

Is kyphoplasty better than vertebroplasty in restoring normal mechanical function to an injured spine?

Jin Luo, Wendy Bertram, Davinder Sangar, Michael A Adams,

*Deborah J Annesley-Williams, Patricia Dolan

Department of Anatomy, University of Bristol, Bristol, U.K.

*Department of Neuroradiology, Queen's Medical Centre, Nottingham, U.K.

Funding sources: Funded in the U.K. by Action Medical Research, and by the Hospital Saving Association Charitable Trust. Materials for vertebroplasty and kyphoplasty were supplied free of charge by Stryker (UK) and by Kyphon Inc., respectively.

Corresponding author:

Dr Patricia Dolan,
Reader in Biomechanics,
Department of Anatomy,
University of Bristol,
Southwell Street,
Bristol BS2 8EJ,
U.K.

Trish.Dolan@bris.ac.uk

Tel: +44 (0) 117 9288363

Fax: +44 (0) 117 9254794

Conflict of interest statement: all authors have no conflict of interest.

Abstract

Introduction: Kyphoplasty is gaining in popularity as a treatment for painful osteoporotic vertebral body fracture. It has the potential to restore vertebral shape and reduce spinal deformity, but the actual clinical and mechanical benefits of kyphoplasty remain unclear. In a cadaveric study, we compare the ability of vertebroplasty and kyphoplasty to restore spine mechanical function, and vertebral body shape, following vertebral fracture.

Methods: Fifteen pairs of thoracolumbar “motion-segments” (two vertebrae with the intervening disc and ligaments) were obtained from cadavers aged 42-96 yrs. All specimens were compressed to induce vertebral body fracture. Then one of each pair underwent vertebroplasty and the other kyphoplasty, using 7 ml of polymethylmethacrylate cement. Augmented specimens were compressed for 2 hr to allow consolidation. At each stage of the experiment, motion segment stiffness was measured in bending and compression, and the distribution of loading on the vertebrae was determined by pulling a miniature pressure transducer through the intervertebral disc. Disc pressure measurements were performed in flexed and extended postures with a compressive load of 1.0-1.5 kN. They revealed the intradiscal pressure (IDP) which acts on the central vertebral body, and they enabled compressive load-bearing by the neural arch (F_N) to be calculated. Changes in vertebral height and wedge angle were assessed from radiographs. The volume of leaked cement was determined by water displacement.

Results: Vertebral fracture reduced motion segment compressive stiffness by 55%, and bending stiffness by 39%. IDP fell by 61-88%, depending on posture. F_N increased from 15% to 36% in flexion and from 30% to 58% in extension ($P < 0.001$). Fracture reduced vertebral height by an average 0.94 mm and increased vertebral wedging by 0.95° ($P < 0.001$). Vertebroplasty and kyphoplasty were equally effective in partially restoring all aspects of

mechanical function (including stiffness, IDP, and F_N) but vertebral wedging was reduced only by kyphoplasty ($p < 0.05$). Changes in mechanical function and vertebral wedging were largely maintained after consolidation, but height restoration was not. Cement leakage was similar for both treatments.

Conclusions: Vertebroplasty and kyphoplasty were equally effective at restoring mechanical function to an injured spine. Only kyphoplasty was able to reverse minor vertebral wedging.

Key words: Vertebroplasty; kyphoplasty; spinal deformity; cadaver; spinal mechanics.

Introduction

Vertebral compression fractures are the most common type of osteoporotic fracture, and their relatively early onset compared to other types of fragility fracture [1] makes them a major health concern. In recent years, vertebral augmentation by vertebroplasty or kyphoplasty has been used increasingly to treat such fractures. Vertebroplasty involves the percutaneous injection of bone cement into the fractured vertebral body in order to stabilise the fracture and alleviate symptoms [7]. Kyphoplasty involves inflating a balloon inside the fractured vertebral body in order to reduce the fracture and create a cavity into which cement is then injected [8]. Kyphoplasty appears to reduce the incidence of cement leakage during injection [9,10], and it leads to compaction of bone around the balloon, elevating the fractured end-plate and restoring vertebral body height [11-16]. However, despite these potential advantages, the relative merits of kyphoplasty compared to vertebroplasty remain uncertain, especially when the greater costs of kyphoplasty are considered. Only one randomised controlled trial has compared these techniques clinically, finding only small differences between them [17]. A recent systematic review reported a lower incidence of cement leakage with kyphoplasty, but levels of pain relief, restoration of vertebral height, and risk of adjacent level fracture were similar with both procedures [18].

Some other aspects of kyphoplasty and vertebroplasty have been compared in experiments on cadaveric spines. Intravertebral pressures were lower during kyphoplasty than vertebroplasty [19], and this may partly explain the lower incidence of cement leakage reported clinically [9,10,18]. Studies on isolated vertebral bodies showed that kyphoplasty achieves significantly greater restoration of vertebral body height than vertebroplasty [20,21]. However it is not easy to extrapolate this information to an intact spine, because the height of the anterior column (vertebral bodies and intervertebral discs) influences load-sharing between it and the posterior column of neural arches [22]. Increasing vertebral body height is

likely to increase loading of the anterior column, and this could explain why height restoration in-vitro is not maintained during subsequent repetitive loading [23]. Other problems with experiments on isolated vertebral bodies are that they are not loaded in a natural manner by intervertebral discs, and the results can not be used to explain subsequent fracture at adjacent vertebral levels.

In order to avoid these difficulties, we have developed techniques for performing cement augmentation on cadaveric “motion segments” (two whole vertebrae and the intervening disc and ligaments) and then assessing the mechanical consequences in terms of specimen stiffness in bending and compression, and load-sharing between the augmented vertebra and adjacent structures [24-26]. These techniques have shown that vertebroplasty produces large and consistent changes that largely reverse the adverse changes caused by fracture. However, normal mechanical function is not entirely restored by vertebroplasty, even when large volumes of cement are used [26], suggesting that the shape of the augmented vertebra must be restored as well as its mechanical properties. Kyphoplasty may be better at achieving this than vertebroplasty.

In the present study, we use our techniques to make a comprehensive assessment of the mechanical and shape changes induced by vertebroplasty and kyphoplasty. The specific aim of the study was to compare the immediate effects of the two techniques on restoring a) shape, b) stiffness, and c) load-sharing to an injured spine. Cement leakage was also compared. In order to maximise sensitivity, a matched-pair design was employed in which vertebroplasty and kyphoplasty were applied to adjacent motion segments from the same cadaveric spine.

Materials and methods

Cadaveric material Four male and eight female thoracolumbar spines were obtained from cadavers aged 42-96 years (mean 70 years) that were donated for medical research. Spines

were stored at -20 °C in sealed plastic bags until required for testing. Subsequently each spine was thawed at 3°C and dissected into two or four motion segments. All spinal levels between T7-8 and L3-L4 were used (**Table 1**) with the choice of level being determined by the need to avoid large osteophytes (which interfere with disc stress measurement) and to maximise use of scarce human tissue. After testing, the intervertebral disc was sectioned in the transverse plane and graded for degeneration, using points 1 (non-degenerated) to 4 (severely degenerated) on the scale defined by Adams et al.[27].

