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ABSTRACT

Background Context: Complex testing protocols are required to fully understand the biomechanics of the spine. There remains limited data concerning the mechanical properties of spinal specimens under dynamic loading conditions in six axes.

Purpose: To provide new data on the mechanical properties of functional spinal unit (FSU), and isolated disc (ISD) spinal specimens in six degrees of freedom.

Study Design/Setting: Dynamic, six-axis stiffness matrix testing of porcine lumbar spinal specimens.

Methods: The stiffness matrix testing of lumbar porcine FSU (n=6) and ISD (n=6) specimens was completed in a custom six-axis spine simulator using triangle wave cycles at a frequency of 0.1 Hz. Specimens were first tested without an axial preload, and with an axial preload of 500 N with equilibration times of both 30 and 60 minutes. The study was supported through an institutional grant, and the authors are not aware of any conflicts of interest related to this research.

Results: The stiffness matrices were not symmetrical about the principal stiffness terms. The facets increased all the principal stiffness terms with the exception of axial compression-extension. Significant differences were detected in 15 stiffness terms due to the application of an axial preload in the ISD specimens, including an increase in all principal stiffness terms. There were limited differences in stiffness due to an equilibration time of 30 and 60 minutes.

Conclusions: The assumption of stiffness matrix symmetry used in many previous studies is not valid. The biomechanical testing of spinal specimens should be completed in 6 degrees of freedom, at physiological loading rates, and incorporate the application of an axial preload. The present study has provided new data on the mechanical properties of spinal specimens, and demonstrates that the dynamic stiffness matrix method provides a means to more fully understand the natural spine, and quantitatively assess spinal instrumentation.

INTRODUCTION

The spine is a complex structure with six degrees of freedom (df) at each level, and each level comprising the triple joint structure of the intervertebral disc (IVD) and facet joints. Appropriate biomechanical testing to better understand the natural spine is critical in order to improve the design and performance of spinal instrumentation prior to clinical use, which currently lacks the clinical successes seen in other joints such as the hip and knee [1,2].

Biomechanical testing of the spine often requires complex testing equipment to simulate the movement and loading conditions present in-vivo. Previous studies have shown that the stiffness of the spine is significantly affected by the presence of the facets [3], the application of an axial preload [3-6], the testing frequency [7,8], the moisture condition of the spinal specimen [9-11], the specimen temperature [12], and the number of cycles a specimen is subjected to [10].

There are a number of studies that have investigated spinal specimens in using pure moments in the three rotational axes [13-16], and other studies that have actively tested all six axes [3-6,17]. However, few have tested spinal specimens dynamically in six axes [8], and the only previous dynamic measurement of the full stiffness matrix of a spinal specimen was part of a development study involving a single spinal specimen [18].

The aim of this study was to measure the dynamic, six-axis stiffness matrices of porcine spinal specimens, with and without facets, and under the application of a 0 and 500 N axial preload. A further aim was to assess the effect of the equilibration time of the axial preload on the stiffness matrix of spinal specimens.

MATERIALS AND METHODS

Twelve porcine lumbar functional spinal units were harvested from organically farmed pigs aged between eight and twelve months at the time of slaughter, with masses of approximately 60 kg (Bartlett & Sons Ltd., Bath, UK). The specimens were dissected from longer sections of spine (generally T12-S1) on the day of procurement. Musculature was removed from the specimens. Specimens were randomly split into two groups: functional spinal unit (FSU) specimens; and isolated disc (ISD) specimens. The ligaments and facet joint capsules were left intact on the FSU specimens. The facets of the ISD specimens were entirely removed, leaving the vertebral bodies, the IVD, and the anterior and posterior longitudinal ligaments. Both groups comprised six porcine lumbar specimens: two L1-L2; two L3-L4; and two L5-L6. Once prepared, the specimens were labelled, triple bagged, and frozen at a temperature of -24°C until the day of testing.

On the morning of testing each specimen was left to thaw for three hours at room temperature (20±2°C) in a sealed plastic bag to minimise moisture loss due to evaporation. During the last hour of thawing, the specimen was removed from the plastic bag, and three self-tapping screws were driven into the vertebral bodies at the cranial and caudal ends of the specimen to aid stability when potted in aluminium specimen holders using low melting point alloy (MCP75; Mining & Chemical Products Ltd., Northamptonshire, UK). Water-cooling of the specimen holders was used to prevent thermal damage to the specimen. The cranial end of the specimen was potted first, followed by the caudal end. Care was taken during potting to ensure that the IVD was aligned with the horizontal plane. A previously described custom spine simulator [18] (Figure 1) was used to lower the specimen into the caudal pot, which allowed the alignment to be finely adjusted so that the specimen was in the neutral position, and the centre of the IVD was aligned with the origin of the displacement axes of the spine simulator.

Once the specimen was potted, it was sprayed with 0.9 % saline solution and wrapped in plastic wrap in order to maintain an adequate moisture level, and re-mounted in the spine simulator. A six-axis load cell was mounted between the caudal specimen holder and the baseplate of the spine simulator (AMTI MC3-A-1000, Advanced Mechanical Technology, Inc., MA, USA).

