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A comparative study between axial compression and lateral fall configuration tested in a rat proximal femur model

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Abstract

Background. Conventional testing method for evaluation of rat hip failure force is based on an axial compression approach. However, as the most osteoporotic hip fractures are a result of a lateral fall, it is necessary to establish mechanical testing methods more close to clinical conditions. This study was therefore designed to evaluate the differences in hip mechanical failure force to be tested between 'axial compression' and 'lateral fall configuration' in a rat proximal femur model.

Methods. Eighteen 10-month-old female Wistar rats were body-weight matched and divided into ovariectomized group ($n = 9$) and sham group ($n = 9$). All rats were euthanized 3 months after surgery. The bilateral proximal femora of each rat were excised. The left femur served for testing to failure in a fall configuration fashion while the right femur was tested using axial compression approach. After mechanical testing, the anterior–posterior radiographs were taken to identify the fracture mode and measure the Pauwel's angle between the fracture line and the line perpendicular to the femoral shaft long axis.

Findings. The failure force in fall configuration was significantly correlated with but lower than that tested in axial compression. A comparison between the ovariectomized and sham group showed that the failure force in both fall configuration and axial compression was found significantly higher in the sham group than that in the ovariectomized group. However, the logistic regression analysis revealed that the fall configuration approach had larger discrimination power. Radiographs showed that almost all samples fractured at the base of the femur neck. The Pauwel's angle in fall configuration group was significantly larger than that in axial compression group.

Interpretation. In comparison with the characteristics of axial compression test, the fall configuration approach generated higher shearing stress with larger bending moment to the bone and induced susceptibility to fracture at lower mechanical load. Although the failure force obtained from the two testing configurations revealed significant correlation, the mechanical testing in fall configuration demonstrated higher sensitivity in identifying the estrogen-deficiency induced deterioration of hip mechanical failure force as compared with that in axial compression. These findings suggested the potential application of lateral fall configuration in evaluation of improvement effects of intervention stratagems on hip mechanical failure force involving an ovariectomized rat model.

Keywords: OVX rat; Hip; Mechanical test; Axial compression; Lateral fall configuration

1. Introduction

It has been known that the risk of hip (proximal femur) osteoporotic fracture in elderly persons is not only related to skeletal status (bone quality or bone failure force) but also related to non-skeletal factors associated with fall (NIH Consensus Statement, 2000). The

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bone quality or bone failure force at the hip of living persons can however only be estimated using non-invasive approaches, such as the well-accepted densitometry measurements at hip using dual-energy X-ray absorptiometry (DXA) (Bergot et al., 2002). Invasive mechanical approaches are conventionally used to obtain information on the bone quality or bone failure force directly from human cadaveric hip or from pre-clinical studies using animal models (Cheng et al., 1997; Peng et al., 1994).

Among all types of osteoporotic fractures, hip fracture has been reported to be the one resulting in more disability and mortality in postmenopausal women or elderly persons (Leibson et al., 2002; Melton, 1993). As most hip fracture is a consequence of fall, especially side-fall (Grisso et al., 1991; Cummings et al., 1990), to establish testing method mimicking side-fall is desirable. Ovariectomized (OVX) rat has been regarded as a standard experimental model in research and development of anti-osteoporosis drug (Thompson et al., 1995).

In the most related experimental studies, the mechanical failure force of the rat proximal femur was tested under an axial compressive load parallel to the long axis of the femoral shaft (Søgaard et al., 1994). This test configuration simulates one-legged stance during weigh-bearing in humans, which does not reflect situation in osteoporotic fracture at hip result from side-fall inducing approximately 90% of hip fractures (Grisso et al., 1991; Cummings et al., 1990). In order to simulate side-fall, a lateral compressive testing (fall) configuration has been adopted in human cadaver studies to investigate mechanical failure force of the proximal femur (Lochmuller et al., 2002; Cheng et al., 1997; Lotz and Hayes, 1990).

As the skeleton is anisotropic and its mechanical failure force is dependant on loading direction (Bostrom et al., 2000; Lotz and Hayes, 1990), our hypothesis of the present study was that the maximum or failure force was smaller if the hip was tested using a lateral fall configuration as compared with a certain axial compressive load applied onto the principal or strong axis of the femoral neck. In order to prove that and also investigate how the withdrawing estrogen would affect the test sensitivity, an OVX rat model was used to compare the difference in mechanical failure force of the bilateral proximal femora between the axial compression and lateral fall configuration.