Overview of experiments One of each pair of motion segments from the same spine was assigned to kyphoplasty or vertebroplasty. The upper specimen from each pair was alternately assigned to one or other of the procedures to avoid bias due to specimen size or level. Prior to testing, vertebral body BMD was assessed using dual energy x-ray absorptiometry, as described previously [25]. Each motion segment was then set in plaster and radiographed in the sagittal and frontal planes. An initial “creep” test (1.0 kN compression for two hours) was performed after which the motion segment was fractured and its yield strength determined. A second set of radiographs was taken to allow the fractured vertebra to be identified. Vertebroplasty or kyphoplasty was performed on the fractured vertebra and a third set of radiographs was obtained to demonstrate the area of cement filling. The motion segment was then creep-loaded for a further 2 hrs at 1.0 kN to allow cement consolidation, after which a fourth and final set of radiographs was obtained. The following mechanical properties were compared at each stage of the experiment: compressive and bending stiffness of the motion segment, and the distribution of compressive “stress” within the intervertebral disc. The latter was used to calculate neural arch load-bearing. Changes in vertebral shape (vertical height and anterior wedging angle) were determined from radiographs. Where the mechanical testing procedures have been described previously [24,25], they are described only briefly here.

The study was approved by the Frenchay Research Ethics Committee.

Mechanical testing apparatus Each motion segment was secured in two metal cups containing dental plaster (Ultrahard Die Stone Iso-Type IV, Kerr S.p.A, Italy). The cups were attached to metal mounting plates which enabled the specimen to be loaded on a computer-controlled, hydraulic materials testing machine (Dartec-Zwick-Roell, Leominster, UK). The testing rig allowed complex loading to be applied by means of one or two low-friction rollers (**Figure 1**), the height of which could be adjusted to apply either pure compression (rollers of equal height) or compression combined with flexion (posterior roller lower) or extension (posterior roller higher) [28].

Initial “creep” test When discs lose fluid and height over the course of a day, ligaments become lax reducing the resistance to bending, and nucleus pressure falls so that compressive load-bearing by the annulus and neural arch increases [30-32]. All these effects are reversed when discs are unloaded overnight. However, in post-mortem specimens, which have been unloaded for variable periods following death, excessive disc hydration may lead to abnormal spinal mechanics. At the start of testing, all specimens are therefore subjected to a pure compressive force of 1.0 kN for 2 hr to simulate the diurnal change in intervertebral disc water content and height that occur in life so that disc hydration is brought within the normal physiological range [29].

Stiffness in compression and bending To determine compressive stiffness, each motion segment was compressed at 0.6 kN/s while positioned in 2° of flexion. The maximum compressive load applied was either 1.2 kN or 1.5 kN, depending on specimen size and bone mineral density (BMD). Compressive stiffness was measured as the slope of the tangent to the load-deformation graph at 1 kN. For two specimens from spine #10 that had a particularly low BMD, the maximum compressive load was reduced to 0.75 kN and compressive stiffness was measured at 0.5 kN, to reduce the risk of premature damage. This

approach was justified by the very low yield strength observed in these specimens when they were subsequently loaded to failure (**Table 1**).

Spinal flexion was induced by applying an off-centre compressive force to the motion segment [33]. In these tests, the posterior roller (**Figure 1**) was removed to enable the specimen to flex forwards about its own natural centre of rotation, without prohibiting shearing movements or coupled rotations [33]. Vertebral rotation was measured by attaching 5 mm diameter reflective markers to the apparatus and to pins inserted into the lateral aspect of each vertebral body. Bending moments acting on the specimen were calculated by multiplying the applied compressive force (measured by the Dartec load cell) by the lever arm of that force relative to the geometric centre of the disc [25]. Bending moment-rotation angle graphs were then plotted, and bending stiffness calculated as the slope of the tangent to the graph at 5 Nm.

Stress profilometry and compressive load-sharing A miniature pressure transducer (Gaeltec, Dunvegan, Scotland), side-mounted in a 1.3-mm diameter needle, was used to measure the distribution of compressive stress along the mid-sagittal diameter of the intervertebral disc (**Figure 1**). During “stress profilometry”, each motion segment was subjected to a compressive force of either 1.0 kN or 1.5 kN depending on specimen size and BMD [25,28]. (For the two specimens from spine #10, the compressive force during stress profilometry was reduced to 0.75 kN, for reasons given above.) Stress profiles were obtained with the specimen positioned in 2° of extension, to simulate the erect standing posture [30], and in 2-6° of flexion (depending on specimen mobility) to simulate the flexed postures typically found in light manual work [34].

Stress profiles were analysed to determine the intradiscal pressure (IDP) and the size of anterior (SP_A) and posterior (SP_P) stress peaks (**Figure 1**). A stress “integration” technique [22] was used to compute the overall compressive force acting on the anterior half of the disc

and vertebral body (F_A) and on the posterior half (F_P). These two forces were subtracted from the applied force in order to calculate the compressive force resisted by the neural arch (F_N) [22].

Vertebral fracture Each motion segment was positioned in flexion (2-10° depending on flexibility) to simulate a stooped posture. It was then compressed at 3 mm/s while a graph of compressive load against vertical displacement was plotted in real time. This enabled the compressive load to be removed immediately at the first sign of damage, which was indicated by a reduction in gradient (stiffness). The compressive force applied at this point was recorded as the yield strength. The location of fracture was confirmed from radiographs taken before and after damage.

Vertebral body shape Vertebral body dimensions were assessed for the fractured vertebra from lateral radiographs taken at each of the four experimental stages: pre-fracture, post-fracture, post-treatment, and post-creep. Radiographs containing a linear scale were scanned to create digital images which were analysed using Image J software (National Institute of Health, USA). Vertebral body height was measured at three locations (**Figure 2**). Because the superior and inferior surfaces of the motion segment were secured in plaster, it was not possible to visualise the entire height of the vertebral bodies. Therefore, all height measurements were relative to a horizontal line represented on the radiograph by the metal plate to which the cup holding the specimen was attached (**Figure 2**). Care was taken to ensure that the metal plate was always in the same relative position compared to the X-ray source. Anterior vertebral body height was measured from the most anterior-superior point of the superior endplate margin, and posterior height was measured from the most posterior-superior point of the superior endplate margin. The middle height was measured equidistant between anterior and posterior heights, again at the superior endplate margin [35]. The

“wedge angle” of each fractured vertebral body was measured as the angle between the horizontal reference line and the endplate that was adjacent to the disc (**Figure 2**).

Vertebroplasty Two 11 G vertebroplasty needles were gently tapped into the fractured vertebra via the transpedicular route, one needle being introduced through each pedicle. A radiograph was taken in the sagittal plane to confirm that the tips of the needles were located in the anterior-inferior quadrant of the vertebral body. A frontal plane radiograph, confirmed that the tips of both needles were located in the centre of the vertebral body.

Polymethylmethacrylate (PMMA) bone cement (Spineplex®, Stryker Instruments, Howmedica International, Limerick, Ireland) was prepared by mixing 20 g of powder with 10 ml of monomer liquid, after which 3.5 ml of cement was injected through each needle into the vertebral body. Both needles were then removed, and cement was left to set for 1 hr before another radiograph was taken. This demonstrated the placement of the cement and any leakage. Leaking cement was collected at the end of the experiment and its volume measured by water displacement.