All testing was completed at room temperature (20±2°C). The range of motion (ROM) used for the stiffness matrices was: ±3 mm in anterior-posterior shear (TX); ±1.5 mm in lateral shear (TY); ±0.4 mm in axial compression-extension (TZ); and ±4° in lateral bending (RX), flexion-extension (RY), and axial rotation (RZ). All axes were tested in position control at a single test frequency of 0.1 Hz; this resulted in a testing speed of 1.6°/s in the rotational axes.

Each stiffness matrix comprised six tests, one for each axis. For each test, one axis was cycled through five triangle waves, while the other five axes were held in a stationary position. Position and load data were acquired at 100 Hz for all tests. The first two cycles of each test were considered as preconditioning cycles, and the last three cycles were used to calculate the stiffness at the centre of the superior vertebral body using rigid body transformations and the linear least squares method. The stiffness matrices were not assumed to be symmetric about the principal stiffness terms, resulting in a 6x6 matrix, though some terms would be expected to be negligible due to sagittal plane symmetry (Table 1).

For each specimen, stiffness matrix tests were first completed without an axial preload. An axial preload of 500 N was then applied using load control for an equilibration time of 30 minutes prior to the completion of another stiffness matrix. The 500 N preload was then maintained using load control until 60 minutes after the initial preload application, and a further stiffness matrix test completed.

The order of testing each axis within a stiffness matrix was randomised so as to minimise any residual effects of the previous test(s) on the results of any axis. During testing the axial position was adjusted between each test in order to maintain the correct preload, if required.

Statistical comparisons were made between FSU and ISD specimens under each preload condition, and within the FSU and ISD groups between the preload conditions of 0 N, 500 N with a 30 minute equilibration time, and 500 N with a 60 minute equilibration time. All statistical comparisons were completed using SPSS (IBM SPSS Statistics 19; IBM Corporation, Armonk, NY, USA). Due to the sample size of only six specimens in each group, both parametric and non-parametric statistical methods were considered for the comparisons. Independent t-tests and Mann-Whitney tests were used to compare the FSU and ISD specimens. Repeated measures ANOVA with paired t-tests for post-hoc analysis, and the Freidman test with Wilcoxon tests for post-hoc analysis were used to compare the effect of preload and equilibration time on the stiffness of FSU and ISD groups independently. The differences in the p values between the parametric and non-parametric tests were minor, and as such the parametric tests were used for the presentation of the statistical results (Table 2).

In addition to the statistical tests, the percentage change in the principal stiffness terms was calculated. In this method the baseline stiffness terms were normalised to 0%, and an increase or decrease in stiffness as a result of changed testing conditions was represented by a positive or negative percentage change respectively. This provided a clear way to graphically display stiffness terms in a way that accounted for negative stiffness values, and has been previously used to display 6 df spinal stiffness testing results [8,18].

RESULTS

All tests showed good repeatability during the three cycles over which the stiffness terms were calculated. Many terms in the stiffness matrices exhibited strongly linear relationships, particularly when an axial preload was applied. In specimens with a preload of 500 N, the mean R^2 value in all principal stiffness terms was greater than 0.75 in the ISD group and 0.89 in the FSU group, and was greater than 0.99 in anterior-posterior and lateral shear for both ISD and FSU groups. The R^2 values of non-principal stiffness terms were generally lower than the principal terms, and also demonstrated greater variability.

Negligible Stiffness Terms

For each term of the stiffness matrix a zero error was calculated based on the noise floor of the load cell (±5 N and ±0.25 Nm), and the ROM for each axis. Many of the stiffness terms that were expected to be negligible due to sagittal plane symmetry were low in magnitude, though not all were below the zero error. Of the 17 stiffness terms that would be expected to be negligible, eight were greater than the zero error in FSU specimens, and approximately five in the ISD specimens. This was similar under all preload conditions.

Three stiffness terms that would not be negligible through sagittal plane symmetry were consistently found to be within the zero error for both FSU and ISD specimens: anterior-posterior shear force due to axial compression-extension ($K_{3,1}$); anterior-posterior shear force due to flexion-extension ($K_{5,1}$); and axial torque due to lateral bending ($K_{4,6}$).

Matrix Symmetry

Though some stiffness terms exhibited symmetry about the diagonal of the principal terms, overall the matrices were found to be asymmetric; this was the case for all preload conditions for both FSU (Table 3) and ISD (Table 4) specimens.

Specimen Stiffness

Removing the facets significantly reduced all of the principal stiffness terms with the exception of the axial compression-extension stiffness ($K_{3,3}$), and the lateral bending stiffness ($K_{4,4}$) with a 0 and 500 N (30 min) preload. Six stiffness terms were significantly different in the ISD group compared to the FSU group without an axial preload, and between eight and nine with the 500 N preload (Table 5). Where significance was detected between FSU and ISD specimens in the principal stiffness terms, the stiffness was always lower in the ISD group.

