SHORT COMMUNICATION

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Disc geometry measurement methods affect reported compressive mechanics by up to 65%

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Abstract

Mechanical testing is a valuable tool for assessing intervertebral disc health, but the wide range of testing protocols makes it difficult to compare results from different studies. Normalizing mechanical properties by disc geometry allows for such comparisons, but there is little consistency in the methods by which disc geometry is measured. As such, we hypothesized that methods used to measure disc geometry would impact reported mechanical properties. Disc height and area were measured using computed tomography (CT), digital calipers, and ImageJ to yield three different measurements for disc height and six for disc area. Disc heights measured by digital calipers ex situ were >30% less than disc heights measured in situ by CT, and disc areas measured ex situ using ImageJ were >30% larger than those measured by CT. This significantly affected reported mechanical properties, leading to a 65% reduction in normalized compressive stiffness in the most extreme case. Though we cannot quantitatively correct between methods, results presented in this study suggest that disc geometry measurement methods have a significant impact on normalized mechanical properties and should be accounted for when comparing results.

KEYWORDS
compressive stiffness, disc area, disc height, interverbal disc geometry, measurement methods

1 | INTRODUCTION

Lower back pain (LBP) is a common and often debilitating condition that affects between 70% and 80% of all adults at some point in their lives, with an age-standardized point prevalence of 7.5% in 2017.[1,2] It is estimated that approximately 40% of LBP cases arise from intervertebral disc degeneration,[3] a progressive and irreversible condition in which the disc undergoes a series of structural, biochemical, and mechanical changes.[4,5] Importantly, disc degeneration has strong links to biomechanics and can be triggered by mechanical overloading,[4] so mechanical testing of the disc and its sub-tissues has been extensively conducted.[6–14] However, comparing across different studies can be challenging due to different experimental parameters such as type of test, loading rate and magnitude, hydration, species, and more.[12] Assuming the disc behaves as a single material, normalizing experimental data by disc geometry can provide an easy way to compare across studies and provide material properties that can be incorporated into finite element models.[15,16]

While it has been well established that variations in disc geometry affect disc mechanics,[17–19] there are no standardized methods for measuring disc geometry. The two primary components of disc geometry used to normalize material properties are disc height and area. Disc height is commonly measured by radiography,[15,20–23] computed tomography (CT),[11,12,24–26] magnetic resonance (MR) imaging,[27,28] or

manual methods.[8] Disc area is also measured in a number of ways, from radiography with geometric approximation,[29–32] CT or MR imaging,[27] or bisection of the disc at the mid-transverse plane.[15,23]

As such, the primary aims of this short communication were two-fold. First, we reviewed common methods of measuring disc geometry. Second, we evaluated the influence of disc geometry measurement method on reported compressive mechanics. Our findings highlight the nuances of comparing results across studies while also suggesting promising practices for measuring disc height and area.

2 | REVIEW: METHODS FOR MEASURING DISC GEOMETRY

Geometry is a critical component for understanding disc joint mechanics. Human discs vary in shape due to the spinal cord, and disc size increases from the cervical spine to the lumbar spine.[33] Disc height can also vary by up to 5 mm between the posterior and anterior regions, making it difficult to identify one representative disc height.[34] Furthermore, disc geometry is both sex- and age-dependent, where discs are smaller in females and exhibit sex-dependent changes with age.[20] While disc mechanics have been correlated with age and sex, it is unclear whether these changes are explained by physiological or geometry-based differences.[34]

Disc geometry is not always reported in studies that report mechanical properties. With compressive testing, some studies focus on load-based protocols and results, circumventing the need to report disc geometry. For example, Adams and Hutton investigated[35] gradual disc prolapse using human lumbar motion segments, opting to report peak load, flexion angle, and mode of failure, where flexion angle was measured relative to the plane of compression, rather than to disc height. Similarly, Brinckmann and Horst[36] examined the effect of vertebral body fracture, intradiscal injection, and partial discectomy on disc height and radial bulge, reporting height change of the total motion segment but avoiding the absolute disc height. More recently, our lab reported changes in disc height during creep-recovery testing of bovine discs, where disc height change was assumed as the change in displacement of the mechanical testing device.[37] Around the same time, Feki et al.[38] asserted that disc recovery is governed by osmotic conditions, reporting changes in normalized load and relaxation time without specifying disc geometry or normalization method. Ultimately, studies reporting disc mechanics have continued to be published without normalizing to specimen geometry, making it difficult to compare results.[39–42]

When disc geometry is reported, disc height is commonly measured by 2D or 3D imaging methods. Lateral radiographs are frequently used to determine disc height, which has been used to relate mechanical properties to disc geometry.[15,21,22,43,44] Once radiographs are taken, methods for identifying and reporting disc height vary, while Amonoo-Kuofi[20] reported both anterior and posterior height, Koeller et al.[21] averaged anterior and posterior heights and reported one value. O'Connell et al.[23] reported a singular disc height by measuring the mid-sagittal disc area and dividing the area by the anterior–posterior width.