Kyphoplasty Two Kyphon® 11 G needles were inserted via the transpedicular route into the fractured vertebra to a point about 2 mm past the posterior wall. A guide wire was inserted in the stylus of each 11 G needle to a point about 2-3 mm beyond the tip of the stylus. The 11 G needles were then removed leaving the wires in position. The KyphX® Osteo Introducer® was then positioned over one of the guide wires, and advanced forward until it was at least 4 mm past the posterior wall of the vertebral body. The guide wire and the stylet of the KyphX® Osteo Introducer® were then removed and the KyphX® precision drill used to create a space in the vertebral body to facilitate the insertion of an inflatable bone tamp (KyphX Xpander® Inflatable Bone Tamp, 20/3) which was positioned under the fracture zone. This procedure was repeated on the other side of the vertebra using the second of the guide wires to insert a second inflatable bone tamp. Once both tamps were in place, the balloons were

inflated until their volume reached 3.5 ml, or the maximal pressure of 400 psi was achieved. A radiograph was taken in the sagittal plane to confirm that the balloons were inflated and positioned correctly. Balloons were then deflated and withdrawn from the vertebral body. The PMMA cement was prepared as for vertebroplasty, and 7 ml was injected bi-pedicularly (3.5 ml on each side) into the void in the vertebral body using 5 ml syringes and KyphX[®] bone-filler devices. After injection, the bone-filler devices were removed from the vertebra, and the cement left to set for 1hr. Another radiograph was taken to demonstrate the placement of the cement and any leakage. Cement leakage was measured as for vertebroplasty.

Statistical analysis Intra-observer and inter-observer reliability of vertebral shape measurements obtained from radiographs were evaluated from the intraclass correlation coefficient (ICC). Repeated measures analysis of variance (ANOVA) was used to compare measurements following each intervention, with treatment as a between-subjects factor. Where a significant main effect or interaction effect was found, post-hoc paired comparisons with appropriate Bonferroni adjustment were employed to identify where the differences arose. BMD, degree of disc degeneration, and amount of cement leakage were compared in the two groups using matched pair t-tests. SPSS 14.0[®] was used for all statistical analyses.

Results

Disc degeneration and BMD All discs were grade 2 or 3 on the scale defined by Adams et al.[27] where 1 is non-degenerated and 4 is severely degenerated. BMD values ranged from 0.063 g/cm³ to 0.242 g/cm³ in the kyphoplasty group, and from 0.079 g/cm³ to 0.244 g/cm³ in the vertebroplasty group. There were no significant differences in grade of disc degeneration or BMD between the two groups (**Table 1**).

Vertebral fracture Compressive (yield) strengths ranged from 0.9 - 5.8 kN (**Table 1**).

Radiographs showed that 26/28 specimens sustained a fracture of the lower vertebral body and 2 sustained a fracture of the upper vertebral body.

Cement leakage Vertebroplasty and kyphoplasty were successfully completed in all specimens. Cement leakage was observed in 6 kyphoplasty specimens and 5 vertebroplasty specimens (Table 1). Leakage volumes ranged from 0.5 - 3.5 ml but did not vary significantly between the two groups.

Vertebral body shape Methods used to assess vertebral shape were reproducible, with ICCs ranging from 0.96 - 0.98 for intra-observer error, and from 0.75 - 0.97 for inter-observer error.

Anterior ($P < 0.001$), middle ($P < 0.001$) and posterior ($P < 0.001$) vertebral heights, as well as vertebral wedge angle ($P < 0.001$), all changed during the various stages of the experiment, although there was no main effect of treatment group (i.e. vertebroplasty vs kyphoplasty) on these parameters. Post-hoc paired comparisons showed that mean vertebral body heights (averaged for the three sites) decreased significantly after fracture, by an average 0.94 mm for all 30 specimens (**Figure 3**). This is equivalent to 3-4% of average vertebral body height.

Wedge angle also increased significantly, by an average 1.07° and 0.82° in the vertebroplasty and kyphoplasty groups, respectively, or by 0.95° overall (**Figure 4**). Anterior and middle vertebral body heights were partially restored following both kyphoplasty ($P < 0.05$) and vertebroplasty ($P < 0.05$) but most of this improvement was lost after creep loading (**Figures 3A and 3B**). Vertebral wedge angle was reduced significantly by kyphoplasty, by an average 0.66° ($n=15$, $P < 0.05$) but not by vertebroplasty. The restoration of wedge angle following kyphoplasty averaged 80%, and this was largely maintained after creep loading (**Figure 4**).

Motion segment stiffness Both compressive stiffness ($P < 0.001$) and bending stiffness ($P < 0.001$) varied significantly across the various stages of the experiment, although there was no main effect of treatment group. Fracture reduced compressive stiffness by 53% and 55%

and bending stiffness by 38% and 39%, in kyphoplasty and vertebroplasty groups respectively. Both treatments partially restored compressive and bending stiffness, but there was no significant difference between their effects (**Table 2**).

Stress profilometry and compressive load-sharing Most stress profile and load-sharing parameters varied significantly across the different stages of the experiment (**Table 2**) but there was no significant main effect of treatment group. Therefore data was pooled for the two groups. Averaged pooled data showed that fracture reduced intradiscal pressure (IDP) by 61% in flexion and 88% in extension. Fracture also reduced compressive load-bearing by the anterior half of the disc/vertebral body (F_A), from 52% to 25% in flexion, and from 26% to 9% in extension. Compressive load-bearing by the posterior half of the disc/vertebral body (F_P) was reduced only in extension, from 44% to 33%. As a result of reduced load-bearing by the anterior column, load bearing by the neural arch (F_N) increased after fracture, from 15% to 36% in flexion, and from 30% to 58% in extension. Stress peaks in the posterior disc also increased after fracture, from 0.18 to 1.97 MPa in flexion and from 1.01 to 2.64 MPa in extension.

All of these fracture-induced changes were partially reversed by both kyphoplasty and vertebroplasty, and with the exception of neural arch load-bearing (F_N), these treatment effects were maintained or enhanced following 2 hr of creep loading (**Table 2**). There were no significant interaction terms, indicating that restoration of mechanical properties following cement augmentation was not significantly different for vertebroplasty and kyphoplasty.

Discussion

Summary of findings Compressive fracture reduced vertebral body height and increased anterior wedging. Fracture also reduced motion segment stiffness in compression and bending, decompressed the nucleus pulposus of the adjacent intervertebral disc, and

transferred compressive load-bearing from the anterior column to the neural arch.

Vertebroplasty and kyphoplasty were equally effective in partially reversing these fracture-induced changes, although vertebral wedge angle was restored only by kyphoplasty. Cement leakage during injection was similar for vertebroplasty and kyphoplasty.

Strengths and weaknesses of the study The matched-pair design, in which paired motion segments from the same spine were allocated to vertebroplasty or kyphoplasty, minimised the influence of confounding factors such as age, gender, bodymass, genetic inheritance, vertebral level and BMD. This increased statistical power, enabling small differences to be detected with fewer specimens. Our techniques used to assess the spines' mechanical properties have been validated previously [22,33,36], and the limitations of cadaveric experiments have been considered in detail [37]. Methods to assess vertebral height and wedge angle were shown to have good inter-observer and intra-observer reliability. Vertebral fractures were induced using physiologically-reasonable complex loading in bending and compression that simulated moderately stooped postures in living people [34], and these loading regimes were applied to elderly human spines with ages and BMD similar to the patient population that normally receives cement augmentation. Both vertebroplasty and kyphoplasty were carried out as recommended clinically, and except for the use of the inflatable bone tamp during kyphoplasty, the protocol was standardised so that the same cement volume and bipedicular approach was used in all specimens.

Methodological limitations include measuring height and wedging of vertebral bodies that had one surface embedded in dental plaster. This was done to allow sequential changes to be evaluated without having to remove the specimen from plaster, but it assumes that no changes occur at the embedded endplate. This is a reasonable assumption because the plaster protects the adjacent endplate, so that compressive damage nearly always affects the vertebral body adjacent to the intervertebral disc [24-26]. Cement was injected without the aid of

fluoroscopic imaging, so the experimental cement leakage would have been greater than that seen clinically, where injection is stopped as soon as leaking begins. Nevertheless, the experiment did demonstrate that, when other factors are kept constant, there is no inherent difference in the incidence or amount of cement leakage between vertebroplasty and kyphoplasty.

