Induction of Changes Over time in the Rat Proximal Femur Following Ovariectomy: A Model with Clinical Implications

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Summary

The menopause-related expansion of the proximal femoral marrow cavity is thought to have implications for the long-term cohesion of hip prostheses. This theory would be further strengthened if there was evidence to show that the expansion of the proximal femur marrow cavity takes place after the occurrence of a femoral neck fracture, which, it is often recommended, should be fixed with a hip prosthesis. But till now, the temporal relationship between those two osteoporotic-related changes has not been checked carefully. The objective of the current study was to examine the temporal relationship between the marrow cavity expansion of the proximal femur and the biomechanical deterioration of the femoral neck in a rat model. To do so, a cross-sectional study with multiple time points was carried out on 6-month old Sprague-Dawley rats, which were ovariectomized or sham-operated (as controls). The biomechanical properties of the femur neck and geometrical parameters of the femur shaft were evaluated at 0, 3, 6, 9, 12, 15, 18, and 21 weeks postoperatively, with special reference to the timescale of the observed changes. We found that the maximum load of the femoral neck in ovariectomized rats could bear decreased significantly compared, to that of controls, at 9 weeks postoperatively (p=0.03), while the marrow cavity of the proximal femur in ovariectomized rats turned out to be significantly enlarged at 15 weeks postoperatively (p=0.04). *Conclusion:* Our result demonstrated that the osteoporosis-related marrow-enlarged posterior led to the collapse of femoral neck strength. If the change in postmenopausal women is analogous to that in ovariectomized rats, the menopause-related marrow cavity expansion would be a risk factor for the longevity of hip prostheses.

Introduction

The endocortical envelope area of the proximal femur plays a crucial role in bone-implant fitness. The high bone turnover resulting from estrogen deficiency would accelerate the age-related bone loss (*Ahlborg et al.,2001*). Since the bone loss of the diaphysis mainly occurs at the endosteal surface of the cortex (*Frost,1999*), the medullary cavity would increase in size after menopause (*Hofmann et al.,1989; Robinson et al.,1994; Ahlborg et al., 2004; Takeuchi et al.,1998*). Although it has been suggested that the increases of the medullary cavity after menopause might have some effects on the prognosis of hip prostheses (*Ahlborg et al., 2004*), uncertainty remains as to whether postmenopausal osteoporosis is a contributing factor in the prediction of implant failure. This suggestion would be strengthened if evidence could be obtained to show that the expansion of the endocortical envelope area of the proximal femur takes place after the occurrence of femoral neck fracture. In the present study, a cross-sectional study with multiple time points and an appropriate control group was carried out on ovariectomised (OVX) Sprague-Dawley rats, aiming to explore the hypoth-

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esis that ovariectomy-induced expansion of the endocortical envelope area of the proximal femur takes place after biomechanical collapse of the femoral neck.

Materials and Methods

All procedures involving live animals were performed in accordance with National legislation and the Council of Europe Convention ETS 123.

Seventy five female Sprague-Dawley rats, 6 months old (Shanghai Experimental Animal Center of Chinese Science College, Shanghai, China) and weighting 410±15g, were randomized into 15 groups at the beginning of the study. On the day of surgery, all the rats were anesthetized with an intraperitoneal injection of 5% ketamine hydrochloride (2ml/kg body weight). Five rats were sacrificed to obtain baseline data (0 week postoperative), and the remainder underwent either a sham or genuine ovariectomy operation. For ovariectomy, bilateral dorsal incisions were made on the back of the rat under sterile conditions after anesthesia. Both of the ovaries were identified, the blood vessels were clamped, tied off, and the ovaries were removed. The sham operation was performed following the steps above with the visualization of the ovaries but without clamping or removing of the tissue. Post operation, the rats were housed in groups and fed with sterilized food (Ca: 0.46%; P: 0.38%) (provided by the Animal Centre of Medical School Zhejiang University Hangzhou, China) and tap water. Room temperature was maintained at 23 °C, and the light cycle was 12h light and 12h dark. The sham rats had free access to food and water throughout the experiment, while the food supplied to the OVX rats was restricted to the amount eaten by the sham group in order to minimize the increase in body weight associated with ovariectomy.

Sham and OVX rats were killed with an anaesthetic overdose by intraperitoneal injection of 5% ketamine hydrochloride at 3, 6, 9, 12, 15, 18, and 21 weeks after operation. Success of the ovariectomy was confirmed under autopsy by failure to detect ovarian tissue and by the observation of an obviously atrophied uterus. Both femora, from each rat, were harvested with some soft tissues attached and immersed in 0.9% saline solution. Femur length and the width of the femoral neck were measured by caliper (0.02mm). The cross-section area of the femoral neck was calculated as π x bilateral width x anterior-posterior width x 0.5.Bone mineral density (BMD) of the femur neck was then measured with a dual-energy X-ray absorptiometer (DEXA, LUNAR Radiation, Madison, Wisconsin, USA) using the small-animal program. The region of interest (ROI) included the area from subcapital to the baseline of the femoral neck (Fig 1). An ultra-high resolution scan model was used. Coincident positioning of all specimens for the measurement was ensured. Following scanning, the femora were wrapped in saline-moistened gauze and stored at -20 °C for later biomechanical testing.

