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# Appendix E1

## Specimen Preparation Procedures

Eleven thoracolumbar spines, obtained fresh-frozen from female cadavers 50–70 years of age, were prepared for this study. Each spine was radiographed (Faxitron, HP, McMinnville, OR) to exclude gross pathology, vertebral fractures, or metastatic bone involvement. R.N.A. (Research scientist, 19 years of biomechanical testing experience) and R.A. (7 years of biomechanical testing experience) dissected the spine's musculature with care to preserve the facet capsules, intervertebral discs, and ligaments (ligamentum flavum, anterior-and posterior-longitudinal, supraspinous, and interspinous ligaments). Once dissected, the spine was wrapped in saline soaked gauze and stored in double plastic bags at -20°C.

## Quantitative Computed Tomography (QCT) Protocol

Each spine was thawed overnight at 4°C, the spine was vacuum-degassed for 6 hours at 37°C. Under the guidance of A.M. (8 years of medical image analysis experience), R.A. CT scanned each spine (Aquilion 64, Toshiba Medical, USA) using a standard clinical spine CT acquisition parameters for living humans (125kV, Matrix 512*512, slice thickness 0.5 mm). The CT image volume was reconstructed using a field-of-view of 16 cm to yield an image voxel of (0.31*0.31*0.5)mm (Fig E1). A six-chamber calcium hydroxyapatite (HA) phantom (0–1.5 g/cc$^3$, CIRS, Norfolk, Va) provided bone density calibration (Fig E1). R.A. and R.N.A. segmented each spine to obtain three-level spinal motion units (T6-T8, T9-T11, T12-L2 and L3-L5), yielding 44 thoracic, thoracolumbar, and lumbar spinal units in total.

## Mechanical Test Procedures

### Establishing the Segment's Kinematic Instantaneous Center of Rotation (ICR)

Due to anatomic and kinematical differences among human spines and to provide consistent initial loading conditions while keeping the application of coupled moments to a minimum, (R.A. and R.N.A.) applied the test loads at the segment's ICR (15). To locate the ICR, each spine segment was mounted on the compression test device (Fig 1a[ID]FIG1[/ID]) with the hydraulic test system (DDC 4000, Interlaken, MN) used to apply a compressive load of 100N. Using the mechanical X-Y table (0–15 mm range, (0.01 mm) accuracy, for both axes), the position of the spine was adjusted with respect to the long axis of the test system until the load cell (MC5–5000, AMTI, MA) recorded a minimum value for the sagittal (flexion-extension) and lateral (bending) moments. A permanent pen was used to mark the ICR location on the middle vertebra with respect to its sagittal and coronal planes, which was used for positioning of load application for the mechanical tests.

### Compression

The spine segment was mounted on a compression test device (Fig 1a), secured to the hydraulic test system (DDC, 4000, Interlaken, MN) and an initial load of 25N applied at the ICR (Supplement A2) through a ball and socket joint (Fig 1a) to ensure initial contact. Monotonically increasing compressive displacement was applied at a rate of 5 mm/min until the spine failed, defined as 10%, reduction in the maximum value of compressive force measured by the integrated load cell.

### Compression + Torsion

The compression test set up was modified with the inclusion of a universal joint (Part # 9, Fig 1b), to allow application of combined compression and torsional load permitting for free deformation of the spine along its sagittal and coronal plane during the test. Once an initial load of 25N was applied, the system applied torsional moment at a rate of 0.1Nm/sec. until a value of 3Nm was recorded by the integrated load cell, followed by the application of compressive displacement (5 mm/min) until failure (10% reduction in the maximum value) was recorded by the load cell. We performed posttest fluoroscopic imaging at this point.

For each test, applied displacement and the 6DOF load cell (MC5–10000, AMTI, MA) output (force: compression, anterior-posterior shear and transverse shear; moment: forward and lateral bending, axial torque) were recorded at a rate of 20 Hz per channel with a custom program written using LabView (2010 Full Development System, National Instruments, TX).

## Simulation of Lytic Defect

Tanechii et al (8) presented systematic classification for lytic defects affecting the thoracic and lumbar spine with the defects divided into those effecting the anterior column, the additional involvement of the middle column and all three columns. For this study, (R.N.A.) chose to simulate four defect patterns of increased severity (Fig 2) which were replicated in the thoracic and lumbar spines. For the defect involving the VB, we selected in the range of percent occupation of the body cross-sectional area that were found to be the most clinically confounding for prediction of fracture (8). Under fluoroscopic control (Mini 6600, GE Medical, WI), (R.N.A.) used a mechanical burr and an expanding reamer to create one of the four lytic focus models in the middle vertebrae of the motion unit (Fig 2). The spines were imaged again with CT. The CT image having the largest lytic focus cross-sectional area within the VB was used to compute the defect size measure (Table 1).

