Comparison of In Vivo and In Vitro Adjacent Segment Motion After Lumbar Fusion

Mark B. Dekutoski, MD, Michael J. Schendel, PhD, James W. Ogilvie, MD, John M. Olsewski, MD, Larry J. Wallace, DVM,* and Jack L. Lewis, PhD

Study Design. For in vitro studies, there is no basis for choosing a “load control study” over a “displacement control” study. This study qualitatively compared results from in vitro and in vivo tests, allowing the authors to address the experimental assumptions that in vitro testing contributes to the understanding of the in vivo condition.

Objectives. To compare motion changes at segments adjacent to fusions for in vitro and in vivo tests.

Summary of Background Data. Investigators have measured the effects of spinal fusions on the adjacent segment in a human cadaver model and found greater adjacent facet joint load after fusions. Others have found significant increases in motion and facet loads at segments adjacent to in vitro lumbar sacral and long fusions, when the same range of motion was repeated before and after immobilization of lumbar segments.

Methods. L2-L3 motion was measured in vitro by an instrumented spatial linkage under load and displacement control before and after immobilization of segments (L3-L7). In vivo, L2-L3 motion was measured while animals walked on a treadmill. L3-L7 was fused and the L2-L3 motion testing was repeated. The change in in vivo adjacent segment motion was qualitatively compared with the in vitro change under “load” and “displacement” control.

Results. Under “load” control, in vitro facet motion did not significantly change after immobilization, whereas under “displacement” control, the facet motion significantly increased from 2.2 ± 0.4 mm to 4.1 ± 0.6 mm. Post-instrumentation, in vivo L2-L3 facet motion increased significantly. This change in vivo related better to the changes seen in the in vitro “displacement” control test than to the in vitro “load” control test. [Key words: adjacent segment, canine, in vivo motion, intervertebral motion, lumbar fusion; Spine 1994; 19:1745-1751]

Clinically, degeneration of motion segments adjacent to a spinal fusion has become a significant problem. Follow-up studies have demonstrated that adjacent segment problems account for a significant proportion of the 20% to 40% poor long-term results seen after lumbar spine fusion for degenerative conditions. Significant pathology at these levels is the indication for surgical immobilization, yet little is known about the effect this has on the overall functional capacity of the patient, the function of the lumbar spine, the load and motion within the remaining motion segments, and the metabolism of disc and facet cartilage. Experimental methods are invoked to assist in the understanding of this clinical problem. Interpretation of in vitro animal or human cadaveric data depends on the answers to two questions. First, is the bovine or canine spine comparable to the human spine? Second, is the in vitro testing condition comparable to the in vivo condition?

The canine has been used for in vivo lumbar spine investigations of cartilage and disc biochemistry after fusion. Recently, surgical artifact was shown to be a significant problem of in vivo motion measurement and surgical manipulation. Schendel et al noted that surgical exposure results in significant loss of motion and ankylosis as early as 3 weeks postoperatively. Others have noted the stabilizing effects of soft tissue scarring, and these studies have emphasized the need for adjacent segment biomechanical investigations (concurrent with biologic studies) in which the mechanics of the joint are known and are not altered by the hypertrophic osseous scar overgrowth noted in canines and rabbits. Gait pattern for the functional quadruped affects spinal motion in the canine, baboon, goat, and rabbit models. Despite a quadruped gait, the canine has been documented to exert significant axial facet load during gait. Except for noninvasive human testing, no adequate bipedal experimental model exists.