As technology has become more accessible, more research groups have applied imaging techniques such as CT or MR imaging to measure disc height. Newell et al.[11,12,26] employed CT imaging to measure disc height for both bovine and human discs. O'Connell et al.[27,28] used MR imaging to measure disc height and disc height loss, which was used to calculate internal strains under axial compression. CT is particularly useful in the case of small animal models, where μCT can provide high-resolution images. Boxberger et al.[24] used μCT to identify localized heights within the rat disc, and with the additional use of contrast agents, Lin et al.[45] created 3D surface renderings of both mouse and rat discs. Additionally, CT and MR imaging can be conducted in vivo. For example, Espizona-Orías et al.[46] used CT to map changes in disc height with axial rotation, Martin et al.[47] used MR to measure diurnal changes in disc height, and Zhong et al.[33] applied MR to characterize nucleus pulposus and annulus fibrosus geometry at different lumbar levels.

It is important to consider the timing of geometry data collection during the sample preparation protocol. In some cases, the whole intact spine is imaged,[11,23,27] while in other cases, only the prepared motion segment is imaged.[12] Further, some groups may measure disc height manually after excising the disc.[8,48,49] This may contribute to differences in disc height, as discs may expand when surrounding boundary constraints are removed.[50]

Like disc height, disc area is an important metric for normalizing loads. X-ray, CT, and MR imaging provide useful tools for accurate characterization of disc area. While transverse imaging methods allow for direct measurements of disc area, deriving disc area from lateral images relies on assumptions about disc shape (i.e., circular or ellipse).[29–32,51] When imaging methods are not available, a few different methods have been applied. Koeller et al.[22] determined disc area by, "tracing the disc contour onto a sheet of paper, and determining the area by planimetry." More commonly, the disc area is measured after removing the disc from the vertebral bodies.[8,48,49] Similarly, O'Connell et al.[23] and Beckstein et al.[15] sectioned discs in the mid-transverse plane using a microtome, then imaged the disc and used ImageJ to calculate the area. While O'Connell et al.[23] was published to compare animal model disc geometry, publications have since used these data to normalize loading protocols to specific stress values.[34]

Given that methods for measuring disc height and area vary widely, we hypothesized that discrepancies in disc geometry contribute to large differences in reported mechanics, which may affect the development and accuracy of computational models that use these values. To test this hypothesis, we evaluated three different methods for measuring disc height and six methods for measuring disc area. Axial compressive testing was conducted, and material properties were calculated using different disc geometry measurement methods.

3 | METHODS

Organically raised skeletally mature bovine caudal spine segments were acquired from a local abattoir ($n = 4$). Surrounding musculature was removed and the top four vertebrae were cut at the mid-transverse

plane to create three bone-disc-bone motion segments (CC1-CC2, CC2-CC3, and CC3-CC4). Bone-disc-bone motion segments were potted in polymethyl methacrylate to ensure plano-parallel loading surfaces (n = 12). During dissection and potting, dehydration was prevented by wrapping discs in saline-soaked gauze.

3.1 | Geometry measurement

After joint segment preparation, disc geometry was measured at six different points (Table 1). Once potted, joint segments were CT scanned to collect disc height and area before hydration (Pre.CT, i.e., pre-swelling condition, 0.092-mm resolution, Figure 1A). Segments were then hydrated in 0.15 M phosphate-buffered saline for 18 h at 4°C and immediately rescanned to measure disc height and area after swelling (Pos.CT, i.e., post-swelling condition). Within 20 min of the Pos.CT scan, joint segments were mechanically tested, as described below. After testing, disc width was measured in situ with calipers (In.Cal, i.e., in situ caliper measurement), and the disc was assumed to be cylindrical. Three measurements were taken and averaged to generate a single measurement for diameter. The disc was then carefully excised using a scalpel, and disc height and width were remeasured (Ex.Cal, i.e., excised caliper measurement). The excised disc height and width were each measured at three locations

