Intervertebral Disc Apparent Torsional Modulus Is Elevated In Lubricin Knockout Mice

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INTRODUCTION:
Lubricin, also called superficial zone protein (SZP) or Proteoglycan 4 (PRG4), was first identified as the boundary lubricant in synovial fluid. In diarthrodial joints, lubricin is produced by superficial zone chondrocytes and synovial lining cells. It is present in the superficial layer of articular cartilage and coats the articular surface, decreasing surface friction. Lubricin has also been found surrounding the collagen bundles of the meniscus, in the substance of the anterior cruciate and lateral collateral ligaments, and surrounding tendon fascicles in multiple locations, such as the rotator cuff and finger flexors. In these locations, lubricin is thought to facilitate interfascicular motion and prevent adhesion formation in tendons. A recent study examining caprine intervertebral discs found that lubricin is localized between the lamellae of the outer annulus fibrosus. Lubricin could potentially facilitate interlamellar lubrication when rotary loads are applied to the disc, a role that would be consistent with its function in other collagenous tissues.

The purpose of this study was to evaluate the mechanical consequences of lubricin deficiency on the mechanical properties of the intervertebral disc using the lubricin null mouse Prg4−/−. Apparent torsional modulus, which is a measure of stiffness normalized to disc geometry, was compared between wild type (WT) Prg4+/+ and lubricin null (KO) Prg4−/− specimens. We hypothesized that the absence of lubricin would result in greater disc apparent torsional modulus in KO animals compared to WT.

METHODS:
Lumbar spines were obtained from mice that were harvested according to an approved IACUC protocol. The KO specimens were obtained from a colony of Prg4−/− mice maintained on the BL6 background strain. Lumbar L1-2 and L3-4 segments from adult mice 2-9 months of age were used for mechanical testing: WT (n=21) and KO (n=14). AP and lateral radiographs of each spine were obtained prior to dissection, confirming the absence degenerative disc disease.

Prior to testing, the surrounding soft tissues and posterior elements were removed to isolate the L1-2 and L3-4 vertebral body-disc-vertebral body motion segments. The motion segments were tested in torsion using an ELF 3200 (Bose, Framingham, MA). Prior to testing, each specimen was fixed within the test frame by gluing the ends of the bone to the frame. After one vertebral body was glued in place, the frame was adjusted to accommodate the height of the motion segment. The other vertebral body was then glued in place. During testing, the superior vertebra was rotated relative to the fixed base vertebra while the torque cell and angular rotations were recorded.

The torsional stiffness and the apparent torsional modulus were calculated according to the methods of Elliot and Sarver. The torsional stiffness (K, N-mm/rad) was calculated via linear regression of the torque-rotation curve (M-Θ) up to 20 degrees of rotation. The yield torque and yield rotation values were also recorded. The apparent torsional modulus (G₀, MPa) was then calculated using equation 1:

\[ G₀ = \frac{K}{J} \]

where K is the measured stiffness, h is the disc height (mm), and J is the polar moment of inertia (mm⁴). J was defined by Equation 2:

\[ J = \frac{1}{6}W_{\text{ap}}W_{\text{ax}}W_{\text{az}} - \left[ N_{\text{ax}}N_{\text{az}} - N_{\text{ax}}N_{\text{az}}^2 \right] / 64 \]

where Wₐₐ is the anteroposterior width, and Wₐₐ is the lateral width of the outer disc as measured using digital calipers, and N is the width of the nucleus pulposus. This was estimated to be 0.2 of the total disc area and located centrally in the disc.

The apparent torsional modulus, yield torque and yield rotation were compared between groups using paired, two-tailed t-tests. Immunohistochemistry on an additional set of discs were performed to determine if lubricin was present in the annulus fibrosus of the WT and absent in the KO discs.

RESULTS:
Immunohistochemistry verified that lubricin was present in the the annulus fibrosus of the WT and HET specimens, but not in the KO specimens. Yield torques and yield rotations did not significantly differ between groups (p>0.05) (Table 1). The mean value for the yield rotation among all groups was 34.2 degrees. Calculating the apparent torsional modulus from data up to 20 degrees rotation was therefore less than 60% of the yield rotation. Mean K values were consistent with linear decay: 0.94-0.97. Apparent torsional modulus was significantly increased in the KO group compared to WT (p=0.014) (Figure 1).

### Table 1: Yield point torque, yield point rotation, and stiffness (±standard deviation)

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>KO</th>
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<tr>
<td>Torque (N-mm)</td>
<td>1.97(±0.75)</td>
<td>2.60(±1.30)</td>
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<tr>
<td>Rotation (deg)</td>
<td>35.6(±16.6)</td>
<td>32.5(±6.6)</td>
</tr>
<tr>
<td>Stiffness (N-mm/rad)</td>
<td>2.88(±1.3)</td>
<td>3.85(±2.0)</td>
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Figure 1: Apparent torsional modulus calculated at 20 degrees (MPa)

DISCUSSION:
The results of our study confirm the hypothesis that the absence of lubricin results in greater apparent torsional modulus of the mouse intervertebral disc. These findings are consistent with the proposed function of lubricin as an interfascicular lubricant in highly collagenous tissues. Future investigations will focus on the age related consequences of the lubricin deficiency on intervertebral disc mechanics and spinal alignment in older WT and lubricin null animals. It is interesting to note that patients affected by camptodactyly-arthropathy-coxa vara-pericarditis syndrome (CACP), which is recapitulated in the lubricin null mouse, may develop spinal deformities including scoliosis, kyphosis, or lordosis. One limitation of this study is that it did not include specimens greater than 9 months of age. Future investigations will focus on the age related consequences of the lubricin deficiency on intervertebral disc mechanics and spinal alignment in older wild type and knockout animals. To our knowledge, no prior study has investigated the role of lubricin on the mechanical function of the intervertebral disc. Another issue to be examined in future studies is what role altered lubricin expression or inflammatory damage may play in disc disease in individuals not afflicted with CACP.

REFERENCES: