Published in final edited form as:

Spine J. 2009 February ; 9(2): 105–114. doi:10.1016/j.spinee.2007.09.006.

Mature Runt Cow Lumbar Intradiscal Pressures And Motion Segment Biomechanics

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Abstract

Background Context—The optimal animal model for in vivo testing of spinal implants, particularly total or partial disc replacement devices, has not yet been determined. Mechanical and morphological similarities of calf and human spines have been reported; however, limitations of the calf model include open growth plates and oversized vertebrae with growth. Mature runt cows (Corrientes breed) may avoid these limitations.

Purpose—This study compared vertebral morphology and biomechanical properties of human and runt cow lumbar motion segments.

Study Design—In vivo disc pressure measurements were obtained in six mature runt cows at L4–5. In vitro evaluation was performed on these same segments and repeated on twelve human motion segments.

Methods—Disc pressures were measured in vivo in runt cow (Corrientes breed) L4/5 discs using a percutaneous transducer with the animal performing various activities. These motion segments were then harvested and morphologic and biomechanical evaluations (disc pressure in compression, flexibility tests to 7.5 Nm) were performed on both cow and male human L2/3 and L4/5 segments.

Results—The transverse lumbar disc dimensions were slightly smaller for (mixed gender) cow vs. (male) humans, but were within the range of reported (mixed gender) human values. The mean $\pm$ SD disc height was smaller for runt cow (7 ± 1 mm) vs. human discs (13 ± 2 mm, $p < 0.001$). The vertebral bodies of the cow were approximately twice as tall as the human. In vitro testing revealed significantly greater disc pressure response to applied axial loading in the runt cow vs. humans (1.27 ± 0.18 kPa/N vs. 0.84 ± 0.15 kPa/N respectively) but similar overall stiffness (2.15

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± 0.71 kN/mm vs. 1.91 ± 0.94 kN/mm, respectively). Runt cow and human segments flexibility curves were similar with the following exceptions: runt cow stiffness was ~40% greater in torsion ($p < 0.05$), runt cow segment lateral bending motion was greater vs. humans (range of motion by 30%, neutral zone by 100%; both $p < 0.05$) and flexion range of motion tended to be smaller in runt cow vs. human specimens (by ~40%, $p = \text{NS}$). In vivo, the standing disc pressure in the runt cow was 0.80 ± 0.24 MPa.

Conclusions—Although no animal replicates the human motion segment, the runt cow lumbar spine had a number of biomechanical and morphological measurements within the range of human values. The closed physes and temporally stable morphology of the mature runt cow may make this model more suitable vs. standard calf models for human intradiscal implant studies.

Introduction

Interest in lumbar spine arthroplasty has increased because spinal fusion for end-stage degenerative disc disease may result in decreased range of motion and accelerated deterioration of the remaining adjacent discs, particularly when multiple disc levels are fused[1–3]. Disc arthroplasty devices must be durable and able to continue to function for the duration of the patients' lives of which most would have implantation during their middle ages. The durability requirements for spinal disc arthroplasty thus far exceeds that of fusion devices, which only need to survive until fusion is obtained, and joint arthroplasty of the appendicular skeleton, which usually applies to the elderly patient with limited lifespan. Animal models may be useful in the design and testing of spinal disc replacements. Optimal in vivo testing should use a mature mammal with lumbar disc morphology and in vivo loading that approximate those of the human spine.

Domestic mammal spines have often been used for in vitro testing of lumbar spinal fusion implants because motion segment load-displacement relationships are somewhat similar to human data[4–9]. But most of these animals, such as the dog, sheep, goat and pig, have vertebra and disc anatomy which are substantially smaller than that of humans[10–15]. Lumbar calf spines are of similar size to human spines, but often fail at relatively low testing loads because the growth plates are still cartilaginous[7;16–18].

There is also controversy as to whether the appropriate animal model for in vivo testing should have an upright posture[19]. Some believe that spinal loading depends on upright posture and have recommended the use of kangaroos or nonhuman primates. However, there are difficulties with these “erect” animal models; as most are scarce, expensive, difficult to work with and are in reality primarily quadruped [20]. Because of their small disc height and diameter, prior studies using nonhuman primates have necessitated routine removal of substantial portions of the endplates in order to fit even miniaturized disc implants[20;21]. Furthermore, similarities in vertebral anatomy and trabecular orientation suggest similarities in the predominant axial forces in bipeds and quadrupeds[19].