TABLE 1 Methods of geometry measurement

Name	Method	Outcome
Pre.CT	Pre-swell CT scan	Area, height
Pos.CT	Post-swell CT scan	Area, height
In.Cal	Disc measured in situ with calipers	Area
Ex.Cal	Disc measured ex situ with calipers	Area, height
Ex.ImJ	Disc measured ex situ with ImageJ	Area
Mt.ImJ	Disc microtomed, measured with ImageJ	Area

and averaged to collect a single value for height and width. Next, the transverse plane of the excised disc was imaged with a millimeter scale (Ex.ImJ, Figure 1B). Lastly, the disc was refrozen, and a freezing stage microtome was used to cut the disc to the mid-transverse plane for imaging (Mt.ImJ, Figure 1C).

Disc height and area were collected from CT scans using a custom MATLAB code (version R2019b).[27] Briefly, disc area was measured at the mid-transverse plane and disc height was measured by dividing the mid-sagittal cross-sectional area by the disc width. ImageJ (version 1.52q) was used to calculate disc area from images of excised and microtomed discs, where images were taken using a 12-megapixel wide camera with a 26-mm focal length, positioned approximately 8 inches above the sample. All manual measurements were made with digital calipers.

3.2 | Mechanical testing

Short-duration mechanical tests were conducted in air at room temperature (<10 min, ~22°C, MTS 858 MiniBionix, MTS Systems Crop.). Specimens were wrapped in saline-soaked gauze to prevent dehydration.[12] A 50 N compressive preload was applied for 5 min to mimic in vivo intradiscal pressure of a healthy individual while laying down.[12] Based on disc geometry measurements, the preload resulted in resting compressive stresses that were within the range of human intradiscal pressure while lying down, but were slightly lower than the resting stress for bovine discs.[52] The specimen was then compressed at 0.5 Hz between 0 and 10% strain for 15 cycles (data acquisition at 50 Hz). Strain inputs for mechanical testing were determined using Pre.CT height. The last loading cycle was used for data analysis. A no-sample test was conducted to determine machine compliance within the relevant loading regime (0.03 μm/N) and data were adjusted accordingly.

Conversions to stress and strain were calculated using different combinations of geometry measurement methods. Stress–strain
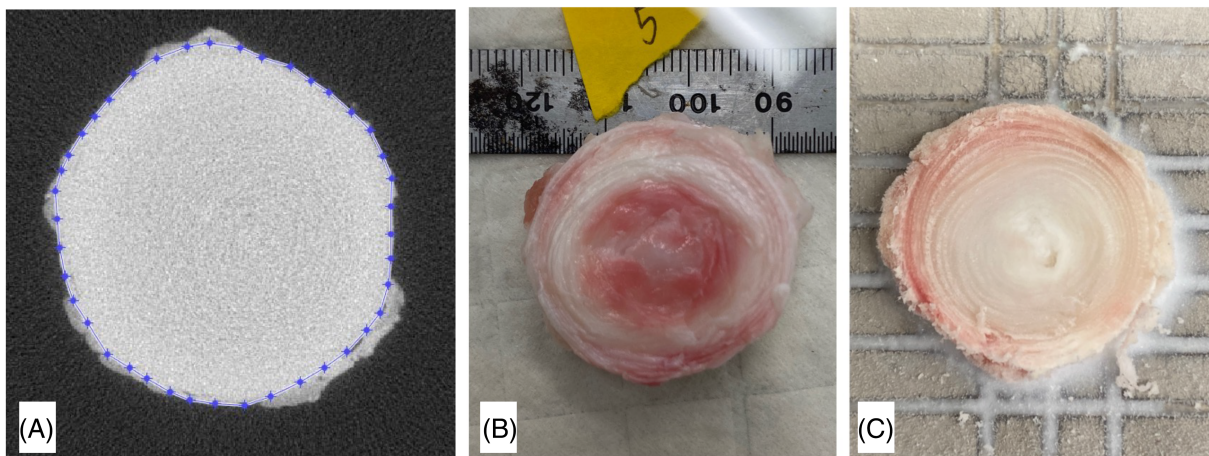