Experimental results of in vitro models vary according to testing methods. With fusion, it is believed that the motion, previously distributed over several lumbar segments, will be transferred to the open segments and increase the motion and load of these segments. Lee and Langrana investigated the effects of spinal fusions on the
adjacent segment in a human cadaver model loaded axially before and after immobilization. These motion data were analyzed by a mathematical model; for this load control experiment, an increase in stress on the adjacent unfused segment was noted. With their in vitro load control/mathematical model, posterior fusions were found to cause greater adjacent facet joint load than anterior fusions.\textsuperscript{14} Yang et al used the same model with compression-torsion loads and found no significant increase in stress at the adjacent level.\textsuperscript{25} Ha et al found significant increases in motion at segments adjacent to an in vitro lumbar-sacral fusion. They also observed changes in resulting contact patterns of the facet joint in the adjacent segment. These changes occurred when the canine cadaveric spine was forced to reproduce the same range of motion before and after immobilization of lumbar motion segments.\textsuperscript{9} In another canine cadaver study designed to examine the effects of the length of fusion (or the number of open segments between the end of the instrumentation and the sacrum), Nagata et al found facet loads were increased at all mobile segments and not just at the segment adjacent to the instrumentation.\textsuperscript{15} This last study also was a displacement control experiment.

There is no basis for choosing a “load control study” over a “displacement control” study. Furthermore, for the in vivo condition, it is not known whether an animal will attempt to reproduce the same range of motion post-fusion or whether the animal will just apply the same load to the segments. In vitro animal testing is designed to “model” the in vivo state. The present study sought to evaluate in vitro testing methods and compare the results with results from in vivo testing.

We hypothesized that for the in vitro experiment, an increase in motion at the segment adjacent to the immobilization would be noted during a standard activity and that this increase would be confirmed by the in vitro experiment under displacement control.

## Materials and Methods

This investigation was carried out in canine in vitro and in vivo models of a L3–L7 fusion/imobilization.

### In Vitro Materials and Methods

Four cadaveric canine spines (weight, 27 to 42 kg) were obtained from killed animals used in radiology studies. Specimens were obtained immediately ex vivo, en bloc with soft tissues from T8 to the pelvis, and stored at \(-20\) C. Specimens were thawed to room temperature immediately before they were used and were maintained moist with a 0.9 mol/L NaCl solution and kept on ice during preparation. Muscle and soft tissue were maintained intact, except over the tips of the spinous processes of L2, L3, and the superior articular processes of L3 bilaterally. Specimens were transected at the body of the ilium and at T10–T11 for mounting in polymethylmethacrylate (PMMA). Polymethylmethacrylate fixation was supplemented with screws that transfixed the sacroiliac joints and the T11–T12 motion segment. Motion was permitted over nine segments between T12–T13 and L7–S1. Wood screws (standard 3/8 × 2.5 inch) were placed into the vertebral bodies of L3, L5, and L7 parallel to the end plates for later use. Two strands of piano wire were passed down the spinal canal, through the ligamentum flavum at L7–S1, and out through the immobilized/mounted T10–T11 interspace. These wires were later used to apply an axial load. Dual 2.5 mm Schanz pins (Synthes, Paoli, PA) were inserted into the spinous processes of L2 and L3, and an instrumented spatial linkage (ISL) was mounted on these pins.

The specimen was mounted in a 6-degree-of-freedom load testing device to allow for displacement of the spine orthogonal to gravity. A load cell was attached to an extension arm on the proximal end of the specimen. It measured the loads that were applied to the L1 end of the specimen to move the specimen from neutral to a specified end point, corresponding to a total lumbar spine range of motion of ±30° (Figures 1A, B).

Each set of tests consisted of five repetitions of 30° of flexion, extension, and right and left lateral bending, with and without 100 N of axial load. Data from ISL potentiometers and the load cell were recorded during movement from the neutral position to the 30° angulation end point. These data were collected via a computer-controlled data acquisition system (Asyst Software Technologies, Inc., Rochester, NY) in an IBM (Armonk, NY) PS/2-80 microcomputer.

Testing of the unfused spine was followed by application of PMMA to the ventral screws and rods that formed the immobilization between segments L3–L5–L7. After this immobilization was completed, the test sequence already described was repeated.