In a pilot study, the authors found that the mature runt (“mini-”) cow and male pig (boar) lumbar spines had a number of anatomical size similarities to the human lumbar spine particularly with respect to the intervertebral disc[22]. Surgical access to the lumbar spine of

the runt cow is simple relative to that in the boar, and may therefore be a more suitable animal model for the study of human disc prostheses[23].

The purpose of this study is to compare and contrast runt cow and human lumbar anatomy, in vitro and in vivo intradiscal pressures, and multi-axial load-displacement properties. One hypothesis of this study is that the disc of the mature runt cow, which is similar to human size, has intradiscal pressures similar to human (e.g., 0.5 to 1.2 MPa range while standing) [24–28]. The combined in vitro and in vivo results would validate that the runt cow may be a suitable in vivo animal model for study and treatment of human lumbar intervertebral conditions.

Methods

In vivo cow intradiscal pressure testing

After approval by the institutional animal care and use committee, six adult runt cows (Corrientes breed, 3 female and 3 male, size and age in Table 1) were obtained for study. Each cow was premedicated with xylazine (0.02 to 0.05 mg/kg, intramuscularly [IM]), and anesthetized with ketamine (2–4 mg/kg, intravenous [IV]) and Pentothal (3–4 mg/kg, IV, to effect.) Buprenorphine (0.005–0.01 mg/kg, IM) was administered to provide preemptive analgesia and then each animal was intubated and maintained in anesthesia with isoflurane or sevoflurane inhalant anesthetic.

The animal was placed in a semi-sternal recumbent position and its L4–5 disc was identified under fluoroscopy. After application of local anesthetic, a 1.5 mm diameter trocar was percutaneously placed from a dorsolateral approach into the disc. A cannulated guide tube was placed over the trocar and secured in the annulus. The trocar was removed, and through the guide tube a calibrated intradiscal pressure transducer (1.3 mm diameter × 20 cm length, <5% error, Medical Measurements Incorporated, Hackensack, New Jersey) was placed into the L4–5 disc using a technique previously described[28], but modified to allow wireless data acquisition. Biplanar fluoroscopy was used to confirm transducer placement within the central region of the disc.

Opening intradiscal pressure values were obtained with the animal at rest and the changes related to ventilation (0.02 to 0.05 MegaPascal, MPa) and spontaneous breathing (mean 0.05 MPa, range 0.01 to 0.16 MPa) were recorded to determine their artifact. The animals were transferred to a 3 × 5 m pen and allowed to recover (typically 30 to 90 minutes) after which intradiscal pressure measurements were again obtained with the animal lying holding its head up, standing, and walking. After satisfactory data acquisition, animals were sedated with xylazine (0.2 mg/kg IM) and ketamine (2–4 mg/Kg), and then euthanized with an overdose of phenobarbital.

Morphometric data

After in vivo testing, the lumbar cow spines were harvested and stored at –20° C. Male human L2–3 (n=6) and L4–5 (n=6) motion segments were obtained from an ongoing study[29]. Radiographs and bone mineral densities (DEXA, GE Lunar Medical Systems, Madison, WI) were obtained for the runt cow and human spines. Morphometric data for

both runt cow and human specimens were obtained from radiographs and direct measurements using calipers. This included vertebral endplate diameters in coronal and sagittal planes, L4/5 intervertebral lordosis (Cobb technique), disc and vertebral body height, and transverse process length (Figure 1). The error between radiographic and direct measurements was 0.7 mm on average and ranged from 0 to 3 mm. After flexibility testing, the motion segments were disarticulated and the discs cross-sections digitally photographed with a standardized distance marker. The nucleus, annulus, and total disc cross-sectional area were measured using image-analysis software (Scion Corporation, Frederick, MD). Disc degeneration was categorized over five grades using the Thompson classification (I = normal to V = severe degeneration)[30]).

In vitro cow and human intradiscal pressure testing and calibration

Explanted L4–5 bovine motion segments were isolated (longitudinal and posterior ligaments, disc and facet capsules were left intact) and the ends potted for in vitro testing. Intradiscal pressure measurements were conducted as described above unloaded and under applied axial loads. Axial loads were applied in compression using a servopneumatic load frame (Enduratec, Minneapolis, MN) with all rotations and off-axis translations fully constrained. A peak compression load of 1500 N was applied in a sinusoidal fashion at a mean rate of 80 N/s. Three load cycles were applied with a 10 second dwell time between cycles; the third cycle was used for data analysis.