2. Methods

2.1. Animals and groupings

A total of 26 female Wistar rats were used for this study and assigned for the following two tests to study the method error and the sensitivity of the two distinguished testing methods described below. Ethics approval was obtained for this animal experiment from the Ethics Committee of the Chinese University of Hong Kong.

2.1.1. Evaluation of the method error

Twenty 10-month-old female Wistar rats (mean body weight of 440 g) were purchased from SIPPR-BK Experimental Animal Ltd. Shanghai, China, and randomized into 'Fall Configuration' (FC) group ($n = 4$) and 'Axial Compression' (AC) group ($n = 4$) for testing the potential variations in hip mechanical failure force. After general euthanasia intraperitoneally (100 mg/kg body weight Ketamine and 5 mg/kg body weight Xylazine, respectively), bilateral femora of each rat were excised, wrapped with gauze saturated saline, and stored at −20° before the mechanical test on the bilateral femora with identical testing configuration for calculating error of testing methods. The method error was calculated from the variation between the paired femora using the following formula:

$$\sqrt{\frac{\sum \boldsymbol{d}_i^2}{2\boldsymbol{n}}},$$

where $\boldsymbol{d}_i$ is the relative difference between the values of each pair and $\boldsymbol{n}$ is the number of pairs (Jamsa et al., 1998; GlÜer et al., 1995). More details on the testing configurations were described below.

2.1.2. Evaluation of method sensitivity in OVX rat model

Eighteen 10-month-old female Wistar rats were obtained from the same experimental animal supplier. All rats were fed with standard rat chow (SIPPR-BK Experimental Animal Ltd., Shanghai, China) that contained 0.93% calcium, 0.65% phosphorus and 3.0 IU of Vitamin D per gram, with free cage activity. After acclimation for 1 month, they were divided into two weight-matched groups ($n = 9$, each), including SHAM Group (Sham-operated and oral vehicle) and OVX Group (ovariectomized and oral vehicle). All animals were held in a central animal house at 22° with 12-h light and 12-h dark cycles. Three months after OVX, rats were euthanized (intraperitoneally, 100 mg/kg body weight Ketamine and 5 mg/kg body weight Xylazine, respectively) and bilateral femora of each rat were excised, wrapped with gauze saturated with saline, and stored at −20° before mechanical test.

2.2. Mechanical testing at hip

Before mechanical testing, rat femora were taken from the freezer and thawed overnight at room temperature. The following two distinguished testing configurations were used to compare differences in mechanical failure force of the bilateral proximal femora.

2.2.1. Test with lateral fall configuration (FC)

The left femur was used for testing mechanical failure force in a loading configuration designed to simulate a lateral fall using a modified custom-made testing device based on Jamsa T's design (Jamsa et al., 1998) (Fig. 1). The distal end of the femur was clamped using a modified attachment system without ante- or retroversion. The femur was then fixed against rotation around the diaphysial long-axis. Polymerization reagents (UREOL 5202-1A, UREOL 5202-1B, Filler DT-082. Ciba Specialty Chemicals, Hong Kong Limited, HK) were mixed pro rata (1:1:2) in a silicone tube. The distal end of the femur was then inserted into the silicone tube for hardening. The clamp system was tightly adjusted at the distal end for fixation. In addition, the height of the clamp and the specimen stage were adjustable for fixing the femoral shaft angle at 10° between the femoral shaft and the horizontal supporting metal plate and at an internal rotation angle of femoral neck at 15° by referring to the design using in human cadavaric studies (Jamsa et al., 1998; Cheng et al., 1997). A sheet of rubber was used and placed underneath the trochanter to prevent it from crushing during testing as suggested before (Backman, 1957). In order to avoid stress concentration on the contact interface between femoral head and the metal indenter, a concave plastic cup composed of polyethylene was fused onto the end of the metal indenter, which provided best fit for contact surface of femoral head (Figs. 1 and 2). A 2.5 KN load cell was used and a 5 N preload was applied to the femoral head and then tested continually to failure at a testing speed of 2 mm/min using a material test machine (H25KS Hounsfield Test Equipment Ltd. Redhill, Surrey, UK). The failure force was obtained and analyzed using built-in software (QMAT Professional Material testing software from Hounsfield).

