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Biomechanical evaluation of the New Zealand white rabbit lumbar spine: a physiologic characterization

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Abstract Physiologic motions of the human, sheep, and calf lumbar spines have been well characterized. The size, cost, and ease of care all make the rabbit an attractive alternative choice for an animal lumbar spine model. However, comparisons of normal biomechanical characteristics of the rabbit lumbar spine have not been made to the spines of larger species. The purpose of this study was to establish baseline physiologic kinematic data for the rabbit lumbar spine. Ten skeletally mature New Zealand white rabbit osteoligamentous spines were obtained. L4-L7 spine segments were harvested and mounted. Multi-directional flexibility testing was performed by applying pure moments up to 0.27 Nm. Resulting rotations were measured using an Optotrak system. Data were analyzed for each intervertebral level in the three planes of rotation. The three levels tested had roughly similar range of motion (ROM). The mean (SD) angular ROMs in flexion for L4-L5, L5-L6, L6-L7 were 12.10° (2.59°), 12.38° (2.70°), and 15.17° (3.22°), respectively. The ROMs in extension were 5.86°

(1.21°), 5.58° (1.48°), and 6.13° (2.03°). Lateral bending and axial rotation were roughly symmetric due to the symmetric nature of the spine. For right lateral bending, the ROMs were 8.25° (2.44°), 4.96° (1.70°), and 4.25° (1.20°). For left axial rotation, the ROMs were 1.23° (1.16°), 0.35° (0.61°), 0.87° (0.64°). Neutral zone (NZ) was on average 60% (29%) of ROM for the motions studied. The physiologic ROM of the New Zealand white rabbit lumbar spine was found to be similar between the rabbit and human. This relatively conserved physiologic flexibility supports the use of the rabbit as a model of the lumbar spine for kinematic studies. However, the overall NZ was found to be a greater percentage of ROM in the rabbit than the corresponding percentage in the human (60% as compared to 25%). This suggested that the rabbit lumbar spine has a greater laxity than that of the human.

Key words Spine · Lumbar spine · Biomechanics · Animal model · Rabbit

Introduction

There are many variables that affect the results of lumbar fusions. Particularly with the advent of novel methods of instrumentation and the purification of bone-stimulating growth factors, the orthopedic surgeon is faced with many

surgical options. Objective comparison of these many variables is imperative. Not only must existing fusion modalities be critically evaluated, but novel modalities must also be compared to those already in use.

Outcome studies are an ideal way to compare the efficacy of fusions. However, it is difficult to normalize study groups in this type of clinical trial. Furthermore, baseline

data must be established before techniques can be moved to the clinical setting.

Animal studies are an effective means of addressing these limitations. Outcomes of such studies are extrapolated to human scenarios. Of significance, the more closely animal models mirror clinical scenarios, the more confidence can be placed in such extrapolations. As this implies, models must be designed to address specific clinical questions [9]. Animal size, cost, and ease of care are also issues to be considered.

Multiple animal models have been established to study the spine. One mode of model validation has been to compare physiologic motions of animal spines to those of the human spine. Multidirectional flexibility testing has been used for such determinations. For example, Wilke et al. have studied the physiologic motions of sheep [18] and calf [17, 19] spines. Resulting values are compared to baseline human values [14]. Wilke et al. concluded that the physiologic motions of the sheep and calf are roughly similar to those of the human, and thus reasonably model human spine kinematics.

Boden and colleagues have developed and extensively published on a spine model based on a smaller animal, the New Zealand white rabbit [1, 3, 13, 14]. This methodology was developed specifically to study intertransverse process fusions. The surgical technique used by this group is similar to that used clinically. The observed nonunion rate of 33% with autografting mirrors clinical outcomes. Boden's group did perform pull-apart testing of the resulting fusion masses. In comparison to control spinal segments, fused segments had 90% greater tensile strength

and 40% greater stiffness. However, physiologic biomechanical characterization in response to functional loading was not performed before or after performing fusion surgery.

As with the sheep and calf, there are significant anatomic differences between rabbit and human spines. The rabbit has seven lumbar vertebrae as compared to the human five. The rabbit spine has evolved to facilitate ambulation as a quadruped as opposed to humans who have evolved to facilitate bipedile ambulation. Furthermore, the rabbit is significantly smaller than any animal for which physiologic spine motion has been previously studied.

The purpose of this study is to establish objective physiologic data of the normal rabbit spine. In particular, the lower lumbar spine needs to be characterized to further interpret studies based on the rabbit model, which focuses on the L5-L6 intervertebral level.

Materials and methods

Specimens

Ten skeletally mature New Zealand white rabbit cadaveric spines were obtained. As noted above, this species has seven lumbar vertebrae. Previous studies have focused on the rabbit L5-L6 intervertebral level. It was thus determined appropriate to study this level as well as one level above and one level below (L4-L5 and L6-L7). Osteoligamentous L4-L7 specimens were harvested en bloc. Specimens were dissected of all soft tissues except for ligaments and joint capsules. Specimens were stored at -20°C wrapped in saline moistened gauze and sealed in double plastic bags until testing was performed. Such storage conditions have previously been shown not to affect the outcome of standard biomechanical testing [10].