The preload increased the stiffness of between four and five of the principal stiffness terms in the FSU specimens, and all six in the ISD group (Table 6). A total of 15 terms were significantly different due to the application of a preload in the ISD group; this was the case after an equilibration time of both 30 and 60 minutes. The comparisons of the 0 and 500 N preload were similar irrespective of the equilibration time; only two differences in significance were found in the FSU specimens, and none in the ISD specimens. However, whilst only two terms were significantly different between the comparison of the equilibration times of 30 and 60 minutes in the FSU specimens, there were eight in the ISD specimens, including all the principal stiffness terms with the exception of the anterior-posterior shear stiffness term ($K_{1,1}$).

The percentage change in stiffness of the principal stiffness terms reflected the results of the statistical analyses. Irrespective of the preload, the removal of the facets reduced the mean stiffness in flexion-extension ($K_{5,5}$) and axial rotation ($K_{6,6}$) by over 50% (Figure 2). Shear stiffness terms were also significantly reduced by removing the facets but the mean percentage changes were smaller (5-25% for both $K_{1,1}$ and $K_{2,2}$).

Although there were significant differences in the absolute values of the principal stiffness terms of FSU and ISD specimens, the effect of applying the 500 N preload resulted in similar percentage increases in the principal stiffness terms (Figure 3). The application of the 500 N preload caused the mean stiffness to increase in both FSU and ISD specimens by 200-350% in axial compression-extension ($K_{3,3}$), 200-500% in lateral bending ($K_{4,4}$), and 65-165% in flexion-extension ($K_{5,5}$). Although the increase in stiffness due to preload in anterior-posterior shear ($K_{1,1}$), and axial rotation ($K_{6,6}$) was significant for both FSU and ISD specimens at both 30 and 60 minutes equilibration times, the mean increase was only 10-25%. The application of the 500 N preload only caused a significant difference In lateral shear stiffness ($K_{2,2}$) in ISD specimens but this difference equated to a mean increase in stiffness of 35-40%.

DISCUSSION

The aim of this study was to measure the dynamic, six-axis stiffness matrices of porcine spinal specimens, with and without facets, and under the application of 0 and 500 N axial preloads. The results demonstrated a large increase in spinal stiffness due to the application of a physiological preload, and the role of the facets in providing increased stiffness and stability to the spine in all principal stiffness terms with the exception of axial compression-extension. The stiffness matrices were also found to be asymmetric about the principal stiffness terms. This study has provided the first comprehensive dynamic stiffness matrix data of spinal specimens.

As with many biomechanical tests, the present study does have certain limitations associated with the specimens used, the testing method, and the data analysis. These limitations are outlined in the relevant sections of the discussion below.

Stiffness Calculation method

The stiffness terms were calculated using the linear least squares method over the entire positive-negative cycle of each translation or rotation. There are two limitations of using this method: stiffness asymmetry is not accounted for; and the potential S-curve of the neutral and elastic zone is not accounted for. The two principal stiffness terms that would be affected by asymmetry are anterior-posterior shear ($K_{1,1}$), and flexion-extension ($K_{5,5}$). The mean (SD) R^2 value irrespective of specimen type or preload condition was 0.995(0.002) and 0.808(0.209) for $K_{1,1}$ and $K_{5,5}$ respectively, with the large standard deviation in $K_{5,5}$ caused primarily by one ISD specimen which had very low stiffness in flexion-extension, and as such exhibited a low R^2 value. Costi et al. [8] completed stiffness tests of human cadaveric ISD specimens at various test frequencies, and found that the stiffness over a ROM of ±2° was similar in flexion and extension. In regard to the neutral and elastic zone, many stiffness terms in the present study did not exhibit such a stiffness profile, and with the application of a physiological preload all principal stiffness terms exhibited R^2 values greater than 0.75, and commonly there was no easily definable neutral zone.

It was, therefore, justifiable to use the same method to calculate all stiffness terms due to the limited published data for the dynamic stiffness matrices of spinal specimens. However, it may be possible in future studies to perform a detailed examination of the optimal method of calculating each stiffness term, and whether additional matrix terms are required to account for asymmetry in in the positive and negative phases of a cycle.

Negligible Stiffness Terms and Matrix Asymmetry

Some stiffness terms expected to be negligible were found to have values greater than the zero error calculated for the spine simulator. It may be that more specimens are required to average out the asymmetry of individual specimens and any misalignment during potting and mounting the specimens. Further studies using a larger number of specimens should provide a clearer understanding of the practical zero error for stiffness matrix testing using the spine simulator of the present study.

In addition to negligible terms due to sagittal plane symmetry, Gardner-Morse and Stokes [5] identified that the anterior-posterior shear force due to axial compression-extension ($K_{3,1}$) was negligible. This was found to be the case in the present study, along with two other stiffness terms ($K_{5,1}$ and $K_{4,6}$).