Another limitation is the relatively small changes in height and wedge angle resulting from fracture. In life, damaged vertebrae can continue to be loaded immediately after fracture, exacerbating height loss and vertebral wedging. We have demonstrated this in-vitro when old motion segments were subjected to sustained loading following fracture [38,39]. These findings showed that creep deformity was greatly accelerated following fracture especially in vertebrae with low BMD. In order to minimise any confounding effects of fracture severity on the outcome of the two augmentation procedures, we therefore removed the load from the specimen at the first sign of damage to ensure that the extent of damage was similar in all specimens. However, this meant that the fracture-induced changes in vertebral shape were often less marked than those reported clinically. It would be reasonable to suppose that if the injuries had been greater, then the restoration of shape following vertebroplasty and kyphoplasty would also have been greater, but it is not clear how this might have changed the *relative* ability of the two techniques to restore vertebral body shape.

Relationship to other studies Figure 3 shows that vertebroplasty and kyphoplasty increased vertebral height by a similar amount, whereas previous in-vitro studies found kyphoplasty to be superior in this respect [20,21,23]. As suggested in the Introduction, the previous results could have been distorted by using isolated vertebral bodies [20,21] or disc-vertebral body units [23] rather than motion segments. Also, the previous studies used digital calipers to measure heights around the vertebral rim [20,23], or CT to assess mid-sagittal heights [21], whereas the present study assessed vertebral height changes from sagittal radiographs.

Specimens tested previously were all from female donors with a higher average age than in the present study, so specimen size and BMD could have been lower. Cement volumes varied between the two procedures [20,21] and tended to be greater than in the present study. Fracture severity was also greater in previous studies, with vertebral body height loss ranging from 2.8 mm [20] to 6.6 mm [21] compared to 0.94 mm (approximately 3%) in the present study. The mechanical effects of vertebroplasty depend on BMD [25,40-42], percentage cement fill [26,43-45] and fracture severity [25,46], so methodological differences between the present and previous studies could explain why they reported different effects on height restoration.

Augmented specimens in the present study lost much of the newly-restored height during subsequent compressive loading. This consolidation effect has been observed before in-vitro when augmented disc-vertebral body units were subjected to cyclic loading, and consolidation was found to be greater in specimens treated with kyphoplasty [23]. The authors speculated that consolidation is encouraged by compaction of trabecular bone adjacent to the cement-filled cavity created by the tamp in kyphoplasty; but consolidation is discouraged in vertebroplasty because injected cement can flow between trabeculae to form a single load-bearing column between the end-plates. This latter effect was not evident in the present study, possibly because cement volume was limited to 7 ml (equivalent to 25% cement fill [26]) so that unfilled voids would be left near the endplates, which then allow consolidation during subsequent loading. The similar amount of consolidation following both procedures in the present experiment may be attributable to using the same volume of cement (7 ml). Consolidation effects could be magnified in living patients who suffer more severe fractures, so any restoration of vertebral height and shape following augmentation in-vivo should be followed-up over time.

The mechanical effects of kyphoplasty proved to be remarkably similar to those of vertebroplasty, with both procedures restoring motion segment stiffness and spinal load-sharing by a similar amount (**Table 2**). Previous studies likewise reported no differences in restoration of bending stiffness [47] and intradiscal pressure [48] following the two procedures. However, the effects on compressive stiffness appear to be more variable, with one study reporting larger increases following kyphoplasty [20] and another reporting larger increases following vertebroplasty [23]. These conflicting results may reflect differences in the method used to determine specimen stiffness, or differences in the volume of cement injected because cement volume strongly influences compressive stiffness [40,43,45].

Where kyphoplasty did perform better than vertebroplasty in our study was in reducing the small vertebral body anterior wedge angle, and this advantage was maintained after consolidation. A similar (non-significant) trend was reported in a study on isolated vertebral bodies [21]. The lack of any significant change in vertebral body shape following vertebroplasty confirms our previous experience in cadaver motion segments [25]. Clinical studies suggest that vertebroplasty *can* improve vertebral shape and spinal kyphosis [49,50] but this could be because shape is measured in-vivo with the spine under load, so the injected cement is actually preventing further load-induced wedging rather than restoring (unloaded) shape. The combined evidence from clinical and cadaveric studies indicates that kyphoplasty is better than vertebroplasty in restoring vertebral shape under zero load, but the advantage is smaller under load-bearing conditions, and may be diminished subsequently by consolidation.

Explanation of results Mechanical changes following vertebral fracture arise from the increased deformability of damaged bone, which allows increased vertical deflection of the vertebral endplates under load [51] and a consequent loss of intradiscal pressure (IDP) in the nucleus pulposus of adjacent intervertebral discs [24,25,52]. Reduced IDP causes compressive load-bearing to be transferred to the annulus fibrosus [52], leading to high

concentrations of compressive stress acting on the vertebral body margins. Reduced IDP also allows the annulus to bulge radially, like a “flat tyre” [53], reducing disc height and increasing compressive load-bearing by adjacent neural arches [54]. All these changes in motion segment mechanics (Table 2, columns 1-4) would probably be exaggerated in vivo if more severe fractures were sustained [25]. Vertebroplasty and kyphoplasty both support the vertebral endplates, restoring IDP and neural arch load-bearing back towards normal levels [24,25,55]. These mechanical effects depend largely on the volume of injected cement, and so were similar in the present study for vertebroplasty and kyphoplasty. In contrast, the extra ability of kyphoplasty to restore vertebral shape (for the same volume of injected cement) could be due to the inflated tamp creating space for cement to be injected more anteriorly, where height loss is greatest. In the present study, the slightly greater anterior height restoration following kyphoplasty, although not significant, may have contributed to the observed reduction in vertebral wedging.

In life, increased vertebral wedging as a result of fracture would act to increase the force vector acting anteriorly on the spine leading to increased extensor muscle forces in order to maintain spinal stability in upright postures. The resulting increase in spinal loading may accelerate vertebral wedging at the fractured level [38] and induce anterior wedging at adjacent levels, especially where vertebral BMD is low {Pollintine, 2009 #7780}. Over time, this may result in progressive spinal deformity and all its associated problems. If cement augmentation can reduce or reverse vertebral wedging then it may help to avoid these mechanical changes and to prevent the development of kyphotic deformity.

Unanswered questions and future research The present experiments reveal only the short-term mechanical effects of cement augmentation. Clinical studies are required to assess how long-term cement consolidation, and bone remodelling, can influence longer term responses.

Acknowledgements

This work was funded by a grant from Action Medical Research U.K and the Hospital Saving Association Charitable Trust. Materials and equipment for vertebroplasty and kyphoplasty were provided by Stryker (UK) and by Kyphon, Inc.