FIGURE 1 Representative image of a disc (A) on a CT scan, (B) after removal from the vertebral bodies (Ex.ImJ) and (C) after microtoming (Mt.ImJ)
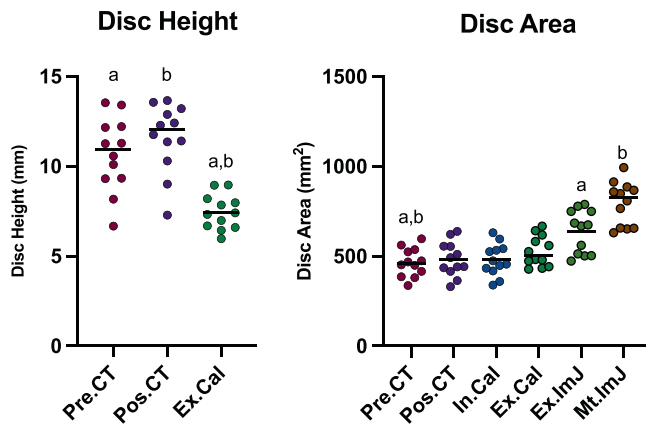
FIGURE 2 Disc height and area measurements. Groups with the same letters represent statistically significant differences ($p \leq 0.01$). Black bar denotes group median.

curves were generated, and normalized compressive stiffness was calculated as the slope of the linear region between 0.4 and 0.6 MPa.[15]

3.3 | Statistical analysis

Because geometry data were paired and sample sizes were small, normality was not assumed. As such, comparisons between groups were made using Friedman tests with Dunn's multiple comparisons adjustment. Comparisons were primarily made to Pre.CT disc measurements, as pre-testing scans without swelling were most commonly reported in the literature. Statistical significance was assumed at $p \leq 0.05$. Statistical analyses were conducted using Prism (Graphpad, version 9.1.2). Data are presented as median (interquartile range).

4 | RESULTS

4.1 | Disc height

Disc height was collected for Pre.CT, Pos.CT, and Ex.Cal measurements. Pre.CT disc heights (10.9(2.8) mm) were not significantly different than Pos.CT disc heights (12.02(1.9) mm; $p > 0.3$), despite 18 h of swelling. Ex.Cal disc heights (7.4(1.4) mm) were significantly lower than both Pre.CT and Pos.CT disc heights ($p \leq 0.01$; Figure 2).

4.2 | Disc area

While the disc remained constrained by vertebral bodies, disc area remained consistent (Pre.CT, Pos.CT, and In.Cal). Once the disc was excised, disc area increased. Ex.ImJ disc area was 37% greater than Pre.CT disc area ($p < 0.01$), and Mt.ImJ disc area was 57% greater than Pre.CT disc area ($p < 0.01$; Figure 2). While Ex.Cal disc area was 9% greater than Pre.CT disc area, this was not significantly different due to the high number of comparisons (15 comparisons, $p = 0.25$).
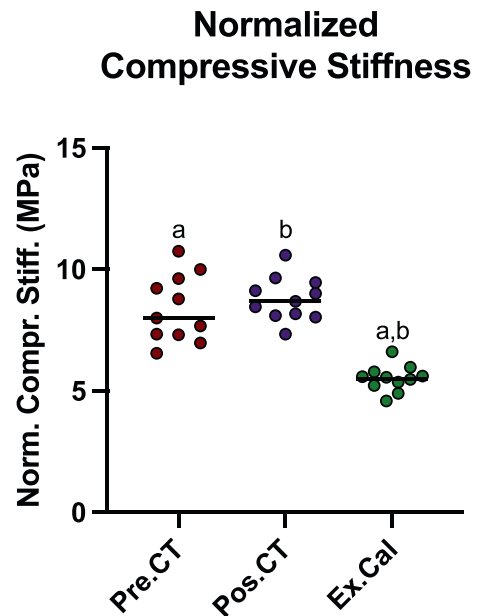

FIGURE 3 Normalized compressive stiffness calculated with Pre. CT, Pos.CT, and Ex.Cal geometry. Groups with letters represent statistically significant differences ($p \leq 0.01$). Black bar denotes group median.

4.3 | Mechanics

Due to variations in disc height between samples, the maximum applied load varied between 285N and 1250N (494(477) N). When normalized to stress and strain using Pre.CT values, maximum stress was inconsistent between samples, ranging from 0.74 to 2.91 MPa. Stress–strain curves were also inconsistent when normalized by Ex. Cal values, with maximum stress ranging from 0.60 to 2.52 MPa.