2.2.2. Test with axial compression (AC)

The right proximal femur was used and tested under an axial compression configuration. The femoral shaft

Fig. 1. Fall configuration designed to test the proximal femur subject to failure.

Fig. 2. The axial compression parallel to the femoral shaft vertical axis for testing the proximal femoral strength.

of each specimen was embedded in a specially constructed fixation device using above mentioned polymerization reagents, and aligned vertically to the compression indenter (Fig. 2). All other testing conditions remained the same as described above for the FC approach.

2.3. X-Ray examination of fracture mode

After mechanical testing, anteroposterior view radiographs were taken for all specimens using high sensitive Kodak film and a Cabinet X-Ray System (Specimen Radiography System, Faxitron 43855C, Fraxitron X-Ray Corporation, Wheeling, IL, USA) under an exposure of 40 kV/30 min. The fracture line in sub-region of proximal femur was identified and the obliquity of the fracture line interpreted by Pauwel's angle between the fracture line and the line perpendicular to the femoral long axis was measured from the digitalized radiographs using the Image Analysis software (Adobe Photoshop 7.0) (Garden, 1961) (Fig. 3).

2.4. Statistics

Statistical analysis was performed with SPSS v.10.0 software. Mechanical testing values were expressed in mean and standard deviation (SD). Student's paired t-test was used to compare the failure force and the failure mode interpreted in Pauwel's angle. Bivariate linear Pearson's correlation coefficient and corresponding determinant coefficient related to failure force were calculated for FC and AC test. One-way analysis of variance (ANOVA) was used to compare the failure force in FC test or AC test between the SHAM group and the OVX group. Logistic regression was used to identify failure force between FC test and AC test that was able to best identified form SHAM (Healthy) or OVX rats. Statistical significance was set at $P < 0.05$.

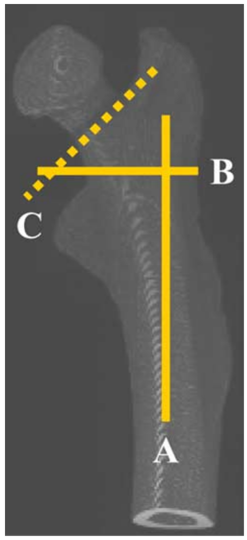

Fig. 3. The obliquity of the fracture line based on the definition of Pauwel's angle. A line: the femoral shaft axis; B line: the line perpendicular to the femoral shaft axis; C line: the fracture line Pauwel's angle; α: the angle between B line and C line.

3. Results

3.1. Related to the establishment of testing methods

The 'method error' determined from the paired samples was 6.1% for FC and 5.2% for AC, respectively. The fracture occurred at the basicervical region of the proximal femur in both FC and AC group (Fig. 4). The Pauwel's angle in FC group was significantly larger than that in AC group (62–66° vs. 52–56°, $P < 0.05$). The failure force of proximal femur was significantly lower in FC group than that in AC group (79.84(5.04) N vs. 92.80(6.76) N, $P < 0.05$) (Fig. 5). Nevertheless, the failure force of both testing configurations revealed significant correlation ($r = 0.79$, $R^2 = 0.62$, $P < 0.05$) (Fig. 6).

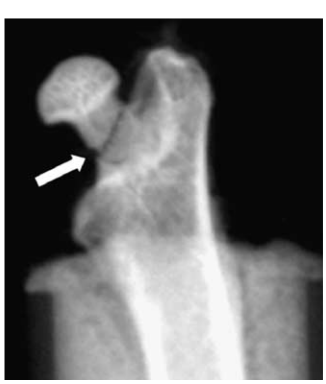

Fig. 4. Representative fracture mode from 'fall-configuration test'. White arrow: the fracture line.

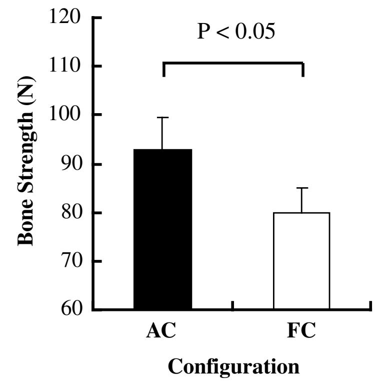

Fig. 5. Plot of loading configurations versus corresponding femoral neck failure force. AC: axial compression. FC: fall configuration.