References

1. Meunier PJ, Delmas PD, Eastell R, McClung MR, Papapoulos S, Rizzoli R, Seeman E, Wasnich RD 1999 Diagnosis and management of osteoporosis in postmenopausal women: clinical guidelines. International Committee for Osteoporosis Clinical Guidelines. *Clin Ther* **21**(6):1025-44.
2. Silverman SL 1992 The clinical consequences of vertebral compression fracture. *Bone* **13 Suppl 2**:S27-31.
3. Cockerill W, Lunt M, Silman AJ, Cooper C, Lips P, Bhalla AK, Cannata JB, Eastell R, Felsenberg D, Gennari C, Johnell O, Kanis JA, Kiss C, Masaryk P, Naves M, Poor G, Raspe H, Reid DM, Reeve J, Stepan J, Todd C, Woolf AD, O'Neill TW 2004 Health-related quality of life and radiographic vertebral fracture. *Osteoporos Int* **15**(2):113-9.
4. Fechtenbaum J, Cropet C, Kolta S, Horlait S, Orcel P, Roux C 2005 The severity of vertebral fractures and health-related quality of life in osteoporotic postmenopausal women. *Osteoporos Int* **16**(12):2175-9.
5. Phillips FM 2003 Minimally invasive treatments of osteoporotic vertebral compression fractures. *Spine* **28**(15 Suppl):S45-53.
6. Galibert P, Deramond H, Rosat P, Le Gars D 1987 [Preliminary note on the treatment of vertebral angioma by percutaneous acrylic vertebroplasty]. *Neurochirurgie* **33**(2):166-8.
7. Galibert P, Deramond H 1990 [Percutaneous acrylic vertebroplasty as a treatment of vertebral angioma as well as painful and debilitating diseases]. *Chirurgie* **116**(3):326-34; discussion 335.

8. Garfin SR, Yuan HA, Reiley MA 2001 New technologies in spine: kyphoplasty and vertebroplasty for the treatment of painful osteoporotic compression fractures. *Spine* **26**(14):1511-5.
9. Fourney DR, Schomer DF, Nader R, Chlan-Fourney J, Suki D, Ahrar K, Rhines LD, Gokaslan ZL 2003 Percutaneous vertebroplasty and kyphoplasty for painful vertebral body fractures in cancer patients. *J Neurosurg* **98**(1 Suppl):21-30.
10. Phillips FM, Todd Wetzel F, Lieberman I, Campbell-Hupp M 2002 An in vivo comparison of the potential for extravertebral cement leak after vertebroplasty and kyphoplasty. *Spine* **27**(19):2173-8; discussion 2178-9.
11. Dudeney S, Lieberman IH, Reinhardt MK, Hussein M 2002 Kyphoplasty in the treatment of osteolytic vertebral compression fractures as a result of multiple myeloma. *J Clin Oncol* **20**(9):2382-7.
12. Kasperk C, Hillmeier J, Noldge G, Grafe IA, Dafonseca K, Raupp D, Bardenheuer H, Libicher M, Liegibel UM, Sommer U, Hilscher U, Pyerin W, Vetter M, Meinzer HP, Meeder PJ, Taylor RS, Nawroth P 2005 Treatment of painful vertebral fractures by kyphoplasty in patients with primary osteoporosis: a prospective nonrandomized controlled study. *J Bone Miner Res* **20**(4):604-12.
13. Ledlie JT, Renfro MB 2006 Kyphoplasty treatment of vertebral fractures: 2-year outcomes show sustained benefits. *Spine* **31**(1):57-64.
14. Lieberman IH, Dudeney S, Reinhardt MK, Bell G 2001 Initial outcome and efficacy of "kyphoplasty" in the treatment of painful osteoporotic vertebral compression fractures. *Spine* **26**(14):1631-8.
15. Shindle MK, Gardner MJ, Koob J, Bukata S, Cabin JA, Lane JM 2006 Vertebral height restoration in osteoporotic compression fractures: kyphoplasty balloon tamp is superior to postural correction alone. *Osteoporos Int* **17**(12):1815-9.

16. Voggenreiter G 2005 Balloon kyphoplasty is effective in deformity correction of osteoporotic vertebral compression fractures. *Spine* **30**(24):2806-12.
17. Liu JT, Liao WJ, Tan WC, Lee JK, Liu CH, Chen YH, Lin TB 2009 Balloon kyphoplasty versus vertebroplasty for treatment of osteoporotic vertebral compression fracture: a prospective, comparative, and randomized clinical study. *Osteoporos Int.*
18. Hulme PA, Krebs J, Ferguson SJ, Berlemann U 2006 Vertebroplasty and kyphoplasty: a systematic review of 69 clinical studies. *Spine* **31**(17):1983-2001.
19. Weisskopf M, Ohnsorge JA, Niethard FU 2008 Intravertebral pressure during vertebroplasty and balloon kyphoplasty: an in vitro study. *Spine* **33**(2):178-82.
20. Belkoff SM, Mathis JM, Fenton DC, Scribner RM, Reiley ME, Talmadge K 2001 An ex vivo biomechanical evaluation of an inflatable bone tamp used in the treatment of compression fracture. *Spine* **26**(2):151-6.
21. Hiwatashi A, Sidhu R, Lee RK, deGuzman RR, Piekut DT, Westesson PL 2005 Kyphoplasty versus vertebroplasty to increase vertebral body height: a cadaveric study. *Radiology* **237**(3):1115-9.
22. Pollintine P, Przybyla AS, Dolan P, Adams MA 2004 Neural arch load-bearing in old and degenerated spines. *J Biomech* **37**(2):197-204.
23. Kim MJ, Lindsey DP, Hannibal M, Alamin TF 2006 Vertebroplasty versus kyphoplasty: biomechanical behavior under repetitive loading conditions. *Spine* **31**(18):2079-84.
24. Farooq N, Park JC, Pollintine P, Annesley-Williams DJ, Dolan P 2005 Can vertebroplasty restore normal load-bearing to fractured vertebrae? *Spine* **30**(15):1723-30.

25. Luo J, Skrzypiec DM, Pollintine P, Adams MA, Annesley-Williams DJ, Dolan P 2007 Mechanical efficacy of vertebroplasty: Influence of cement type, BMD, fracture severity, and disc degeneration. *Bone* **40**(4):1110-9.
26. Luo J, Daines L, Charalambous A, Adams MA, Annesley-Williams DJ, Dolan P 2009 Vertebroplasty: only small cement volumes are required to normalise stress distributions on the vertebral bodies. *Spine* (In press).
27. Adams MA, Dolan P, Hutton WC 1986 The stages of disc degeneration as revealed by discograms. *J Bone Joint Surg [Br]* **68**(1):36-41.
28. Adams MA, McNally DS, Dolan P 1996 'Stress' distributions inside intervertebral discs. The effects of age and degeneration. *J Bone Joint Surg Br* **78**(6):965-72.
29. McMillan DW, Garbutt G, Adams MA 1996 Effect of sustained loading on the water content of intervertebral discs: implications for disc metabolism. *Ann Rheum Dis* **55**(12):880-7.
30. Adams MA, Hutton WC 1980 The effect of posture on the role of the apophysial joints in resisting intervertebral compressive forces. *J Bone Joint Surg [Br]* **62**(3):358-62.
31. Adams MA, Dolan P 1996 Time-dependent changes in the lumbar spine's resistance to bending. *Clinical Biomechanics* **11**(4):194-200.
32. Adams MA, McMillan DW, Green TP, Dolan P 1996 Sustained loading generates stress concentrations in lumbar intervertebral discs. *Spine* **21**(4):434-8.
33. Zhao F, Pollintine P, Hole BD, Dolan P, Adams MA 2005 Discogenic origins of spinal instability. *Spine* **30**(23):2621-30.
34. Dolan P, Earley M, Adams MA 1994 Bending and compressive stresses acting on the lumbar spine during lifting activities. *J Biomech* **27**(10):1237-48.