The ISL was calibrated using a calibration device and op-
timization program that calculated parameters needed to define the transformation matrix between the two ends of the ISL. To provide local coordinate systems, the transverse and spinous processes of L2 and L3 were digitized as reference points using a calibrated three-dimensional digitizer. Mounting bars on the ends of the ISL were digitized to define ISL local coordinate systems. After the bony coordinate systems and ISL local coordinate systems were defined, the constant transformation matrices between these systems were calculated. Vertebral body motion of L2 relative to L3 was determined from the ISL transformation matrices and the constant bone to ISL end transformation matrices determined earlier.

Vertebral body motion and facet motion of L2–L3 were compared between the completely mobile state (no immobilization) and the adjacent segment state (immobilized from L3–L7) using a paired Student's t test.

In Vivo Materials and Methods. Four adult male canines (weight, 33 to 39 kg) were trained on a leash and treadmill. Posteroanterior, oblique, and lateral radiographs were obtained to assess maturity, to screen for osseous anomaly, and to screen for roentgenographic evidence of facet arthrosis or disc space narrowing. With fluoroscopic guidance and under a general anesthesia (15 mg/kg intravenous sodium thiamylal) two parallel 2.5 mm Schanz pins were placed into the spinous processes of L2 and L3 through a cutaneous incision as per the methods of Schendel et al.17 The pins were cut (so that ≈10 mm protruded from the process) and capped (Synthes), and the wound was closed. The dorsal soft tissues, fascia, and muscle were not disrupted as per the methods of Schendel et al.17 One week later, the animals were anesthetized via inhalation anesthetic (halothane and nitrous oxide), the wounds were opened, pins were exposed, and an ISL was attached to the pins. The animals recovered and walked voluntarily in the test area until they were steady. The ISL was connected to the data collection hardware and the animals were walked voluntarily with a leash on a treadmill at 2.0 mph. Data were collected over 5 second periods during each maneuver via an analog to digital convertor and custom software. After data collection, the animals were anesthetized so the ISL could be removed, the pin caps could be replaced, and wound could be closed.

One week after ISL testing, the animals were given perioperative antibiotics and anesthetized, and L3–L7 was instrumented/fused by a dual approach (Figures 2A, B). The dual approach was necessary because of the surgical artifact present at all exposed levels. If the interspinous wiring alone was used, the adjacent segment (segment being studied) would have experienced some motion artifact because of fusion overgrowth from the surgical exposure. Therefore, a lateral plate was used near the adjacent segment to avoid exposure of the posterior musculature and subsequent surgical artifact. First, a dorsal midline approach to the spinous processes of L4–L7 was conducted. The supraspinous and interspinous ligaments were left intact. No facet capsules were exposed. For immobilization, an interspinous process wiring technique with dual longitudinal Steinmann pins (½ and ¾ inches) was used. Then, a unilateral, paraspinous approach to L3 and L4 was performed. Periosteal exposure was limited to that necessary for vertebral body plate application. The dorsal nerve roots of L3 and L4 were identified and preserved. The dorsal nerve root of L2 was neither exposed nor destroyed. A four- or five-hole 3.5 mm DC Plate (Synthes) was contoured to the posterolateral bodies of L3 and L4. Two cortical screws were placed by standard technique into each of the vertebral bodies (L3 and L4). Immediate postoperative radiographs were taken to assure proper alignment of the implants. Animals were given analgesics and antibiotics, housed, fed, and cared for according to the National Institutes of Health guidelines for animal care.

The ISL motion testing protocol was repeated 1 and 12 weeks post-instrumentation. Animals were evaluated roentgenographically at 1 month intervals for evidence of implant failure, disc degeneration, and facet arthrosis. After the 12 week interval motion test, the animals were killed and the position of the ISL to vertebral bodies was measured via a calibrated three-dimensional digitizer. Motion data were calculated per the protocol of Wood et al.24 Facet excursion was measured from computer animation of the facet movements. Comparison of pre-instrumentation, 1 week post-instrumentation, and 12 weeks post-instrumentation facet motion was conducted by paired Student's t tests.