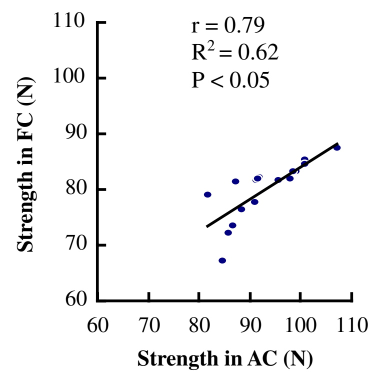

Fig. 6. The relationship between the rat femoral neck failure force in axial compression (AC) and that in the falling configuration (FC). ($n = 18$).

3.2. Related to the comparison between OVX and SHAM group

One-way ANOVA showed that the failure force in both configurations was significantly higher in the Sham group than that in the OVX group, tested using either FC approach (82.87(2.37)N vs. 76.04(5.00)N, $P < 0.05$) or AC test (95.99(7.39)N vs. 88.80(2.81)N, $P < 0.05$) (Fig. 7).

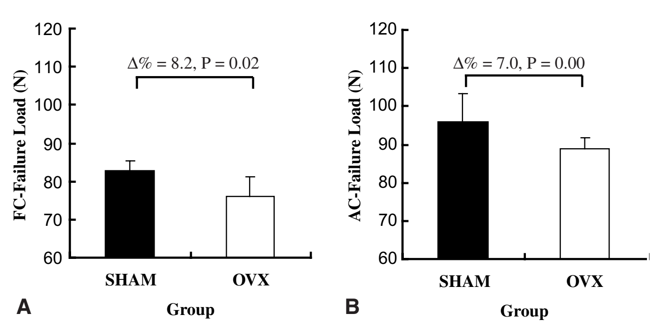

Fig. 7. (A) Failure force of proximal femur compared between SHAM and OVX groups tested in axial compression (AC). (B) Failure force of proximal femur compared between SHAM and OVX groups tested in falling configuration (FC).

Table 1
Logistic regression analysis on failure force tested between OVX and SHAM groups in AC or/and FC models

Method	Variables	B	SE	Chi-square model	*P*-value	Correct class (%)
Enter	AC-failure force	−0.23	0.12	6.22	0.01	78
Enter	FC-failure force	−0.70	0.34	12.41	0.00	83
Stepwise	*Step1*					
	AC-failure force	0.09	0.18	12.65	0.00	83
	FC-failure force	−0.84	0.49			
	Step2					
	FC-failure force	−0.70	0.34	12.41	0.00	83

Note. AC: axial compression. FC: fall configuration. Enter: input all the variables in the block in a single step. Stepwise: variable entry and removal examines the variables in the block at each step for entry or removal. B: regression coefficient of constant. SE: standard error of B. Chi-square model: corresponding statistic value.

Logistic regression analysis was performed between the OVX and SHAM group when ovariectomy was used as a dichotomic-dependent variable and the failure force in FC and in AC test was treated as independent variables normalized for the standard deviation of the subject group's variation. Firstly, the two independent variables were tested separately by 'Enter' method, which showed that each of them was able to discriminate animals correctly in a similar capacity, within in a small range between 78% and 83%. When the two independent variables were analyzed using 'Stepwise' method, only the failure force from the FC test was preserved in the regression model (Table 1).

4. Discussion

The present study was designed for the first time to compare the sensitivity between the two distinguished testing methods, i.e. 'Fall Configuration (FC)' and 'Axial Compression (AC)' for evaluation of failure force of the proximal femur in an OVX rat model. As hypothesized, lower failure force was revealed in the FC testing design. This was explained by difference in loading orientation and bone structural adaptation, i.e. loading onto the principal or strong axis in AC design and onto the non-principal or weak axis of proximal femur in FC design, which was also showed in a previous human cadaveric study, in spite of functional difference between human (biped) and rat (quadruped) (Lotz and Hayes, 1990). This may also be supported by experimental studies, which demonstrated variations in mechanical failure force in association with anisotropic property of bone beam specimens obtained from different angles to the bone long axis (Bostrom et al., 2000). However, the present study also revealed a significant 'intrinsic' correlation ($r = 0.79$, $P < 0.05$, $R^2 = 0.62$) in failure force between the two loading configurations, which implied that around 38% of the variation was attributed by the difference in structural anisotropy in association with the two distinguished loading directions. Interestingly, the curve fit of failure force between AC and FC (refer to Fig. 6) revealed that the lower part of the AC-value (<90 N) showed disproportionately greater decrease in the FC-value. As the primary objective of the present study was to compare the difference in failure force between AC and FC, the sample size may be not large enough to substantiate different curve behavior related to the sensitivity of the different testing methods. Yet, this deserves further curve fit study with large sample size.