In three animals the cannulas were still well fixed in the annulus from the in vivo testing, which was conducted using posterolateral access, and were tested with the sensing needle passed through this cannula. The other three motion segments had the needles inserted using the posterolateral approach simulating that in vivo. All motion segments were also tested with the needle placed anteriorly, and with the needle sensing surface oriented both vertically and horizontally; artifact due to anterior vs. posterolateral access and vertical vs. horizontal needle orientation was less than 4%. Manual bending of the transducer cannula produced artifactual readings ranging from 0.005 to 0.02 MPa. The 12 human cadaver motion segments were tested in vitro using identical methods but, because needle access direction (anterior vs. posterolateral) appeared to have little influence, specimens were tested only using the simpler anterior needle access.

The applied load vs. intradiscal pressure relationship was always linear in both the cow and human motion segments ($R^2 = .99$). Accordingly, a linear load vs. pressure curve was fit for each animal. The slope and offset from this curve were recorded for each animal and were used to convert the bovine in vivo pressures to estimated in vivo axial loads.

In vitro cow and human flexibility testing

Flexibility testing (sagittal and lateral bending and axial torsion) was conducted in an identical fashion for both cow and human motion segments. Unconstrained moments were applied sinusoidally to 7.5 Newton-meters (Nm) at 0.03 Hz, with an additional 100 N axial compressive load applied coincident with the long axis of the distal vertebrae. Three preconditioning cycles were used for each test and the fourth cycle was used for data analysis. A 10 second pause held at 0 Nm occurred at after each moment cycle to allow for

viscoelastic recovery. Rotation data were recorded using a video motion measurement system (VICON, Oxford Metrics, Oxford, UK) by tracking three reflective markers mounted on each vertebra. Marker translations were converted to anatomic rotations using Euler angle formulations. Range of motion and neutral zone were calculated as previously described[31]. Initial stiffness was calculated as the slope of the flexibility curves in the low-stiffness neutral zone, and the elastic zone stiffness was calculated in the higher stiffness elastic zone region. Rotation data were sub-sampled at 1.0 Nm increments, and the rotation at each increment was averaged to yield a mean $\pm$ standard deviation for plotting moment-rotation curves (Figures 2–4).

Statistical Analysis

Statistical analysis was performed to compare cow and human vertebrae in terms of range of motion, neutral zone, stiffness and dimensional measurements. Comparisons were made using the student's t-tests with significance considered at $p < 0.05$. The error bars in the graphs represent plus or minus one standard deviation (SD).

Results

All the young adult runt cow discs were well hydrated whereas the middle-aged human discs varied in their degree of degeneration (Table 1). Measurements of runt cow endplate diameter were within approximately 20% of the human values measured in this study. The superior endplates of the cows were dome shaped, whereas human endplates were comparably more planar. The transverse dimensions of the cow endplates were slightly smaller than those for human segments. Within this small group of runt cows the weight was 5% greater and the endplates were 4% larger for the males relative to the females. The runt cows had significantly larger vertebral body axial height, longer transverse processes, smaller disc height and less lordosis relative to the human motion segments.

Overall, the runt cow flexibility profiles were similar in shape to those observed in the human motion segment, but tended to differ in magnitude (Figures 2–4). For the number of animals tested, the combined flexion/extension motion segment properties were not statistically different between humans and runt cows in terms of neutral zone, initial stiffness and elastic zone stiffness (Figures 5–6). The mean range of motion of the human specimens tended to be greater than that of the runt cow in both flexion ($5.7 \pm 3.2^\circ$ vs. $3.3^\circ \pm 0.8^\circ$) and extension ($2.9 \pm 2.0^\circ$ vs. $2.1^\circ \pm 0.6^\circ$), but these differences did not reach a level of statistical significance with this sample size ($p = 0.174$). In lateral bending, the runt cow had a significantly greater range-of-motion ($p = 0.022$). This difference was primarily due to the runt cow's large neutral zone, which was significantly greater than that of the human motion segment ($p = 0.001$). In axial torsion, there tended to be more motion in the human vs. runt cow motion segments; this difference was marginally significant in terms of elastic zone stiffness only ($p = 0.046$).

Over the entire axial compression loading curve the mean stiffness was 2.15 ± 0.71 kN/mm and 1.91 ± 0.94 kN/mm for the cow and human motion segments respectively (Figure 7a). From the mean load-displacement curves, the stiffness in the high load region (>500 N applied) 2.27 kN/m and 2.24 kN/m for the cow and human motion segments respectively.