The stiffness matrices were found to be asymmetric, this matches with developmental research completed using the same methodology as the present study [18]. Almost all published literature on the stiffness matrices of spinal specimens has assumed symmetry, which makes it impossible to compare with previous studies. The only other study that has not assumed symmetry used a static loading protocol, only published the matrices of one specimen in an intact state and after a total disc replacement, and used a different method to calculate the stiffness terms [19]. It is therefore difficult to assess whether any differences in symmetry compared to this study are due to the testing protocol, the calculation methods, or a combination of the two.

Specimen Stiffness

Previously published stiffness matrices using porcine [3,4] and human cadaveric [5] specimens have demonstrated a large variability in the stiffness values. The results of the present study compare reasonably with these data, though the magnitudes of the principal stiffness terms were at the lower end of the range in the literature. The previous studies have tested specimens quasistatically whilst submerged in a saline bath at a temperature of 4°C. Several authors have shown that the stiffness of spinal specimens are significantly affected by both testing frequency [7,8], and temperature [12], and such differences in the testing protocols are likely to affect the resulting stiffness matrices.

Comparing the results of the present study with the multi-axis testing of Costi et al. using human cadaveric ISD specimens [8] and ovine ISD specimens [11] submerged in a saline fluid bath at a temperature of 37°C suggests that the magnitude of the principal stiffness terms of porcine ISD specimens is generally greater than that of ovine specimens but lower than that of human cadaveric specimens. Costi et al. [8,11] submerged specimens in a 37°C saline fluid bath to maintain the moisture levels of the specimens, whereas the present study sprayed specimens with 0.9% saline and wrapped them in plastic wrap at room temperature. Both methods are understood to maintain constant moisture levels over normal testing periods [9,10], though the temperature of 20±2°C used in the present study is below that in vivo, and may have had an effect on the specimen stiffness. The testing temperature of 20±2°C is within the range recommended by Wilke et al. [20] for spinal testing, though Bass et al. [12] have shown that the stiffness of the anterior longitudinal ligament increased by approximately 38% when tested at 21.1°C compared to 37.8°C. Therefore, future studies should ideally perform stiffness matrix testing at body-temperature to more closely resemble in vivo conditions.

Such a wide variety of stiffness data demonstrates the large effect of specimen type, and testing conditions, on the mechanical properties of spinal specimens. Animal specimens can reduce inter-specimen variation compared to human cadaveric testing, and this can be valuable in efficiently assessing variables of interest. Porcine spines have been reported to exhibit geometric similarities to the human spine [21,22], and qualitative similarities in mechanical properties under pure moment testing without an axial preload [23,24]. Porcine spinal specimens may, therefore, provide a suitable alternative to human cadaveric specimens, though care must be taken in directly relating such specimens with human spine, and the clinical scenario.

FSU vs ISD Specimens

The facets and ligaments provide stability in all six degrees of freedom, as well as limiting the ROM. Previous studies have used both FSU and ISD specimens for biomechanical testing. The FSU more clearly resembles the overall clinical scenario, but at the expense of being able to clearly identify the mechanical properties of individual structures. The present study has confirmed previous results to this effect, and it is recommended that the shielding effect of the facets be considered when designing test methodologies to investigate the mechanical properties of the IVD.

Preload and Equilibration Time

The assessment of preload and equilibration time clearly demonstrated two things. Firstly, that, as previously published, an axial preload has a significant effect on the stiffness of spinal specimens. This was found to be the case for all principal stiffness terms, as well as many off-axis stiffness terms. Secondly, that there was not a large difference between an equilibration time of 30 and 60 minutes. It is, therefore, critical in determining the physiologically relevant mechanical properties of spinal specimens, and in completing the efficacy testing of spinal instrumentation, that a physiological preload is applied to specimens.

These results suggest that, for the preparation and testing methods used in the present study, 30 minutes is a sufficient time to equilibrate a specimen prior to testing. Whilst significant differences were found between equilibration times of 30 and 60 minutes for five of the six principal stiffness terms of the ISD specimens, not all the differences were increases in stiffness or were large in magnitude: the mean change was a decrease of 0-4% for three terms ($K_{1,1}$, $K_{2,2}$, and $K_{6,6}$), which relate mainly to the elastic response of the annulus fibrosus, and the reduction in these terms is likely to be due to creep from the prolonged equilibration time; the mean change was 6.5% for axial compression-extension ($K_{3,3}$), which reflects a combined response to the fluid flow in the nucleus pulposus and the elastic properties of the annulus fibrosus; greater increases (mean changes of 40-60%) were seen in the lateral bending stiffness ($K_{4,4}$), and flexion-extension stiffness ($K_{5,5}$), which strongly relate to the fluid flow behaviour of the nucleus pulposus. Therefore, when testing of specimens sprayed with 0.9% saline and covered in plastic wrap, one should adopt an equilibration time long enough to stabilise the load, yet short enough that fluid loss from the IVD and creep of the IVD tissue is minimised. Longer equilibration times would be possible if a fluid bath was used, as the dynamic exchange of fluid is possible in such an environment [9], and this should be adopted for future studies.