Specimen preparation

Radiographs were taken of each specimen to ensure that no underlying abnormalities or injuries were present. The superior (L4) and inferior (L7) vertebrae were potted in resin mounts, with the L5-L6

Fig. 1 **A** Anteroposterior and lateral radiographs of rabbit lumbar spine mounted for biomechanical testing. **B** Schematic of specimen as seen from the lateral perspective. Note the motion detection flags mounted on L5, L6, and both the upper and lower mounts

Fig. 2 Pure moments are applied to the specimen via pneumatic pistons to the headpiece attached to the upper vertebra. Motion detection flags designed for an optoelectronic motion measurement system are applied to each vertebra

intervertebral disc oriented in the horizontal position. Screws were placed into the border vertebrae for additional fixation in the resin mounts. Bolts were also imbedded in each mount to allow fixation of the lower vertebra to the testing table and to apply pure moments to the upper vertebra via a headpiece.

The upper and lower mounts (representing L4 and L7) were fitted with Plexiglas motion-detection flags on the lateral aspect of the specimen. L5 and L6 were fitted with similar flags attached to the vertebral bodies via pairs of 0.062-in. k-wires. Each flag was equipped with three non-co-linear infrared light emitting diodes designed for detection by an optoelectronic motion measurement system (Optotrak, Northern Digital, Waterloo, Ontario, Canada) (Fig. 1). The accuracy of the Optotrak measurement system has been determined in an earlier study [5]. The standard deviation and mean of the error were found to be 0.140° and -0.014° respectively.

Three-dimensional flexibility testing

To determine the multidirectional flexibility of the specimens, six pure moments (flexion and extension, left and right lateral bending, and left and right torsion) were applied (Fig. 2). These moments were applied to the top mount via a headpiece while the bottom mount was fixed to the testing table. The masses of the headpiece, upper mount, and L4 (uppermost vertebra) were summed and counterbalanced from the center of gravity of the combined masses. This was accomplished with a weight suspended from above with a pulley that was free to follow the motion of the headpiece below. This method of testing has been established and previously published for human specimens [11, 12, 20]. The specimens were kept moist with normal saline throughout testing.

Human specimens are loaded to a maximum of 10 Nm in the studies referred to above. It was determined appropriate to decrease the testing moment applied to the rabbit spines in a body mass proportional fashion. Thus, a maximum moment of 0.27 Nm was selected for testing. The idea of loading based on body mass has been used in prior experiments [16].

Further validation of the selected testing moment was obtained from preliminary reproducibility experiments. Range of motion was found to be reproducible to 0.81° (0.68°) [mean (standard deviation)] with the maximum testing moment of 0.27 Nm. This was felt to be within the error of the system and to indicate that no sig-

nificant injury was produced by the loading protocol. Conversely, gross injury was observed with loading to 0.40 Nm.

The loading protocol involved loading in a stepwise fashion to the maximum load. Each step (0.00, 0.09, 0.18, and 0.27 Nm) was sequentially applied for 30 s. A total of three load/unload cycles was performed for each motion studied and data were gathered from the final loading cycle. This protocol was established to minimize error due to the effects of creep. Each specimen was sequentially tested in flexion, extension, right lateral bending, left lateral bending, right axial rotation, and then left axial rotation.

Data analysis

Intervertebral rotations were calculated for each level in reference to a single zero point, which was obtained at the onset of testing for each specimen. There were six main motions, corresponding to the six moments applied. For each direction of motion, range of motion (ROM) and neutral zone (NZ) were analyzed. ROM is defined as the displacement from initial neutral position of the specimen to that at the maximum load. NZ is defined as the motion from the initial neutral position to that at the unloaded position of the specimen at the beginning of the third load cycle. Results of all ten specimens were averaged and standard deviations were calculated.

Results

Flexibility data were gathered for the ten rabbit spines. Data were analyzed as noted above. Figure 3 represents the motions of the L5-L6 intervertebral level as seen in the stepwise loading protocol. This is shown as representative of the three levels studied in this experiment. As seen in the figure, a significant portion of the motion for each direction of applied moment was due to the NZ, with a gradual increase in displacement with subsequent loading up to ROM with the application of 0.27 Nm.

Data for all levels studied are shown in Table 1. Flexion and extension are independent study parameters. Lateral bending and torsion are expected to be symmetric due

Fig. 3 Mean (SD) measures of each main motion of the L5-L6 intervertebral level of the rabbit spine. Marked are the flexion neutral zone (NZ) and range of motion (ROM) as examples of parameters drawn from the testing protocol

Table 1 Physiologic motions of the lowest three intervertebral levels of the rabbit lumbar spine loaded to a maximum of 0.27 Nm. Mean (SD) represented in degrees (NZ neutral zone, ROM range of motion)