The intradiscal pressure response was significantly greater in the cow (1.27 ± 0.18 kPa/N) relative to the human (0.84 ± 0.15 kPa/N, $p < 0.001$, Figure 7b). Human pressure response under axial loading was significantly lower in degenerated specimens (disc grade 3, 0.67 kPa/N) than those in normal motion segments (disc grade 2, 0.93 kPa/N, $p = 0.02$), whereas between levels (L23 versus L45) there was no significant difference ($p = 0.5$).

In vivo cow L4–5 intradiscal pressures for various activities are shown in Figure 8. Intradiscal pressures were lowest in sedentary positions, greater in static activities that actively used back muscles, such as holding the head up, and greatest during dynamic activities such as walking or pushing off with the legs. In vivo loads as estimated from the individual specimens' in vitro load vs. pressure "calibration" curves averaged 167 N (range 88 – 292 N) when sedated and lying prone, 256 N (75 – 409 N) lying sedated in the pen, 437 N (385 – 958 N) when lifting head, 632 N (385 – 955 N) when standing, and reached a mean peak load of 2585 N (1313 – 4375 N) during ambulation.

Discussion

This comprehensive comparison of cow and human lumbar segment morphology and biomechanics has two unique features: first, this model evaluated a mature animal with closed physes and stable morphology; second, in vivo disc pressures were evaluated, which has not been previously published for quadrupeds. In this discussion, the authors also validate the human results by comparison to prior human in vitro studies.

Anatomic measurements found the anterior disc height of intact human lumbar discs of the present study to be similar to those previously reported[32–36]. Many anatomical human measurements of the present study of males, Table 1, found a mean size slightly greater than prior reports, which typically reported measurements from combined male and female spines[33;37;38].

Disc height values were less for the cow, but were comparable to those seen in humans with disc degeneration (Table 1). Lordosis between adjacent L4 and L5 endplates was significantly less in the cows than for the humans of this study and that reported in prior human studies ($p < 0.01$)[35;39;40]. Morphometric data pertinent to disc implant studies found that the runt cow and human both had oval shaped endplates. The cow endplate dimensions were smaller than the male humans of the present study; however, they were of similar size in diameter and cross sectional area to prior mixed gender human reports [32;33;36–38;41–44]. The ratio of disc nucleus to annulus in cows was slightly less than that for the human specimens in this study, but is close to previously reported human values[45]. Although the endplates were oval for cow and human, the waists of the bovine vertebral bodies were slightly triangular in cross-section. The transverse process lengths of the cows were 4 to 5 times as long as for the humans, which suggest that the cows may experience greater bending moments due to the processes acting as long levers. This idea is supported by the high transient pressures seen in vivo in this study.

The bone mineral density of the bovine vertebrae were within the range predicted for mature animals of the size tested in the present study[46]. Anteroposterior DEXA scans found 25%

greater bone density in cows compared to humans ($p = \text{NS}$). Lateral scans found a greater difference in apparent density; this difference may be underestimated by the smaller coronal plane diameter in the bovine vertebra. This may be due in part to the greater cortical thickness of the bovine vertebral body which the authors noted qualitatively, and has been described previously [16]. Discrepancies in the DEXA score are less likely to be due to trabecular bone density since a previous study using quantitative computed tomography relating compressive mechanical testing found calf trabecular bone characteristics to be similar to that of young human specimens[47].

Axial compression loading with intradiscal pressure monitoring of the human specimens found stiffness to be in the range (2 – 6 kN/mm) of prior reports[34;44;45;48–50]. In vitro disc pressure response to applied axial loading in the human motion segments was in agreement with previous human studies[24;51;52]. Decreased disc pressure for degenerated discs, as found in the present study, has been noted previously[53–59].

Human flexibility results of the present study were similar to prior reports. The range of motion and neutral zone were well within the range of previous studies[31;45;49;60–63;63–65]. Bending stiffness of the human specimens was also similar to previous studies which have typically found initial stiffness in the range of 0.5 to 1 Nm/degree and elastic zone stiffness of 2 to 4 Nm/degree[31;45;49;60;61;63;63;64].