The application of an axial preload to spinal specimens has been recommended by Wilke et al. [20], yet many multi-axis tests of spinal specimens published in the literature, particularly pure moment tests, have not used a physiological preload [14-16]. It is likely that such tests have been completed without a preload due to the limitations of testing equipment to adequately perform multi-axis tests with the high loads resulting from the preload application. However, without a preload it is difficult to relate the data of intact specimens to the natural spine in vivo, or the likely performance of spinal instrumentation in the clinical environment.

CONCLUSIONS

This study has demonstrated that dynamic stiffness matrix testing can be used effectively assess the mechanical properties of spinal specimens in 6 df. The key finding of this study was that the dynamic stiffness matrices of spinal specimens are not symmetrical about the principal stiffness terms. Comparisons with previously published data suggest that the stiffness of the porcine specimens used in the present study may be lower than that of human cadaveric specimens. However, porcine and other animal specimens nevertheless provide a useful means to complete biomechanical tests of spinal specimens without the difficulties associated with human cadaveric testing.

It is proposed to develop standardised spinal testing methods that mechanical characterisation tests of spinal specimens, and efficacy tests of spinal instrumentation, should be carried out in six axes, at a testing speed equivalent to normal activities, over physiological ROMs, with an appropriate axial preload, and at a temperature and moisture condition representative of the in vivo environment. Furthermore, careful consideration of appropriate spinal specimens is recommended in the design of efficacy tests for spinal instrumentation to prevent possible shielding effects of additional structures, and provide the best means to analyse the instrumentation compared to the structures they are designed to replace or provide stability to.

A drive toward standardised tests will allow a means to compare the results of different studies effectively, and focus future research on improving our understanding of the spine and spinal interventions.

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FIGURES

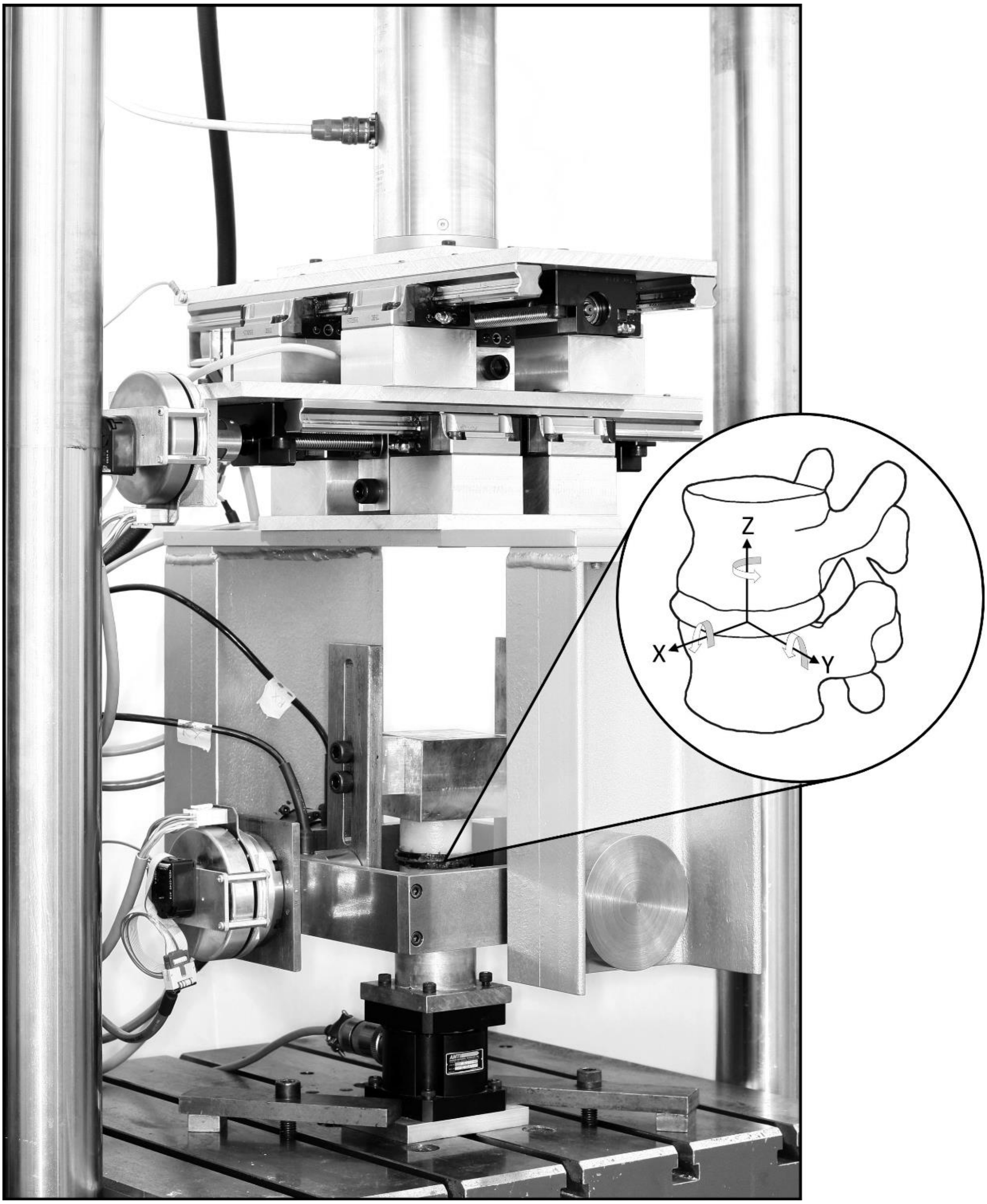

Figure 1: The custom spine simulator with a synthetic specimen mounted, and the orientation of the displacement axes relative to a functional spinal unit (inset)