## Computation of Bone Mineral Density (BMD)

Applying the standard protocol of Lang et al (12), a volumetric region excluding the vertebral endplates was segmented for each CT-imaged vertebral body, the segmented region projected onto the coronal plane and bone mineral content (BMC) computed (A.M. and R.A.) from the density-weighted image derived using values computed from the HA phantom. Vertebral BMD was computed by normalizing BMC by the projected area.

## Prediction of Vertebral Strength with Computed Tomography Structural Analysis Protocol (CT-SAP)

To allow prediction of vertebral strength, we first segmented the geometry of individual vertebrae from the CT image data. Second, the trabecular and cancellous bone were assigned elastic modulus-based on a calibrated CT gray level to bone density curve and third, employing engineering principles, the predicted failure load was computed for the affected spine.

## Segmenting Vertebral Geometry from the CT Image Data

For each spine, an operator independent segmentation algorithm developed in our Laboratory (unpublished work) and implemented in Matlab (2009b, Mathworks, Natick, MA), was used to segment the individual vertebral levels from the scanned CT volume file. In brief, for each CT image, the algorithm first assesses the degree and distribution of noise within the image followed by the application of region-based adaptive fuzzy C-Means clustering method to segment the entire osseous structure of the vertebra, ie, the vertebral body, pedicles, laminae, facets and lateral and posterior processes. Incorporating prior knowledge of the human vertebral bone architecture (trabecular thickness and intertrabecula distance) the algorithm segments the individual cancellous and cortical bone compartments within the boundaries of the segmented vertebral geometry. Finally, by accounting for the sharp transition between the high intensity vertebral end-plate and the low intensity adjacent disks and by segmenting the facet joints up to the joint space, the entire series of vertebral levels including the body and posterior elements are segmented. This approach was repeated until all of the vertebrae, including the separation of the intervertebral disk space and facet joints, are segmented (Fig E1).

## Assigning CT Gray-Level to Bone Material Properties

We calibrated CT density to calcium concentration using a six-chamber calcium hydroxyapatite (HA) phantom (0–1.5 g/cc$^3$, CIRS, Norfolk, VR), imaged with the spine (Fig E1). The values of apparent bone density, ($\rho_{app}$) were estimated from the density of the combined mineral and organic phases of bone by dividing by the value of bone mass ash fraction ($f_{ash}$ = 0.66 (1)) to yield ($\rho_{app} = \rho/0.66$) (2). $\rho_{app}$ values were assigned to the segmented bone (3) (Fig E1) and a density threshold of 1.123 (g/cm$^3$) (4), was used to delineate each voxel as either cancellous or cortical bone (Fig E1). Empirically-derived density-to-modulus relationships (2,4) were used to compute and assign elastic modules (GPa) (4,5), Equation 1 (Fig E1). Any value < 0.2 (g/cc$^3$) was assigned as marrow, and hence a modulus of 0 GPa.

$$\text{Eq 1} \quad \begin{Bmatrix} E_{Cancellous} = 0.82\cdot\left(\rho_{app}\right)^2 + 0.77 & \left|\rho_{app} < 1.123\right. \\ E_{Cortical} \quad = 21.91\cdot\left(\rho_{app}\right) - 23.5 & \left|\rho_{app} > 1.123\right. \end{Bmatrix}.$$

## Computing Vertebral Strength

Figure E2 details the sequence of steps used to predict the strength of a vertebral segment. The approach is derived from the concept of composite beam theory under combined loading. We performed, on slice-by slice basis, analytical computation of the variation in localized vertebral strength at the native resolution of the CT acquisition ((0.31*0.31*0.5)mm). Reconstructing the full volume of the segmented vertebra, the resulted integral curve provided an evaluation of the volumetric change in vertebral strength. Note that we did not aim to predict either the exact location of failure within the vertebra or its exact value, but aim to provide a threshold value. In detail:

## Computing Vertebral Axial Rigidity

Axial load carrying capacity for the entire section (body + posterior elements) was computed by summing the contribution of all the bone pixels within the cross-section, Equation 2, with da being the pixel size (mm).

Eq 2 $\text{Axial rigidity} = \int \mathrm{E}\left(\rho_{\text{app}}\right) da$ .

To account for the effect of the spatial variance in bone density within the cross-section, the ordinate of the section's material weighted center of gravity ($CG_W$) was computed from Equation 3.

Eq 3 $\text{M}odulus - weighted\ centroid\,(CG_W) = \dfrac{\int \mathrm{E}\,(\rho_{\text{app}})\text{xda}}{EA}$ .