This report is the first to study the morphology and biomechanics of the lumbar spine of the Corrientes breed mature runt cow. The intradiscal pressure response to applied loading for the cow was slightly greater than for the human spines but similar to a prior animal (pig) study[66]. The axial stiffness of the runt cow motion segments was slightly greater than for the human specimens (2.00 versus 1.85 MN/m overall, 3.3 MN/m for both at loads > 500 N). The difference in cow versus human, and the difference due to degree of degeneration within the human discs is most likely due to nucleus hydration, greater integrity of the annulus in the animal and greater osmotic pressure in the cow nucleus due to the type and size of proteoglycans, which also may reflect the age of the disc[67;68]

Our mature cow range of motion results were similar to one prior calf spine study[6] but slightly less in axial rotation compared to another study[7;9]. Compared to humans, the runt cows had slightly less range-of-motion in flexion/extension (especially flexion), significantly greater range-of-motion and neutral zone in lateral bending, and significantly greater axial torsion stiffness. Limitations of the present study are the small number of animals tested and the varying degree of disc degeneration of the human specimens. Although none of the human specimens had advanced disc degeneration, even moderate degeneration in the human specimens may have contributed to their flexibility variability; this resultant variance in human specimen range of motion may have masked actual population differences. Post-hoc analysis of the data based on degeneration level (“minimal to mild” vs. “moderate” dehydration) revealed slightly increased flexion-extension mean range of motion for the “minimal to mild” discs. This difference was not statistically significant ($p=0.3$). A sample size analysis using the standard deviation of nearly 50% of the mean observed in this study, ~45 samples would be required to detect a significant range of motion difference of 30% between minimal and moderate dehydration groups.

In vivo cow intradiscal pressures for a number of activities were determined in the present study and values for standing, 0.80 ± 0.24 MPa, were found to be comparable to human studies with reported pressures ranging from 0.55 to 1.2 MPa[24–28]. The mean peak in vivo dynamic pressures during walking were greater in the runt cow (2.3 MPa) compared to the human (0.53 MPa - 0.85 MPa[27]). Compressive loads during various cow activities were estimated using the in vitro intradiscal pressure vs. axial load experiment as a motion segment specific calibration curve. Average runt cow estimated loads were 170 N to 260 N lying at rest, 440 N while holding up the head, 630 N while standing, and 2590 N (peak) during ambulation. The dynamic loads for the cow were greater than predicted human peak dynamic loads, such as estimated human peak ambulation (1060 N – 1590 N)[27;69]. However, static loads were comparable to previous human estimates for lying at rest (144 to 240 N) and standing (500 to 800N)[26;27;53;69]. Animal studies in baboon and sheep, which have smaller discs, have estimated in vivo loads of 200 N to 800 N for a variety of activities[20;70]. These findings and others[19;71] suggest that an upright posture is not required for axial spinal loading.

In vivo loading in articular joints has been measured in both domestic mammals and humans[72–80]. Based on these studies, in vivo joint contact pressures are estimated to generally range from 1.0 to 2.5 MPa (and up to 10 MPa for peak loading). The findings in the current and previous studies that both fibrous joints (e.g., the disc) and articular joints generally exist in a 0.5 to 2.5 MPa dynamic stress environment may not be coincidental because chondrocytes are metabolically most active in this load range[81–86]. A stress environment that is static, too low, or too high results in decreased chondrocyte proteoglycan production, increased protein degradation, and chondrocyte cell death[85;87–91]. One can hypothesize that the size of a mammalian joint or disc is therefore determined by the stress environment in order to maintain a healthy chondrocyte population.

In conclusion, while no animal replicates the human lumbar motion segment, the runt cow lumbar motion segment is similar to that of the human in terms of transverse disc size, flexibility characteristics in several loading directions, and in vitro and in vivo intradiscal pressures representing static positions and were within the range of those reported previously. However, several differences also exist, including slightly smaller disc sizes, greater vertebral body heights and higher dynamic pressures in the runt cow. These differences and similarities should be considered when choosing an animal model for study of human spinal conditions and treatments. The mature Corrientes runt cow lumbar spine may be useful for the in vivo animal testing of human implants, and particularly intradiscal instrumentation, and provide the advantage of stable morphology in survival studies.

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Figure 1. Radiographic tracings of the runt cow L4-5 motion segment vertebrae. See Table 1 for dimensions.

Figure 2. Flexion-extension flexibility curves (mean $\pm$ standard deviation) for runt cow and human lumbar spine.

Figure 3.
Lateral bending flexibility curves (mean $\pm$ standard deviation) for runt cow and human lumbar spine.

Figure 4.
Axial torsion flexibility curves (mean $\pm$ standard deviation) for runt cow and human lumbar spine.

Figure 5. Range-of-motion and neutral zone for runt cow and human lumbar spine in 3 axis of rotation (mean $\pm$ standard deviation).

Figure 6.