Level	NZ	ROM	NZ	ROM
Flexion/extension				
	Flexion		Extension	
L4-L5	9.69 (2.28)	12.10 (2.59)	-4.15 (1.39)	-5.86 (1.21)
L5-L6	9.90 (2.27)	12.38 (2.70)	-4.08 (1.74)	-5.58 (1.48)
L6-L7	12.52 (2.46)	15.17 (3.22)	-4.43 (2.14)	-6.13 (2.03)
Lateral bending				
	Right lateral bending		Left lateral bending	
L4-L5	6.41 (2.08)	8.25 (2.44)	-8.74 (2.81)	-10.23 (2.83)
L5-L6	3.79 (1.63)	4.96 (1.70)	-6.69 (2.38)	-7.52 (2.43)
L6-L7	3.10 (1.61)	4.25 (1.20)	-4.60 (2.45)	-5.78 (2.67)
Torsion				
	Left torsion		Right torsion	
L4-L5	0.56 (0.66)	1.23 (1.16)	-0.24 (0.93)	-0.83 (1.23)
L5-L6	0.05 (0.51)	0.35 (0.61)	-0.26 (0.43)	-0.73 (0.47)
L6-L7	0.37 (0.55)	0.87 (0.64)	-0.08 (0.53)	-0.41 (0.60)

Fig. 4A–D Comparative NZ and ROM data presented for the lowest three intervertebral levels of four different species. Rabbit data are from this study. Human, sheep, and calf data are drawn from the literature for comparison [18–20]. **A** and **B** represent flexion and extension data; **C** and **D** represent unpaired right lateral bending and left torsion data, as these are roughly symmetric with the complementary left lateral bending and right torque data

to the symmetry of the lumbar spine. The relative differences with these parallel data are comparable to those of the reported human data [20].

ROM and NZ tend toward increased flexion and decreased lateral bending, moving caudad through the levels tested. Between intervertebral levels there were ROM differences in flexion of 3.07° (23%), in extension of 0.55° (9%), in right lateral bending of 4.00° (69%), in left lat-

eral bending of 4.45° (57%), in left axial rotation of 0.37° (44%) and in right rotation of 0.42° (64%). Between intervertebral levels there were NZ differences in flexion of 2.83° (26% average value), in extension of 0.37° (8%), in right lateral bending of 3.3° (75%), in left lateral bending of 4.14° (62%), in left axial rotation of 0.19° (58%) and in right axial rotation of 0.16° (83%). The greatest motion for each level tested was in flexion, with lesser motion in extension and lateral bending and least motion with torsion.

Discussion

The New Zealand white rabbit is increasingly being used as an animal model for evaluating the effectiveness of posterolateral lumbar fusions. Many research groups are currently using this model [2, 4, 7, 8, 15]. Although the biomechanical properties of the fusion mass itself have been studied, the baseline and subsequent alterations in physiologic lumbar spine motion have not yet been established.

The primary purpose of this study was to determine baseline biomechanical flexibility parameters of the New Zealand white rabbit lumbar spine. The presented ROM and NZ data can be used as normative values to which future experiments can be compared.

The secondary purpose of this study was to compare the physiologic motion of the rabbit lumbar spine to that of the human lumbar spine. The rabbit, which is significantly smaller than the human, is being used extensively in the research arena, but has not previously been evaluated in a comparative fashion. The data from Table 1 are presented in comparison to human data [20] in Fig. 4. Physiologic ROM is remarkably similar between the rabbit and human. In fact, the average difference in multiplanar ROM between the two species at the lowest three intervertebral levels was only 2.42° . This is most likely attributable to similarities in the facet architecture and intervertebral disc properties between the two species at the levels being compared.

Of note, the average NZ was a greater percentage of the ROM with the rabbit than with the human: 60% as

compared to 25%. If broken down to the individual planes of motion, the rabbit NZ/ROM percentage was 76% in the flexion/extension plane, 80% in the lateral bending plane, and 31% in the axial rotation plane. Analogous values for the human are 23%, 27%, and 26% respectively. The greater NZ/ROM ratio of the rabbit spine than the human spine suggests that the rabbit has greater laxity. This is particularly true in the flexion/extension and lateral bending planes.

The sheep and calf are the only other animals, to our knowledge, for which physiologic motions of the lumbar spine have been studied [18, 19]. Both were tested with maximum moments of 7.5°Nm . Sheep have seven lumbar vertebrae, while calves have six. The data for the lowest three intervertebral levels are presented in Fig. 4, to compare to the human and rabbit data. These additional data sets show a tendency toward decreased flexion and axial rotation compared to that of the human, but are otherwise similar to the human data.

In comparing the rabbit with the previous sheep and calf data, the multiplanar average ROM differences were 3.01° and 3.31° . Flexion, for both the sheep and calf, was the direction of greatest difference from the rabbit data set. This is shown graphically in Fig. 4. The sheep and calf were found to have NZ/ROM percentages of 16% and 34% respectively, as compared to the human 25% and the rabbit 60%.

Conclusions

In general, physiologic motion of the lumbar spine seems to be relatively conserved among the species in which it has been studied. Although some differences are noted, the ROM of the rabbit lumbar spine closely approximates that of the human lumbar spine. Both the NZ and NZ/ROM percentage of the rabbit lumbar spine are somewhat greater than those of the human lumbar spine. This study provides baseline data to which future kinematic studies of the rabbit lumbar spine may be compared.

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