When Pre.CT geometry values were used to normalize stress and strain, the average normalized compressive stiffness was 8.00(2.10) MPa. This was not significantly different than transforming load and displacement data with Pos.CT values (8.69(1.15) MPa, $p > 0.99$). However, normalized compressive stiffness was significantly lower when load and displacement were transformed with Ex.Cal values (5.12(0.47) MPa, $p < 0.01$; Figure 3).

Figure 4 shows the range of impact that different measurement methods may have on calculated normalized compressive stiffness. When holding height constant, normalized compressive stiffness calculated with Mt.ImJ area was 20% lower than the normalized compressive stiffness calculated with Pre.CT area (8.00 MPa vs. 6.53 MPa, $p < 0.01$, maroon vs. brown), while stiffness calculated with Ex.ImJ area (light green) was 11% lower (8.300 vs. 7.19 MPa, $p < 0.02$). On average, using Ex.Cal disc height resulted in a 33% difference between reported normalized compressive stiffness when compared to Pre.CT height (Figure 4, light gray vs. black-bordered bars). Surprisingly, the difference between normalized compressive stiffness calculated with Pre.CT height and Ex.Cal height was not significant when Mt.ImJ area was used (28 comparisons, $p = 0.20$). In the worst case, normalized compressive stiffness calculated with Pre.CT

disc height and area was 65% higher than when calculated with Ex.Cal disc height and Mt.ImJ disc area ($p < 0.01$).

5 | DISCUSSION

Mechanical testing is an important tool for assessing disc health and function but making comparisons between studies can be challenging. The primary aim of this study was to highlight the wide range of methods used to measure disc geometry while characterizing the effect of disc geometry measurement method on reported mechanical properties. In doing so, we highlighted one of the issues associated with making comparisons across experimental studies.

Bovine disc heights reported in the literature vary widely from ~5.5 mm to over 9 mm, partly due to differences in measurement approach (Table 2).[8,11,12,15,23] Our Pre.CT and Pos.CT disc height values were closest to those reported by Beckstein et al. (9.18 ± 0.65 mm,[15] mean ± standard deviation), who similarly utilized fluoroscopic imaging of the disc joint. However, CT or X-ray imaging does not always correspond to greater disc heights. Disc height measured

from CT images of intact bovine tails (6.80 ± 0.90 mm and 6.90 ± 0.35 mm from Newell et al.[11] and O'Connell et al.,[23] respectively) are closer to disc height measurements reported after excising the disc (7.01 ± 1.23 mm and 7.4(1.4) mm from Bezci et al.[8] and this study, respectively). This may be due to lateral disc expansion once surrounding tissue is removed, releasing residual stresses.[50] Lastly, Ex. Cal disc heights were significantly lower than Pre.CT and Pos.CT disc heights ($p \leq 0.01$; Figure 2). An inherent limitation of the Ex.Cal measurement is an inevitable loss of disc tissue during the excision process, where preservation of the disc concavity is quite difficult. This highlights a significant challenge in manual measurement with calipers, which may result in lower reported disc height.

While disc area also varied by measurement method, values remained consistent while the disc was bound by vertebral bodies. Pre.CT (460(119) mm^2), Pos.CT (467(125) mm^2), and In.Cal (465(106) mm^2) areas agreed well with existing literature where the disc was measured in situ with calipers.[12] The consistency of In.Cal measurements with Pre.CT and Pos.CT area is likely due to the circular shape of the bovine disc. Using a similar approach for non-caudal discs, which have a kidney bean shape, would likely present a more challenging approximation and increase the chance for area measurement discrepancies. Area increased once the disc was excised and imaged, resulting in significantly higher measurements partly because disc boundary delineation is more challenging on an image. The highest disc area was reported when the disc was microtomed to the mid-transverse plane and measured using ImageJ (828(215) mm^2), which agrees well with publications that utilized the same method.[15,23] However, most bovine studies do not report specimen age, breed, or weight, which may also contribute to variations in disc geometry.