35. McKiernan F, Jensen R, Faciszewski T 2003 The dynamic mobility of vertebral compression fractures. *J Bone Miner Res* **18**(1):24-9.
36. Chu JY, Skrzypiec D, Pollintine P, Adams MA 2008 Can compressive stress be measured experimentally within the annulus fibrosus of degenerated intervertebral discs? *Proc Instn Mech Engrs* **222**(Part H: J Engineering in Medicine):161-70.
37. Adams MA 1995 Mechanical testing of the spine. An appraisal of methodology, results, and conclusions. *Spine* **20**(19):2151-6.
38. Luo J, Pollintine P, Dolan P, Adams MA 2009 Vertebral deformity: an atraumatic mechanism involving micro-damage and creep. International Society for the Study of the Lumbar Spine, Miami, USA.
39. Pollintine P, van Tunen M, Luo J, Brown M, Dolan P, Adams M 2009 Time-dependent compressive deformation of the ageing spine: relevance to spinal stenosis. *Spine* (In press).
40. Graham J, Ahn C, Hai N, Buch BD 2007 Effect of bone density on vertebral strength and stiffness after percutaneous vertebroplasty. *Spine* **32**(18):E505-11.
41. Heini PF, Berlemann U, Kaufmann M, Lippuner K, Fankhauser C, van Landuyt P 2001 Augmentation of mechanical properties in osteoporotic vertebral bones-- a biomechanical investigation of vertebroplasty efficacy with different bone cements. *Eur Spine J* **10**(2):164-71.
42. Higgins KB, Harten RD, Langrana NA, Reiter MF 2003 Biomechanical effects of unipedicular vertebroplasty on intact vertebrae. *Spine* **28**(14):1540-7; discussion 1548.
43. Belkoff SM, Mathis JM, Jasper LE, Deramond H 2001 The biomechanics of vertebroplasty. The effect of cement volume on mechanical behavior. *Spine* **26**(14):1537-41.

44. Liebschner MA, Rosenberg WS, Keaveny TM 2001 Effects of bone cement volume and distribution on vertebral stiffness after vertebroplasty. *Spine* **26**(14):1547-54.
45. Molloy S, Mathis JM, Belkoff SM 2003 The effect of vertebral body percentage fill on mechanical behavior during percutaneous vertebroplasty. *Spine* **28**(14):1549-54.
46. Pitton MB, Koch U, Drees P, Duber C 2009 Midterm Follow-Up of Vertebral Geometry and Remodeling of the Vertebral Bidisk Unit (VDU) After Percutaneous Vertebroplasty of Osteoporotic Vertebral Fractures. *Cardiovasc Intervent Radiol*.
47. Wilson DR, Myers ER, Mathis JM, Scribner RM, Conta JA, Reiley MA, Talmadge KD, Hayes WC 2000 Effect of augmentation on the mechanics of vertebral wedge fractures. *Spine* **25**(2):158-65.
48. Ananthakrishnan D, Berven S, Deviren V, Cheng K, Lotz JC, Xu Z, Puttlitz CM 2005 The effect on anterior column loading due to different vertebral augmentation techniques. *Clin Biomech (Bristol, Avon)* **20**(1):25-31.
49. Dublin AB, Hartman J, Latchaw RE, Hald JK, Reid MH 2005 The vertebral body fracture in osteoporosis: restoration of height using percutaneous vertebroplasty. *AJNR Am J Neuroradiol* **26**(3):489-92.
50. Teng MM, Wei CJ, Wei LC, Luo CB, Lirng JF, Chang FC, Liu CL, Chang CY 2003 Kyphosis correction and height restoration effects of percutaneous vertebroplasty. *AJNR Am J Neuroradiol* **24**(9):1893-900.
51. Brinckmann P, Frobin W, Hierholzer E, Horst M 1983 Deformation of the vertebral end-plate under axial loading of the spine. *Spine* **8**(8):851-6.
52. Adams MA, Freeman BJ, Morrison HP, Nelson IW, Dolan P 2000 Mechanical initiation of intervertebral disc degeneration. *Spine* **25**(13):1625-36.

53. Brinckmann P, Horst M 1985 The influence of vertebral body fracture, intradiscal injection, and partial discectomy on the radial bulge and height of human lumbar discs. *Spine* **10**(2):138-45.
54. Pollintine P, Dolan P, Tobias JH, Adams MA 2004 Intervertebral disc degeneration can lead to "stress-shielding" of the anterior vertebral body: a cause of osteoporotic vertebral fracture? *Spine* **29**(7):774-82.
55. Polikeit A, Nolte LP, Ferguson SJ 2003 The effect of cement augmentation on the load transfer in an osteoporotic functional spinal unit: finite-element analysis. *Spine* **28**(10):991-6.

Figure legends

Figure 1. Apparatus used for the mechanical testing of motion segments. The height of the rollers could be adjusted to compress the specimen at various angles of flexion or extension. The posterior roller was removed for tests of bending stiffness. Stress profilometry was performed by pulling a pressure transducer along the mid-sagittal diameter of the loaded disc. A typical stress profile is shown to demonstrate how IDP, SP_A, and SP_P were measured. (A: anterior; P: posterior.)

Figure 2. Measurement of anterior (A), middle (M), and posterior (P) vertebral body height and wedge angle (W) from a lateral radiograph. Vertebral body height was measured relative to a horizontal reference line (H) represented by the baseplate.

Figure 3. Mean anterior (3A), middle (3B) and posterior (3C) vertebral body heights at different stages of the experiment. Post-hoc paired comparisons indicate significant differences from pre-fracture (* $p < 0.05$) and post-fracture (+ $p < 0.05$) values. Error bars indicate the standard error of the mean.

Figure 4. Mean vertebral wedge angles at different stages of the experiment. Positive values indicate vertebral body height is lower anteriorly. Post-hoc paired comparisons indicate significant differences from pre-fracture (* $p < 0.05$) and post-fracture (+ $p < 0.05$) values. Error bars indicate the standard error of the mean.

In life, a reversal of vertebral wedging may have mechanical benefits in helping to restore segment stiffness and stability. In a recent study, we found that creep and elastic deformations of the vertebral body in older spines were exaggerated in the anterior vertebral body where bone mineral density was lowest [Pollintine Bone 09] and that such regional deformations were even more marked following vertebral fracture [38]. Increased vertebral wedging at one level would act to increase the force vector acting anteriorly on the spine leading to increased extensor muscle forces in order to maintain spinal stability in upright postures. The resulting increase in spinal loading may induce anterior wedging at adjacent levels with low anterior BMD leading to progressive spinal deformity and a loss of sagittal balance. Restoration of vertebral shape following fracture may therefore help to improve segmental stiffness and stability and help to avoid the development of progressive kyphotic deformity.

Table 1. Details of the 15 pairs of motion segments tested.

V = vertebroplasty, K = kyphoplasty. ⁺ BMD is shown for the fractured vertebra from each motion segment.