Comparative Analysis. The purpose of this comparative analysis was to gain qualitative insight into the type of control (load control or displacement control) being placed on the
lumbar spine in vivo. Facet displacement data from the in vivo pre- to post-instrumentation state was compared with the in vitro facet displacements for the L3–L7 immobilization. The comparison was made for two in vitro methods. The first was a fixed displacement condition in which the range of motion was kept constant from the pre- to post-immobilization. The second method was a fixed load condition in which the applied load was kept constant from pre- to post-immobilization. Statistical analysis using Student's t test was conducted to determine the significance of motion changes at the adjacent segment after fusion/immobilization and to compare in vivo with in vitro load and displacement states.

Results

Concurrent and previous use of the ISL, with daily calibration, has shown accuracies of 0.3 to 0.5 mm in position and 0.4° to 0.6° in rotation. Use of the spinous process/ISL in vivo motion testing system has been reported to eliminate the surgical artifact noted in previous applications of this instrumentation. Use of a standardized treadmill set-up, pre-training of the animals on the treadmill, and collection of data during periods of continuous gait at a regulated speed have reduced variability of testing conditions.

In Vitro L3–L7 Immobilization

An increase in motion at the adjacent segment (L2–L3) was found for all motions after immobilization of the L3–L7 motion segments (Figure 3). The average primary rotations at the L2–L3 segment of the completely mobile spine were 3.2° ± 1.0° in extension, 2.3° ± 0.6° in flexion, 5.8° ± 1.8° in left lateral bending, and 6.3° ± 1.7° in right lateral bending. The changes shown in Figure 3 resulted in motions being increased by 48% in extension, 79% in flexion, 94% in left bending, and 94% in right bending. After immobilization from L3–L7, the adjacent segment total sagittal plane motion increased from 5.5° to 8.9° (61%), while the total coronal plane range of motion increased from 12.1° to 23.4° (94%). All increases in motion at the L2–L3 segment after the immobilization of segments L3–L7 were statistically significant (P < 0.05). The application of a constant 100 N axial load on the column was found not to affect the motion at the L2–L3 segment before or after the application of the immobilization construct.

The motion of the facet joint also was tracked throughout the in vitro testing so that comparisons could be made with the in vivo results (Figure 4). The average peak facet motion (as tracked in an identical fashion to the in vivo experiments) for the L2–L3 joint of the completely mobile lumbar spine with no axial load was 3.1 ± 0.3 mm in extension, 3.2 ± 0.8 mm in flexion, 2.4 ± 0.5 mm in left lateral bending, and 2.7 ± 1.1 mm in right lateral bending. Note that the facet motion described is that of the right articular process; thus, similar results between right and left lateral bending were not expected. After the segments from L3–L7 were immobilized and the lumbar spine was forced through the same range of motion, the peak motion of the facet joint increased in all motion modes, but not all increases were statistically significant.

Figure 3. This graph illustrates intervertebral rotation at the L2–L3 motion segment before immobilization (open bar) and after immobilization of segments L3–L7 (solid bar). Rotation angle (in degrees) is represented with a standard deviation for the accompanying primary rotation. These data represent the “displacement control” test.

Figure 4. Change in facet excursion after immobilization of segments L3–L7. The open bar shows facet excursion for the L2-L3 joint with a completely mobile spine, and the solid bar illustrates excursion after the immobilization of L3–L7. These data represent the “displacement control” test.
The load required to move the specimens through the 30° range of motions was measured during each maneuver and was found to be 10.0 ± 1.2 N for extension, 6.3 ± 1.0 N for flexion, 7.8 ± 1.2 N for left bending, and 8.2 ± 1.7 N for right lateral bending. The amount of load required to move the specimen through the same range of motion after the segments from L3–L7 were immobilized increased significantly (P < 0.05) for all motion directions. The increases were 7.5 ± 1.5 N in extension, 3.4 ± 2.0 N in flexion, 10.3 ± 2.6 N in left bending, and 12.1 ± 2.7 N in right bending.