Once established, the cross-section density-weighted principal axes ($I_{XX}$ and $I_{YY}$, Fig E1) were computed with respect to the section's $CG_W$. In contrast to previous studies, employing a CT-based coordinate system (5), the axes were computed from the mass tensor (Eq 4).

Eq 4 $I = \iiint_V (x,y,z)\left(\left(x^2+y^2+z^2\right)E - \begin{bmatrix} xx & xy & xz \\ yx & yy & yz \\ zx & zy & zz \end{bmatrix}\right) dxdydz.$

Based on the three-dimensional distribution of mass within the vertebral column, this computation is independent of the CT coordinate system. By computing the rigidity about the modulus-weighted axes of the vertebrae, as aligned with the orientation of the Max and Min axes of the tensor mass, we obviated the need to use the parallel axis theorem (6) to compute the contribution of each pixel as required for a CT-based axes representation. Once established, the bending (**EImax**) and transverse (**EImin**) rigidities, were computed (Eq 5).

Eq 5 $\text{Bending rigidity (EI)Max,Min} = \int \mathrm{E}\ (\rho_{\text{app}})\mathrm{X}^2\ \text{da}.$

### Computing the CT Section Load Carrying Capacity

Based on the assumption that i) the elastic behavior of whole bones correlates with the behavior of bone tissue at yield and ii) that bone on the material level fails at a constant strain independent of density (7), a predicted failure load for each vertebral cross section was estimated (Eq 6).

Eq 6 $Fz = \left[\dfrac{\varepsilon}{\dfrac{1}{E\cdot A} + \dfrac{a\cdot C_X}{E\cdot I_{XX}} + \dfrac{b\cdot D_Y}{E\cdot I_{YY}}}\right] \varepsilon = 0.01.$

With Fz: the axial compressive yield load, My: the applied bending moment at yield (which is a function of Fz, derived empirically), $I_{XX}$, $I_{YY}$: is the second moment of area of the cross section, c: is the distance between the applied force and the modulus weighted centriod, $\varepsilon$: is the yield strain (1% for cancellous bone in compression and 0.8% in tension independent of density) (7). This process was repeated for every cross-section of the vertebra until the complete volume of the vertebra was computed. This produced a curve denoting the change in strength across the complete volume of the segmented geometry. Vertebral strength was then predicted by searching for the cross section having the minimum predicted failure load.

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**Figure E1:** Diagrammatic illustration of the workflow for segmentation of the vertebral segments from clinical CT data set. Using a slice-by-slice approach, an edge-preserving noise filter was applied to the CT data to remove image artifacts, followed by region-based adaptive fuzzy C-Means clustering to segment the cancellous and cortical vertebral bone structure. Finally a binary mask was constructed for the segmented vertebral geometry. At this stage, the cortical and cancellous bones within each cross-sectional CT image were segmented. To obtain a segmentation of the complete vertebral structure, the region-based adaptive fuzzy C-Means clustering was extended to consider spatial neighborhood relations based on prior information on the architecture of vertebral bone, to fully segment the cancellous and cortical bone within the volume of the vertebral body. Taking advantage of cortex and the cancellous bone having higher densities than the soft tissue (disk and ligament), the soft tissues were assigned low weights and the complete vertebra (body + posterior complex) is segmented. This process is repeated until all vertebrae were segmented. A separate process segmented the six chambers of the HA phantom (based on known diameter of each chamber) and, for each CT slice, a linear regression was applied to create slice-localized calibration curve associating the CT gray-level with value of apparent bone density. Within each segmented vertebral volume and, on a slice-by-slice approach, the localized calibration curve was used to assign a density value to the bone and, the bone assigned as cancellous or cortical based on density threshold value. Once assigned, the bone voxel was assigned an elastic modulus value based on empirical formulae. This completed the segmentation and material assignment process performed in an autonomous manner.

**Figure E2:** Diagrammatic illustration of the workflow for computing failure load for each vertebra segmented. With the vertebrae segmented and each bone voxel assigned a density and elastic modulus value, an axial rigidity value was computed (Eq 2) for each cross section. To allow computation of each cross-section sagittal and lateral bending rigidities, a density-weighted center of gravity was computed (Eq 3), and the density-weighted principal axes ($I_{XX}$ and $I_{YY}$) derived from the tensor of mass computed for the vertebral volume (Eq 4). In an analogous manner to an elastic beam under eccentric loads (6), largest distance of bone voxels perpendicular to ($I_{XX}$ and $I_{YY}$) were computed, and the section's bending rigidity about ($I_{XX}$ and $I_{YY}$) computed (Eq 5). Lastly, Equation 6 was used to estimate the cross-section strength. Reassembling the individual predicted strength provides a curve describing the change in predicted strength within the vertebral volume. The lowest value of predicted strength was used as the estimated threshold value for the whole vertebral strength.