Spine	Gender	Age (years)	Spinal level		Disc Degeneration		BMD (g/cm ³) ⁺		Yield strength (kN)		Cement leakage (ml)	
			K	V	K	V	K	V	K	V	K	V
1	Female	80	L2-L3	T12-L1	2	2	0.164	0.191	5.3	4.9	1.5	1.5
2	Male	61	L1-L2	L3-L4	2	3	0.146	0.244	4.5	3.7		
			T9-T10	T11-T12	2	2	0.107	0.101	3.3	2.9		
3	Female	58	T7-T8	T9-T10	2	2	0.120	0.176	3	3.2	3.5	0.5
4	Male	84	T9-T10	T7-T8	2	2	0.128	0.126	1.8	2.3		1.5
5	Female	76	T9-T10	T7-T8	3	3	0.147	0.127	2.7	2.5		
6	Female	42	L3-L4	L1-L2	2	2	0.131	0.114	3.2	2.3	1	3
			T9-T10	T11-T12	2	2	0.123	0.118	2	2.3	3	3.5
7	Male	86	T12-L1	T10-T11	3	3	0.242	0.136	2.2	2.1		
8	Female	85	L1-L2	L3-L4	3	3	0.161	0.136	4.2	3.7		
9	Female	54	T9-T10	T12-L1	2	2	0.135	0.121	1.9	2.1	2	
10	Female	90	L1-L2	T10-T11	3	2	0.063	0.079	0.9	1.1		
11	Female	56	T12-L1	L2-L3	2	2	0.152	0.176	5.3	5.8	3	
			T10-T11	T8-T9	2	3	0.157	0.171	4.9	4.6		
12	Male	96	L2-L3	T12-L1	3	3	0.111	0.128	2.6	2.1		
Mean (SD)		70(16)					0.141 (0.039)	0.144 (0.043)	3.2(1.4)	3.0(1.3)	0.9 (1.3)	0.7 (1.2)

Table 2. Average (SD) results from mechanical tests.

Differences between vertebroplasty (V) and kyphoplasty (K) were not significant for any measured parameters. “p values” indicate differences between the four stages of the experiment (ANOVA). Post-hoc paired comparisons indicate differences from pre-fracture (^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$) and post-fracture (^A $p < 0.05$; ^B $p < 0.01$; ^C $p < 0.001$) values.

	Pre-fracture		Post-fracture		Post-treatment		Post-consolidation		p
	K	V	K	V	K	V	K	V	
IDP- flex (MPa)	1.62 (0.84)	1.39 (1.03)	0.62 (0.42) ^c	0.54 (0.59) ^c	1.38 (0.59) ^c	1.29 (0.51) ^c	1.21 (0.62) ^c	1.18 (0.42) ^c	<0.001
IDP- ext (MPa)	1.61 (0.81)	1.53 (0.79)	0.14 (0.20) ^c	0.26 (0.49) ^c	1.14 (0.68) ^{cC}	0.90 (0.93) ^{cC}	0.88 (0.53) ^{cC}	0.80 (0.86) ^{cC}	<0.001
SP _A – flex (MPa)	1.61 (1.27)	2.22 (2.43)	1.09 (0.93)	1.33 (1.00)	1.29 (1.36)	1.87 (1.48)	1.68 (1.69)	1.99 (1.80)	0.072
SP _A – ext (MPa)	0.33 (0.45)	0.43 (0.39)	0.32 (0.34)	0.44 (0.44)	0.46 (0.54)	0.47 (0.52)	0.49 (0.65)	0.36 (0.45)	0.57
SP _P - flex (MPa)	0.06 (0.11)	0.30 (0.33)	1.72 (1.25) ^c	2.22 (1.11) ^c	0.38 (0.60) ^c	0.45 (0.57) ^c	0.25 (0.28) ^c	0.41 (0.55) ^c	<0.001
SP _P - ext (MPa)	0.79 (1.46)	1.24 (1.14)	2.43 (1.52) ^c	2.85 (1.29) ^c	1.51 (1.31) ^a	2.06 (1.73) ^a	1.44 (1.14) ^B	1.85 (1.13) ^B	<0.001
F _A - flex (%)	53.2 (11.4)	51.0 (18.8)	25.5 (11.8) ^c	25.4 (12.3) ^c	41.9 (11.2) ^{aC}	45.6 (13.7) ^{aC}	44.6 (14.6) ^C	47.6 (14.6) ^C	<0.001
F _A - ext (%)	25.8 (8.8)	27.1 (11.2)	7.7 (4.3) ^c	10.3 (7.7) ^c	21.7 (9.6) ^{cC}	17.6 (11.9) ^{cC}	18.3 (8.5) ^{cC}	15.4 (9.7) ^{cC}	<0.001
F _P - flex (%)	33.9 (10.6)	32.6 (14.1)	34.4 (12.4)	41.9 (13.1)	36.6 (12.1)	33.9 (9.6)	31.8 (13.3)	29.2 (7.9)	0.069
F _P - ext (%)	42.7 (13.1)	45.1 (13.1)	27.9 (13.7) ^a	38.0 (19.2) ^a	39.2 (11.6) ^A	45.0 (15.4) ^A	33.9 (15.3)	38.1 (15.1)	<0.001
F _N - flex (%)	12.9 (8.5)	16.4 (12.6)	40.2 (15.5) ^c	32.6 (16.5) ^c	21.6 (16.5) ^B	20.3 (12.7) ^B	23.6 (17.8) ^{aA}	23.2 (15.3) ^{aA}	<0.001
F _N - ext (%)	31.5 (11.9)	27.8 (15.6)	64.4 (14.6) ^c	51.7 (18.6) ^c	39.0 (11.4) ^C	37.4 (14.7) ^C	47.7 (18.3) ^{cB}	46.5 (15.6) ^{cB}	<0.001
Comp stiff (kN/mm)	3.20 (0.97)	3.19 (0.92)	1.49 (0.22) ^c	1.44 (0.27) ^c	1.86 (0.60) ^{cC}	1.98 (0.49) ^{cC}	2.03 (0.58) ^{cC}	2.16 (0.53) ^{cC}	<0.001
Bend stiff (Nm/deg)	6.77 (1.39)	6.59 (2.38)	4.17 (0.99) ^c	4.02 (1.15) ^c	4.91 (1.26) ^{cA}	4.61 (0.71) ^{cA}	4.99 (1.77) ^c	4.26 (0.72) ^c	<0.001

