).

DISCUSSION These multi-level, hierarchical data (MRI, µCT, Histo) indicate that mechanically induced asymmetric spine growth affects multiple tissues: endplate, bone, AF, NP, and vasculature. Compression provoked IVD distortion and degenerative processes, initiated by CEP thickening that may reduce endplate diffusivity, followed by compression mediated physeal growth inhibition, that resulted in vertebral wedging. Vertebral wedging was accompanied by increased cortical thickness, decreased cortical porosity, and increased bone volume fraction at the vertebral endplate (VEP). These structural changes serve to decrease small molecule transport across the VEP, corroborated by altered Gd contrast diffusion (**Fig 2**), thereby impairing nutrient flow to the NP. Both macroscopic imaging (CT/MRI) and microscopic histological analysis of the asymmetrically loaded IVDs revealed degenerative changes consistent with IVD degeneration observed in human and goat models of IVD degeneration [3].

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding the multi-scale osseous and non-osseous tissue adaptations to asymmetric loading of the spine in growing children and adolescents is essential for the development of guided growth interventions that effectively mediate scoliosis without deleteriously affecting the health of the IVD. These data reveal that asymmetric compressive loading of the spine provoked by a posterolateral tether resulted in vascular remodeling and structural changes to the cartilaginous and bony endplate that reduced small molecule transport into the IVD, which incited IVD degeneration.

REFERENCES: [1] Will RE, et al. 2009, Spine. [2] Stokes IA. 2007, Eur Spine J. [3] Gullbrand, SE. et al. 2017. Osteoarthritis Cartilage.

ACKNOWLEDGEMENTS: This research was supported by The Wyss/Campbell Center for Thoracic Insufficiency (Children's Hospital of Philadelphia)

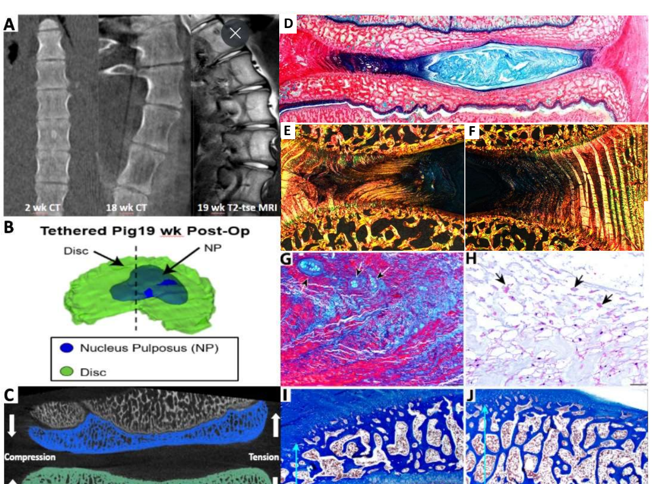

Figure 1. (**A**) CT and MRI coronal views demonstrate progressive vertebral body wedging and NP displacement towards convex side; (**B**) highlighted by 3D reconstruction. (**C**) µCT and histology (**D-J**) at the apex of deformity T13-T14 reveal narrowing of the IVD and of the endplate epiphysis at *concave side (compression)* relative to *the convex side (tension)*. (**C, I, J**) Endplate epiphysis shows narrowing and bone sclerosis at (**I**) concave side relative to (**J**) convex side. (D, G) Non-polarized and (**E, F**) polarized sections demonstrate asymmetric distortion of AF fiber alignment at concave side including fibrillation of inner AF and (**G arrows**) chondroid metaplasia. (**D**) Hisology confirms NP displacement toward convex side observed in MRI (**A**,**B**). NP shows cell necrosis (**H, arrows**).

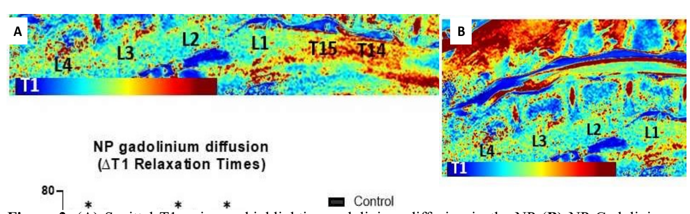

Figure 2. (**A**) Sagittal T1-w image highlighting gadolinium diffusion in the NP (**B**) NP Gadolinium Diffusion depicts significant reductions in Δ*T*1 relaxation times, indicating reduction in small molecule transport into the NP of the tethered levels compared to control discs. (**Black**=Control; **Blue**= 10 weeks Postop; **Yellow**=19 Weeks Postop).

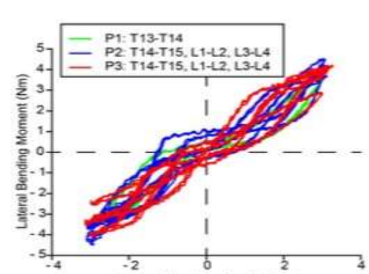

Figure 3. Lateral bending moment vs angular for 7 FSU within the tethered levels from 3 animals demonstrates disc-level mechanical symmetry.