**In Vivo L3–L7 Fusion**

Animals were moving around and comfortable immediately after pinning and ISL testing and within 24 hours of the immobilization surgery. No wound complications or neurologic sequelae were noted for these animals. An increase in hind-end sway was observed during slow walking postoperatively. Increases in vertebral body rotations at the adjacent segment postoperatively were from 1.3° ± 0.2° to 1.6° ± 0.4° in the axial plane, 1.9° ± 0.8° to 2.0° ± 0.3° in the sagittal plane, and 4.2° ± 8.9° to 6.1° ± 1.5° in the coronal plane. Compared with the pre-operative motion measurements, rotation changes were significant (P < 0.01) only in the coronal (right/left) plane for an identical pre- and post-fusion activity of treadmill walking at 2 mph. The significant increase in coronal plane vertebral body rotation was noted consistently at 1 and 12 weeks post-instrumentation/fusion. The excursion of the L2–L3 facet is the length of relative movement of the inferior articular process facet surface in the plane of the superior articular process facet. For all animals tested, the length of excursion increased significantly from 1.8 ± 0.2 mm to 2.9 ± 0.1 mm (P < 0.005) after instrumentation and fusion.

Preoperative oblique radiographs of the L2–L3 “adjacent segment” lumbar facets had no evidence of narrowing or sclerosis. Radiographic evidence of fusion was not evident on posteroanterior, oblique, or lateral radiographs. At 12 weeks post-instrumentation, no radiographic evidence of adjacent segment stenosis or degeneration was noted. Gross anatomic evaluation of the immobilized segments was remarkable for osseous and fibrous ankylosis of the immobilized segments L3–L7. Hypertrophic callus formation was noted over the 3.5 mm DC plate placed on the posteroilateral vertebral body surface of the L3–L4 motion segment. Osseous and fibrous tissue ankylosed the spinous processes, lamina, and posterior instrumentation. No gross evidence of facet ankylosis was noted. All areas of soft tissue that had been surgically altered had increased fibrous connective tissue.

**Comparison of In Vitro and In Vivo Facet Motion**

Results

Changes in in vitro facet motions from the two different loading conditions were compared with the in vivo facet motion changes. The first load condition was a fixed displacement condition where the same lumbar spine range of motion was repeated after immobilization of segments L3–L7. The second loading condition was a fixed bending moment condition determined by the force applied manually to the specimen to achieve the 30° end point/range of motion pre-immobilization. For this in vitro “load” condition, the load equivalent to the load at the end point of the displacement in the completely mobile specimen was determined and defined as the “end-point load.” When the load on the specimen with the immobilization reached the “end point load,” this defined the end point segment motion data for the “load” control condition. The “end point” always was reached before moving through the complete range of motion.

The lateral bending mode was chosen to compare the in vitro and in vivo facet excursion data. This was done because lateral bending showed the most change in vivo and in vitro. Table 1 illustrates the results of the comparison and shows that under in vitro load control, the facet excursion at the L2–L3 joint showed no change, whereas under displacement control, the increases were quite pronounced, although at a higher scale than that shown in vivo. The larger scale of motion changes was attributed to the larger load applied to the larger total range of motion used in the in vitro case.

**Discussion**

Investigation of in vivo lumbar spine biologic changes after fusion previously were conducted in the canine. These studies of cartilage and disc biochemistry after fusion have been conducted without documentation of the alteration in joint mechanics.1,3,4,11,21 Investigations by Wood et al (1992), Buttermann et al (1992), and Schendel et al (1992) elucidated some of the mechanics of the unaltered in vivo canine lumbar spine motion segments.2,17,24 To our knowledge, the only studies of post-fusion in vivo mechanics of the lumbar spine have been kinematic or static assessments of flexion/extension radiographs in humans or canines.3,4,6,16,19 Use of the quadruped canine model allows in vitro and in vivo experimental conditions to be compared. In vitro testing of human or other animal specimens is limited by the ex vivo alteration of soft tissue stiffness and the complex nature of duplicating in vivo loading and physiologic response in an in vitro study.13 Although the present study was conducted in a quadruped animal, it allowed the in vivo and in vitro states to be compared, and thus allowed for a description of the physiologic alterations of mechanics and biology created by immobilization/fusion of lumbar motion segments.