Figure 1

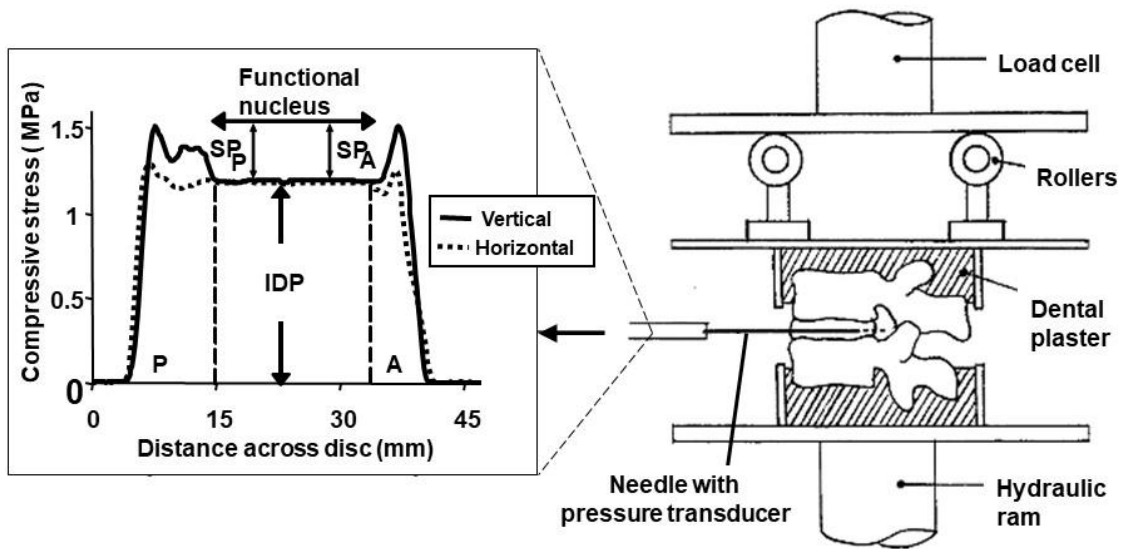


Figure 2

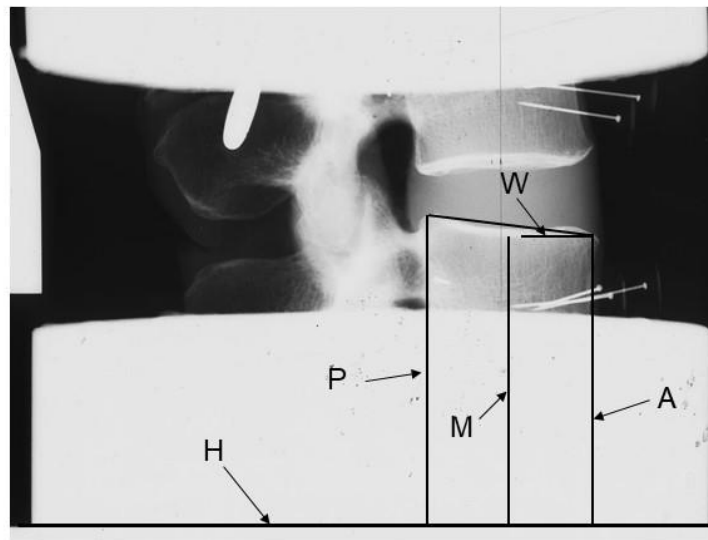


Figure 3A

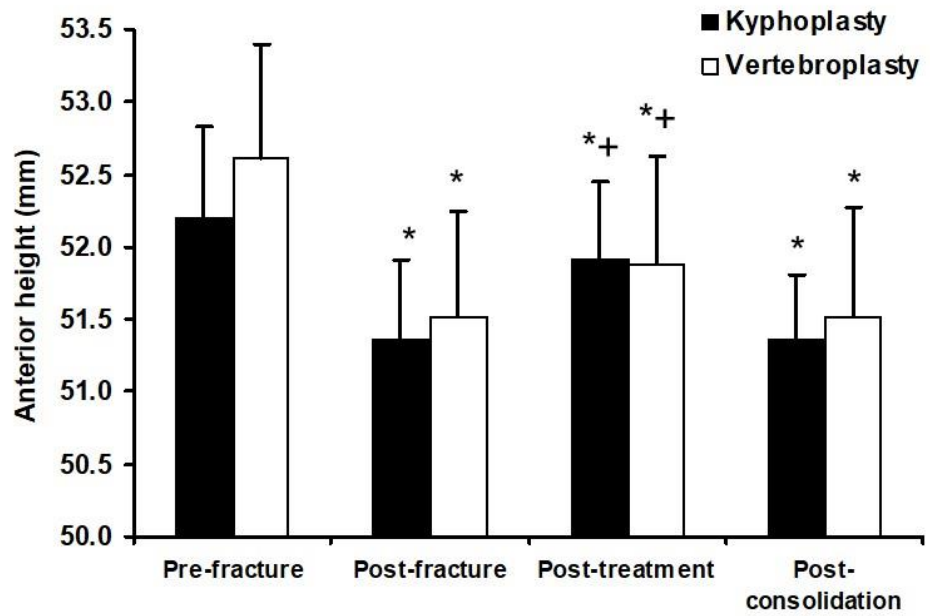


Figure 3B

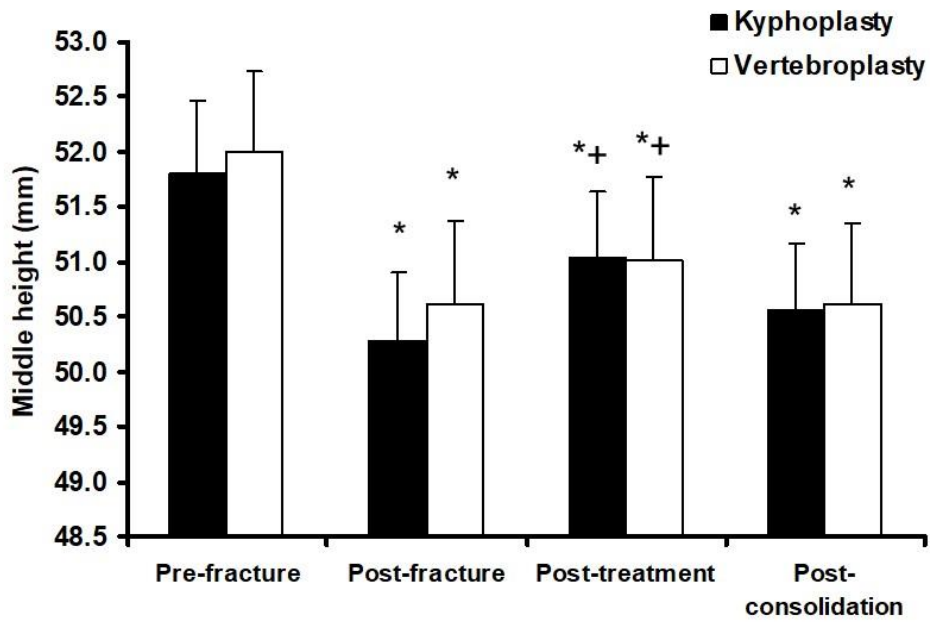


Figure 3C

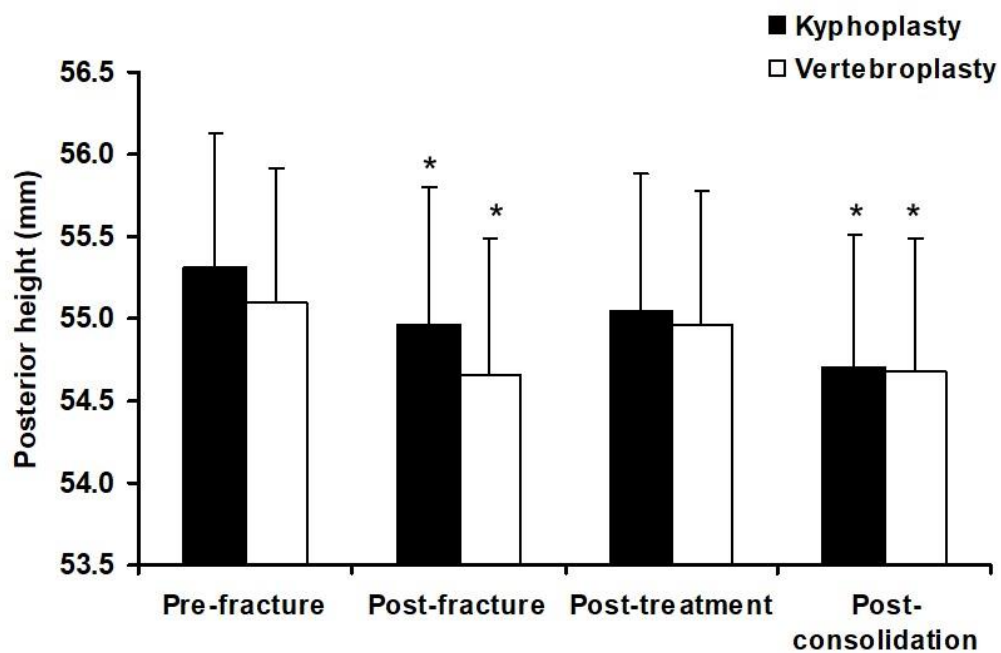


Figure 4