Initial and elastic zone stiffness for runt cow and human lumbar spine (mean $\pm$ standard deviation).

Figure 7. (a, b). (a) Axial load-displacement characteristics and (b) intradiscal pressure of runt cow L4–5 lumbar discs, and human L2–3 and L4–5 lumbar discs (mean $\pm$ standard deviation).

Figure 8.

In vivo runt cow L4–5 intradiscal pressures for various activities. Values represent the mean (+1 SD) values with the exception of Wilke [27], where the median (maximum) of static measurements from each category in Table 1 in that study. The “holding weights” values represent static values for sitting (Polga[28]; Nachemson[24]) or standing (Wilke[27]).

Table 1

Vertebral body and disc space measurements and DEXA score for both human and runt cow specimens. See Figure 1 for corresponding anatomy.

Measure (Drawing Abbreviation)	Runt Cow	Human (male)	Significant Difference	Prior Human Studies, Mean (References)
	Mean (SD)	Mean (SD)	p	
Age (years)	1.9 (0.2)	46.5 (5.7)		
Weight (Kg)	264 (22)	88.7 (26.9)		
Disc Measurements				
Disc height L45 (DH, mm)	7.2 (0.8)	13.3 (2.1)	<0.001	8–14 ^(1,2,10,13,15,17,19)
Ratio of nucleus to total disc area	36% (6%)	41% (4%)	NS	30–50% ^(18,19)
Disc area (cm ²)	13.4 (3.6)	22.1 (4.4)	<0.01	9–26 ^(3,4,14,15,16,17,19)
L45 disc wedging ($\ominus_L$, degrees lordosis)	1.8 (1.6)	8.3 (3.7)	<0.01	15–17 ^(2,5,6)
Disc degeneration (Thompson grade I-V,)	all gr I	4x gr II, 6x gr III, 2x gr IV		
Endplate diameter (mm)				
L4 Coronal (ED-C)	45.5 (2.6)	58.0 (6.8)	<0.01	47–54 ^(4,7,8)
L4 Sagittal (ED-S)	36.0 (3.8)	39.8 (5.5)	NS	33–40 ^(1,4,7,8,10,13)
L5 Coronal (ED-C)	46.8 (2.6)	59.8 (6.2)	0.01	50–53 ^(4,7,8,11,13)
L5 Sagittal (ED-S)	36.2 (4.0)	38.7 (5.0)	NS	33–40 ^(1,4,7,8,10,11,13)
Vertebral body diameter (waist, mm)				
Coronal L4 (VBD-C)	41.7 (3.8)	47.2 (6.5)	NS	41–45 ^(7,9)
Sagittal L4 (VBD-S)	30.0 (3.7)	37.0 (3.9)	<0.01	33–35 ^(7,9)
Coronal L5 (VBD-C)	45.2 (6.2)	51.0 (6.6)	NS	46–48 ^(7,9)
Sagittal L5 (VBD-S)	30.2 (3.5)	36.2 (4.3)	0.02	32–36 ^(7,9)
Other (mm)				
L4 Vertebral body height (VBH)	52.5 (3.6)	27.8 (2.1)	<0.001	24–28 ^(4,7,10,11)
L5 Vertebral body height (VBH)	53.8 (3.3)	28.3 (1.6)	<0.001	23–28 ^(4,7,10,11)
L4 Transverse process length (TPL)	97.0 (16.7)	22.0 (4.5)	<0.001	16.5 ⁽⁴⁾
L5 Transverse process length (TPL)	95.3 (14.2)	20.4 (3.3)	<0.001	23 ⁽⁴⁾
DEXA				
L4 and L5 Vertebral body bone density (anteroposterior, gm/cm ²)	1.529 (0.230)	1.194 (0.225)	NS	1.233 ⁽¹²⁾
L4 and L5 Vertebral body bone density (lateral, gm/cm ²)	1.704 (0.220)	0.855 (0.265)	<0.01	

¹ Tibrewal 1985

² Shao 2002

³ Edwards 2001

⁴ Panjabi 1992

⁵Jackson 1994

⁶Stagnara 1982

⁷Berry 1987

⁸Hall 1998

⁹van Schaik 1985

¹⁰Gilad 1986

¹¹Scoles 1988

¹²Henry 2004

¹³Nachemson 1979

¹⁴Cotterill 1986

¹⁵Koeller 1986

¹⁶Schultz 1979

¹⁷Brinckmann 1991

¹⁸White 1990

¹⁹O'Connell 2006

SD = standard deviation

NS = not significant