Evaluation of the differences between the in vitro and in vivo states is essential to in vitro experimental design and assessment of the validity of in vitro studies. Buttermann et al2 noted that during walking, the canine had an axial load of 50 to 150 N across the lumbar
motion segments, and the facet contact site covered an area about 2.0 mm in diameter, similar to that found for the pre-immobilization specimens in the present study. We had hypothesized that in the in vitro experiment, the application of the axial load would alter adjacent segment motions and facet excursions. We were not able to document any difference in segmental motion or facet excursion for our experimental conditions with and without a 100 N axial load. This contradicts the findings of Janevic et al (1991), who noted an alteration in in vitro lumbar motion segment stiffness upon application of axial load. The inconsistency of these findings probably was the result of the different magnitudes of loads involved and the different geometries of human and canine vertebrae. In the human cadaver study by Janevic et al (1991), the axial loads used were quite large (1000 N), which was appropriate to the human condition. However, the axial loads applied in the present study were relatively small and probably would not induce the stabilizing effects seen in the human cadaver study.

In vivo segmental motion was compared with in vitro segmental motion for two separate conditions. For the in vitro testing, the specimen was moved through a range of flexion/extension or right/left lateral bending while loads at the proximal end of the specimen were measured. This “load” analysis gave the facet excursion for equal pre- and post-immobilization displacement loads. “Displacement” analysis indicated that facet excursion was assessed for an equal end point of total lumbar range of motion (flexion/extension or right/left lateral bending) pre- and post-instrumentation. These changes are noted in Table 1. Based on these data, displacement control appears to simulate the in vivo changes caused by the immobilization of lumbar motion segments. This lends some credibility to the use of in vitro studies to predict in vivo changes. The in vivo motion data also are valuable for designing in vitro studies because the actual physiologic motion now can be used in in vitro studies. This also indicates a role for displacement control studies in which a range of motion is duplicated before and after an experimental alteration to the motion segments.

In vivo and ex vivo canine studies on adjacent segment histology and biomechanics have noted fibrosis and ankylosis after posterior surgical exposure. The modified ISL technique, used in the present study, places spinous process pins under fluoroscopic guidance and limits surgical exposure, thus limiting soft tissue and osseous ankylosis. The facet excursion measured for the normal segments in this study agreed with the values reported by Wood et al (1992). This fusion technique also avoids exposure of the adjacent segment. The postulate that the immediate postoperative increase in adjacent segment motion was likely to decrease over the same time course as our previous surgical artifact animals was anticipated. With this technique, adjacent segment ankylosis was not apparent. Increased motion of the adjacent segment was consistently noted at 1 and 12 weeks post-instrumentation/fusion. This also suggested that the animals may have been attempting to reproduce the pre-instrumentation range of motion in the lumbar spine and did not completely compensate for the loss of mobile segments.

Although increased load and motion are assumed to exist post-fusion at the immediately adjacent open segment, little data have documented that during physiologic function this motion actually increases in the non-pathologic state. Adjacent segment instability with pathologic, degenerative spondylolisthesis has been well described. Although it has been assumed that motion is increased at the adjacent segment, the present study documented increased motion at the adjacent open segment during routine physiologic maneuvers (i.e., walking). This canine model shows great promise as a tool for investigating biologic responses to changes in mechanics of a joint. Future investigations will include examinations of the biologic response to altered mechanics in joints where the tissue is damaged chemically or mechanically.

References

11. Kahanovitz N, Arnozsky SP, Levine DB, Otis JP. The

Address reprint requests to

Michael J. Schendel, PhD
Dept. of Orthopaedic Surgery
University of Minnesota
Box 289, 420 Delaware SE
Minneapolis, MN 55455