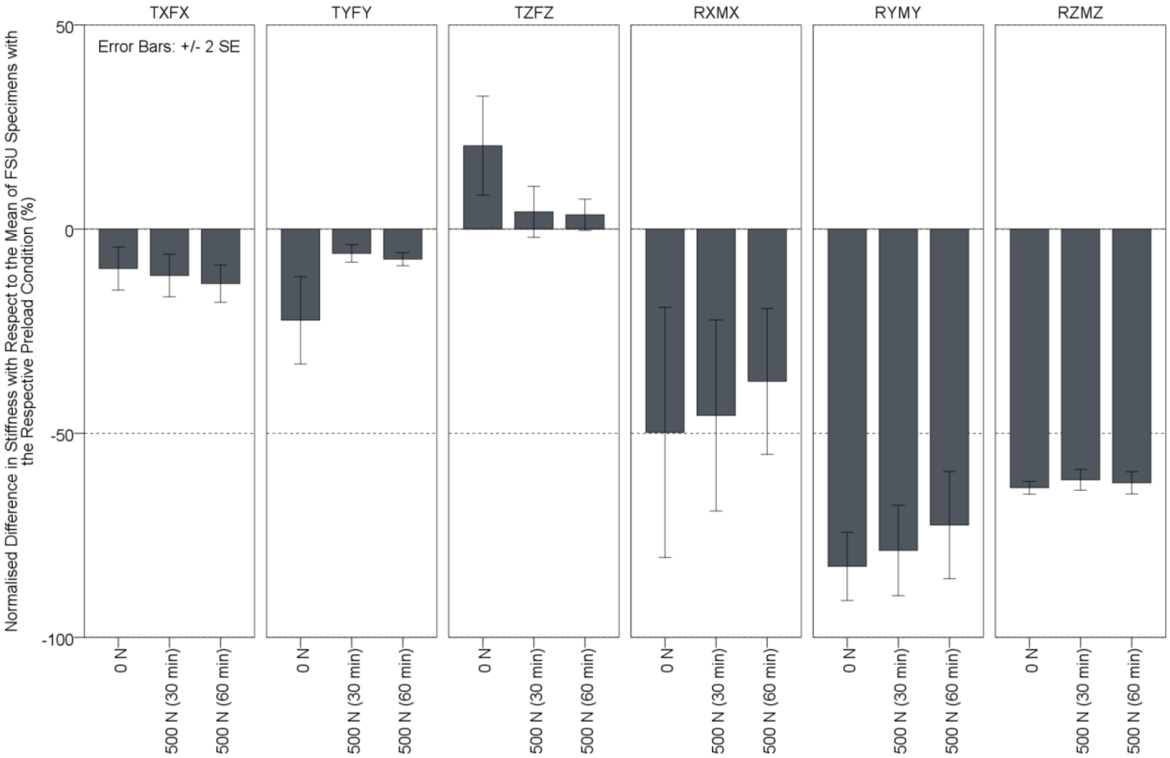

Figure 2: Percentage difference in the principal stiffness terms of ISD specimens compared to the mean stiffness of FSU specimens under the same preload conditions