The increase in disc area and decrease in disc height had a significant effect on lowering the normalized compressive stiffness (Figure 4). When Pre.CT disc height and Mt.ImJ area were used, the compressive stiffness was 6.53(2.09) MPa, which was within one standard deviation of values reported by Beckstein et al.,[15] who applied similar methods for geometry measurement and calculated stiffness at 0.5 MPa. Further, when Ex.Cal disc height and area were used, normalized compressive stiffness was 5.57(0.41) MPa. Bezci et al.[8] applied the same disc geometry measurement methods and reported a toe-region modulus of 2.73 ± 0.81 MPa, which was measured at a lower stress range than used in this study. Taken together,
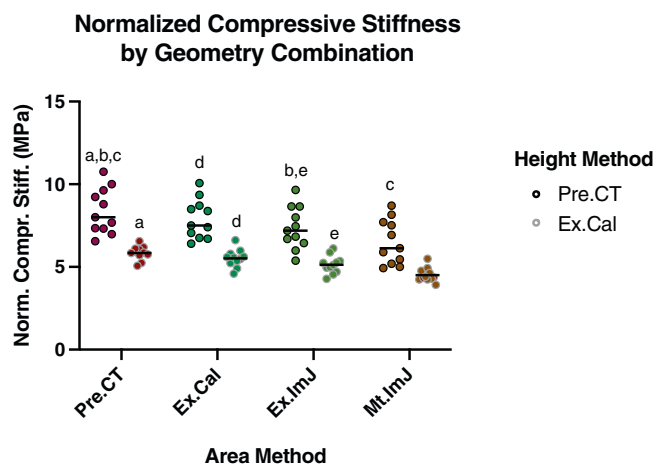

FIGURE 4 Normalized compressive stiffness calculated with different combinations of disc geometry measurement methods. Groups with letters represent statistically significant differences ($p \leq 0.01$). Black bar denotes group median.

TABLE 2 Reported bovine disc geometries

Publication	Height method	Area method	Height (mm)	Area (mm^2)
23	CT of the intact spine	Bisected in the transverse plane with microtome	6.90	622
15	Lateral fluoroscopic image of the joint	Bisected in the transverse plane with microtome	9.18	857
8	Excised and measured	Excised and measured	7.01	491
11	CT of the intact spine	CT of the intact spine	6.80	560[a]
12*	CT of the prepared joint segment	In situ measurement with geometric approximation	6.75	489
			5.48	436
			5.46	439

[a]Approximated using reported disc width.
*Data from three institutions reported in one publication.

these between-study comparisons suggest that trends observed here align with previously reported disc geometry measurement methods and subsequent reported values for disc mechanics.

The relationship between disc geometry and mechanics is expected, but the magnitude of the differences stands out. In a computational analysis of the L3–L4 motion segment, Meijer et al.[18] reported that disc height had the largest influence on motion segment stiffness among non-disc geometric parameters (i.e., endplate width, vertebra height, longitudinal ligament area, and more), with greater disc heights resulting in lower motion segment stiffness. While this may initially appear contradictory to our results it highlights the difference between reporting stiffness defined as force divided by displacement (not adjusted for specimen geometry) and modulus or normalized stiffness.

Even with the clear trends observed, this study is not without limitations. First, each measurement technique was performed by only one researcher, so assessments for intra- and inter-rater reliability were not made, which may further contribute to variability between methods. As such, data reported here serve primarily to highlight a potential source of error, rather than give a definitive conclusion on best practices. Second, measuring the excised disc with calipers was particularly challenging, as the tissue deformed once the calipers applied pressure. While freezing the discs mitigated this deformation, it also led to artificially high measurements due to water expansion. Additionally, this study cannot quantitatively correct for discrepancies between methods, as such a method would likely be institution and researcher specific.

Given these limitations, the trends reported here suggest that CT or X-ray imaging after preparing the motion segment may give the most accurate approximation of disc geometry at the time of testing. If imaging is prohibitive, measuring the excised disc height at the outer annulus may provide the closest approximation of disc height. Similarly, disc area can be measured in situ and approximated based on disc geometry, depending on the species or disc location within the spine (i.e., circle vs. ellipse or lumbar vs. caudal spine).

Mechanical testing has been invaluable for understanding disc health and degeneration, but utilizing existing works requires a thorough understanding of the methods used and their impact on reported values. As most studies report comparisons within a study, differences discussed here do not impact comparative outcomes. However, reliable comparisons between studies are valuable for reducing the need to re-test study groups in future studies. Moreover, reported values are often used by researchers developing computational models to study disease progression or used as target values for tissue-engineered solutions. In summary, findings from this study showed that large differences in normalized compressive stiffness may be attributed to differences in disc geometry and not inherent mechanical properties. Thus, specific details of disc geometry methods should be considered when comparing across studies.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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