As estrogen-deficiency is known to be able to induce osteoporosis, the present study demonstrated the consequence of OVX-induced significant deterioration in mechanical property of the bone at rat proximal femur tested either using AC or FC design. However, 'Stepwise' analysis suggested that the FC design had higher discrimination power or test sensitivity than that of the AC design. This finding may imply a higher risk of fracture if an impact load is applied to the non-principal axis of the skeletons. The lower sensitivity or discrimination power in the axial compression test at rat proximal femur might be explained by the OVX-induced periosteal modeling-dependant bone formation of the basicervical region, which was reported to increase the cross-sectional moment of inertia of the 'principal or strong load axis' (Qin et al., 2005; Bagi et al., 1997). In addition, as compared with the 'non-principle or weak axis' in the FC test, the stress concentration might be more located along the femoral neck axis during the compressive test in the AC test, as this was also evidenced by the Pauwel's angle of the fracture line in the radiographic examinations of the present study, i.e. found more close to the perpendicular line of the femoral neck axis. The potential alterations in the cross-sectional geometry would thus facilitate more compensation for the OVX-induced decrease in the structure-dependent mechanical failure force under the axial compression than that under the lateral compression.

The differences in the failure force found between FC and AC designs might also be explained by the differences in failure mode quantified by Pauwel's angle on radiographs, which was suggested to reflect the magnitude of local shear stress during testing (Nordsletten et al., 1994). Although the fracture line was both found at the basicervical region of proximal femora tested in rats under the two different loading configurations, greater Pauwel's angle was found in FC group as compared with that in AC group. This suggested that the FC design resulted in a combination of higher shear stress characterized with larger bending moment at the femoral neck, whereas the AC approach lead to a combination of compression and smaller bending moment at the femoral neck (Nordsletten et al., 1994).

In order to confirm the reliability of the testing results, the present study evaluated the method error by comparing the data from the bilateral femora using another set of rats. As the repeated measurements for determining the precision error can not be applied to destructive mechanical tests, the paired comparison used in the present study was based on an inherent assumption on an equal mechanical property for bilateral proximal femora of individual rats. This approach has been conventionally used in studies with similar experimental designs in animal models. The 'method error' in our study was 6.1% and 5.2% for FC and AC design, respectively, which were better than that reported by early studies for rat proximal femora under axial compression (Jarvinen et al., 1998; Peng et al., 1994).

The present experimental study also had a few limitations or open questions for further investigations. Firstly, the FC testing design in vitro with continuous loading (rather low compressive force) onto the femoral head does not take into account that fall (sideways or backwards) induced hip fracture in old persons is a result of impact force onto the great trochanter, muscle contraction during falling and damping protection by the soft tissue component around the great trochanter (Robinovitch et al., 1991). Secondly, because of the small size of the rat great trochanter, a FC model with an impact loading was technically not feasible in our current setting as it did not provide test reproducibility in our pilot study. However, the current approach by applying compression onto the femoral head may still create compression onto the greater trochanter as it was used as the supporting point during the testing (refer to Fig. 1). Clinical investigations showed however that the hip fracture occurred either at femoral neck or at trochanteric region (Lochmuller et al., 2002; Cheng et al., 1997), which might be dependent on force direction during fall and this was supported by finite element analysis using human cadavaric femora (Keyak and Falkinstein, 2003). Experimentally, the fracture mode may be determined further by applying the impact force onto the great trochanter. Accordingly, a systemic 'method error' analysis of the new testing configuration with a sufficient sample size should also be established.

5. Conclusions

In conclusion, in comparison with the axial compressive test, the lateral fall configuration test at the rat proximal femur may be regarded as a more sensitive mechanical test in evaluation mechanical properties of the proximal femur in OVX rats, and therefore recommended for use in evaluation of new agents developed for improvement of hip mechanical failure force or for anti-osteoporosis and osteoporotic fractures at hip involving an OVX rat model.

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