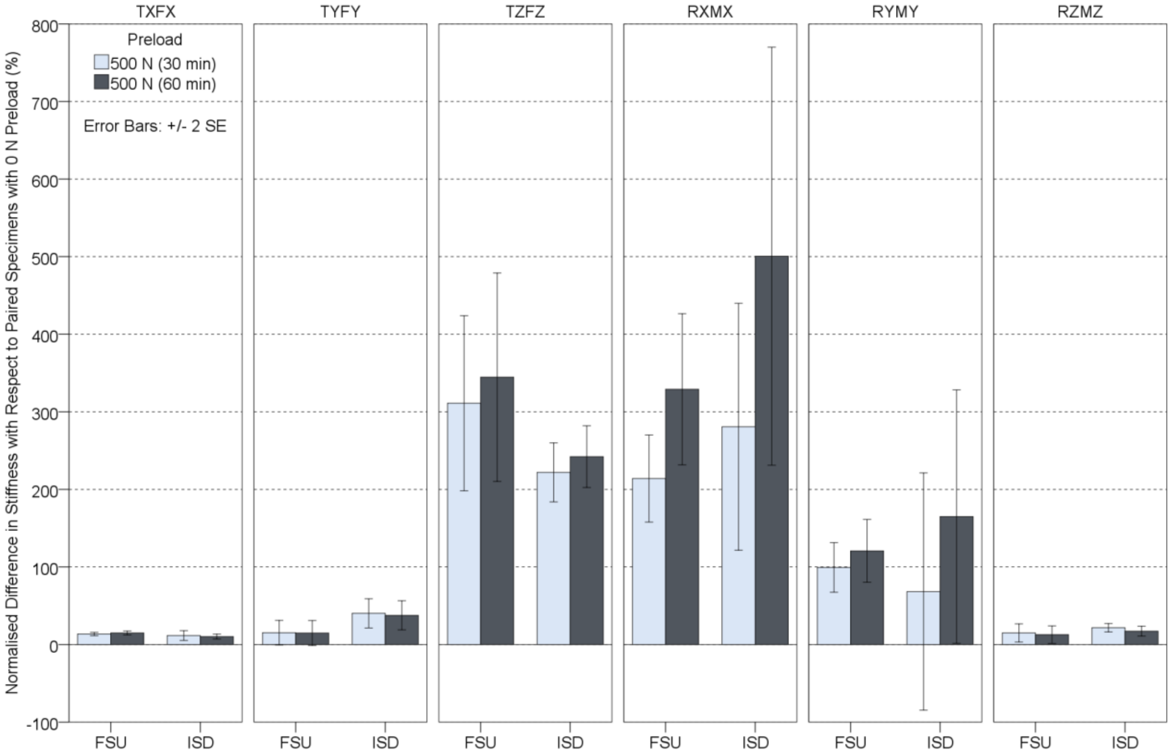

Figure 3: Percentage difference in the principal stiffness terms of specimens with a preload of 500 N and equilibration times of 30 or 60 minutes compared to paired specimens with a preload of 0 N

TABLES

Table 1: Stiffness matrix with principal stiffness terms in bold, and stiffness terms expected to be negligible due to sagittal plane symmetry in italics. Reproduced with permission [18]

Load	TX	TY	TZ	RX	RY	RZ
FX	$\mathbf{K}_{\mathbf{1,1}}$	$K_{2,1}$	$\mathrm{K}_{3,1}$	$K_{4,1}$	$\mathrm{K}_{5,1}$	$K_{6,1}$
FY	$K_{1,2}$	$\mathbf{K}_{\mathbf{2,2}}$	$K_{3,2}$	$\mathrm{K}_{4,2}$	$K_{5,2}$	$\mathrm{K}_{6,2}$
FZ	$\mathrm{K}_{1,3}$	$K_{2,3}$	$\mathbf{K}_{\mathbf{3,3}}$	$K_{4,3}$	$\mathrm{K}_{5,3}$	$K_{6,3}$
MX	$K_{1,4}$	$\mathrm{K}_{2,4}$	$K_{3,4}$	$\mathbf{K}_{\mathbf{4,4}}$	$K_{5,4}$	$\mathrm{K}_{6,4}$
MY	$\mathrm{K}_{1,5}$	$K_{2,5}$	$\mathrm{K}_{3,5}$	$K_{4,5}$	$\mathbf{K}_{\mathbf{5,5}}$	$K_{6,5}$
MZ	$K_{1,6}$	$\mathrm{K}_{2,6}$	$K_{3,6}$	$\mathrm{K}_{4,6}$	$K_{5,6}$	$\mathbf{K}_{\mathbf{6,6}}$

Note: Stiffness terms are derived from a translation (TX, TY, TZ) or rotation (RX, RY, RZ), and an associated force (FX, FY, FZ) or moment (MX, MY, MZ)

Table 2: Comparisons of stiffness matrix results completed using independent t-tests (IND-T), and repeated measures ANOVA with post-hoc paired t-test (RMANOVA)

Comparison	Group 1	Group 2	Group 3	Test
Specimen – 0 N	FSU	ISD		IND-T
Specimen – 500 N (30 min)	FSU	ISD		IND-T
Specimen – 500 N (60 min)	FSU	ISD		IND-T
Preload – FSU – Intact	0 N	500 N (30 min)	500 N (60 min)	RMANOVA
Preload – ISD – Intact	0 N	500 N (30 min)	500 N (60 min)	RMANOVA

Table 3: Matrix FSU02, mean (±SD) stiffness with 500 N (30 min) preload (N, mm, rad). Principal stiffness terms are shown in bold, values within the zero error are shown in italics.

Load	TX	TY	TZ	RX	RY	RZ
FX	**37±0.56**	*2±0.17*	*-3±3.4*	*-8±11*	*-39±55*	*13±12*
FY	*-1±0.17*	**40±0.27**	*4±2.6*	-91±32	*-26±20*	365±72
FZ	31±5.4	-4±2.4	**1,195±27**	-700±557	-6,918±681	-137±96
MX	239±360	708±64	1,612±850	**119,600±23,297**	*-2,367±4,664*	2,022±4,371
MY	-1,280±48	-260±33	-13,486±1032	15,978±6,260	**131,253±8,416**	*-3,191±1,309*
MZ	*-68±15*	351±64	*-354±144*	*-294±4,760*	*990±895*	**124,931±11,427**

Table 4: Matrix ISD02, mean (±SD) stiffness with 500 N (30 min) preload (N, mm, rad). Principal stiffness terms are shown in bold, values within the zero error are shown in italics

Load	TX	TY	TZ	RX	RY	RZ
FX	**33±0.96**	*2±0.13*	*-6±10*	*23±21*	*2±64*	*44±13*
FY	*-1±0.12*	**38±0.43**	*9±3.0*	*15±55*	*4±11*	374±27
FZ	17±5.3	6±3.1	**1,245±37**	-1,741±424	-882±1,092	-202±70
MX	*-78±29*	856±99	2,314±506	**65,000±13,986**	*-969±2,541*	8,651±475
MY	-989±119	-213±20	-1,304±1711	*1,584±3,031*	**27,982±7,271**	*-3,466±809*
MZ	*-46±11*	396±43	*-147±70*	*-3,298±1.412*	*644±216*	**48,220±1,588**

Table 5: Stiffness terms found to be significantly different between the FSU and ISD groups ($p<0.05$). Principal stiffness terms are shown in bold. Dashed cells did not demonstrate significance under the respective preload condition

	Preload and Equilibration Time		
Stiffness Term	0 N	500 N (30 min)	500 N (60 min)
$K_{1,1}$	**0.013**	**0.004**	**0.001**
$K_{1,5}$	-	0.046	0.042
$K_{2,2}$	**0.014**	**0.001**	**<0.001**
$K_{2,3}$	-	0.039	0.012
$K_{3,5}$	0.014	<0.001	<0.001
$K_{4,4}$	**-**	**-**	**0.047**
$K_{5,3}$	0.001	0.001	0.001
$K_{5,5}$	**<0.001**	**<0.001**	**<0.001**
$K_{6,6}$	**<0.001**	**0.001**	**0.001**

Table 6: Significantly different ($p<0.05$) stiffness terms between preload conditions of 0 N (0), and 500 N with equilibration times of 30 minutes (500(30)) and 60 minutes (500(60)) for FSU and ISD specimens. Principal stiffness terms are shown in bold. Dashed cells did not demonstrate significance in the respective comparison

	FSU Specimen Comparisons			ISD Specimen Comparisons		
Stiffness Term	0 & 500(30)	0 & 500(60)	500(30) & 500(60)	0 & 500(30)	0 & 500(60)	500(30) & 500(60)
$K_{1,1}$	**<0.001**	**<0.001**	**-**	**0.014**	**0.001**	**-**
$K_{1,3}$	-	0.011	-	-	-	-
$K_{1,4}$	-	-	-	0.032	0.006	-
$K_{1,5}$	0.004	0.023	-	-	-	-
$K_{2,2}$	**-**	**-**	**-**	**0.003**	**0.005**	**0.011**
$K_{2,3}$	-	-	-	0.012	0.009	-
$K_{2,6}$	-	-	-	0.003	0.005	-
$K_{3,2}$	-	-	-	0.007	0.005	0.047
$K_{3,3}$	**<0.001**	**<0.001**	**0.010**	**<0.001**	**<0.001**	**0.021**
$K_{3,4}$	-	-	-	0.008	0.007	-
$K_{3,5}$	<0.001	<0.001	-	-	-	-
$K_{4,3}$	-	-	-	0.016	0.040	0.009
$K_{4,4}$	**0.002**	**<0.001**	**0.002**	**0.004**	**0.001**	**<0.001**
$K_{4,6}$	-	-	-	0.001	0.036	-
$K_{5,3}$	<0.001	0.001	-	-	-	-
$K_{5,5}$	**0.001**	**0.001**	**-**	**0.018**	**0.009**	**0.005**
$K_{6,2}$	-	-	-	0.010	0.036	0.041
$K_{6,4}$	-	-	-	0.020	0.017	-
$K_{6,6}$	**0.042**	**-**	**-**	**0.001**	**0.003**	**0.010**

For each term of the stiffness matrix a zero error was calculated based on the noise floor of the load cell (±5 N and ±0.25 Nm), and the ROM for each axis (Table 7). The noise floor was calculated using the maximum and minimum values measured over approximately 1 second prior the completion of the first test of each specimen.

Table 7: Stiffness matrix zero error with principal stiffness terms in bold, and stiffness terms expected to be negligible due to sagittal plane symmetry in italics (N, mm, and rad)

Load	TX	TY	TZ	RX	RY	RZ
FX	**±2**	*±3*	±13	*±72*	±72	*±72*
FY	*±2*	**±3**	*±13*	±72	*±72*	±72
FZ	±2	*±3*	**±13**	*±72*	±72	*±72*
MX	*±83*	±167	*±625*	**±3581**	*±3581*	±3581
MY	±83	*±167*	±625	*±3581*	**±3581**	*±3581*
MZ	*±83*	±167	*±625*	±3581	*±3581*	**±3581**