Biomechanical testing was performed according to the method described by Peng *et al.*(*1994*) using Zwiek-Z010 testing systems (Zwick GmbH & Co. Ulm, Germany). The frozen specimens were thawed at room temperature before testing. The femora were cut transversely at distal third of the diaphysis with a handheld saw. The distal end of the proximal part of the femur was inserted into a plaster support with a number of deep holes of different diameters in it. The holes were 5-8 mm in depth and had a gradient angle of 15°. The femoral head was then loaded vertically to failure (Fig. 1) at a displacement rate of 2 mm/minute. All specimens were tested in a coincident position and kept moist throughout testing. Fractures were all induced at the femoral neck and they displayed a load-displacement curve typical for bone, with an initial nonlinear response phase followed by an upward-sloping linear component and then a failure response at the point of break.

After biomechanical testing, the geometrical parameters of the proximal femur shaft were measured at the proximal femur (site 1: lesser trochanter section) and the upper 1/3 section of femoral diaphysis (site 2), as shown in Figure 1. The femora were cut transversely 2mm proximal to site 1 and 2mm

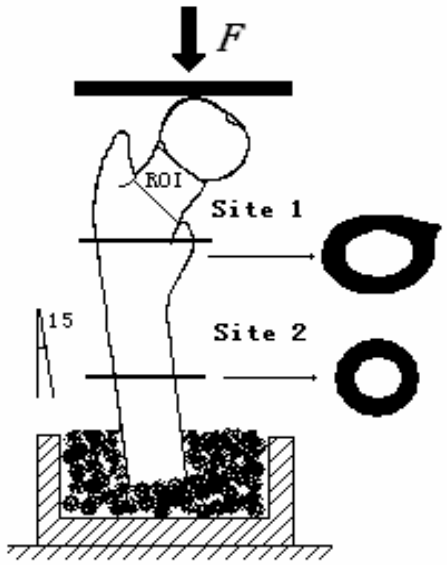

Figure 1. Schematic illustration of the test. ROI: Region of interest for the measurement of the BMD (from subcapital to the baseline of femoral neck). The femoral head was loaded vertically (F) with the femur shaft inserted in the hole of the support at an angle of 15º.

Geometrical parameters were measured at the proximal femur (site 1: lesser trochanter section) and the upper 1/3 diaphysis of femur (site 2).

distal to site 2. The marrow cavity and periosteal surfaces were cleaned, and the cut surfaces were ground to ensure flat and parallel specimens. The bone samples were then decalcified in ethylene diaminetetra-acetic acid (EDTA, 0.5mol/L, pH 7.4) for 24-48 hours to make the surface more suitable for imprinting on paper but without any deterioration in the rigidity. Then the shafts were dehydrated in 95% and 100% ethanol for half hour each and air-dried. The cross-section planes of femoral diaphysis were then stamped onto a paper on a red inkpad. The print of the cross-section was scanned (scan resolution=300px) into a computer file, and the marrow cavity area and total cross-section area were measured with Photoshop 7.0 software (display resolution=1024x768, 150px=1mm^2).

Data were expressed as arithmetic mean ± standard deviation (SD). To justify the pooling of the data obtained from left and right sides, a paired *t*-test was applied between data from each side. Statistical differences between groups were evaluated by an independent sample *t*-test using the SPSS 10.0 analysis software package after checking for the normality of distribution. A P value <0.05 was considered to have statistical difference.

Results

The sham rats were still in growth in the first 6-9 weeks post-operation, judged from the increasing values of body weight, femoral neck size, BMD, biomechanical properties, femoral length and geometrical parameters. Thereafter, these data remained at a relatively constant plateau, which reflects maturity of growth (Fig 2, Table 1, Table 2); and despite food-restriction, the OVX rats gained relatively more weight and femoral length than the sham groups (Fig 2, Table 2). No statistical difference was detected in the cross-section area of the femoral neck (except at 9th week post operation, p=0.025) (Table 1). No statistical differences were detected in BMD, biomechanical strengths and

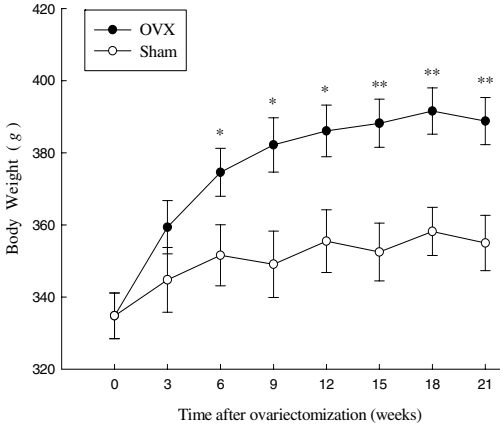

Figure 2. Body weight after operation. Sham: sham-operated rats; OVX: ovariectomized rats. **P*<0.05. ***P*<0.01. sham vs. OVX

Table 1. Bone size, BMD and biomechanical properties of the femoral neck. (Mean±SD)

	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks	18 weeks	21 weeks
Cross-section area (mm^2)							
OVX	4.01±0.97	4.10±0.76	4.19±0.06	4.21±0.86	4.19±0.12	4.21±0.08	4.21±0.11
sham	4.08±0.90	4.08±0.88	4.13±0.06	4.16±0.76	4.18±0.09	4.15±0.09	4.19±0.83
p (t)	0.09	0.50	0.03	0.11	0.74	0.13	0.55
BMD (g/mm^2x10^{-2})							
OVX	20.1±2.2	19.2±2.8	18.2±2.9	17.8±2.3	17.4±1.7	17.8±2.3	18.0±2.3
sham	21.0±1.9	22.5±3.6	21.0±2.6	21.5±2.0	22.1±3.1	21.8±2.3	22.5±2.0
p (t)	0.33	0.03	0.03	0.01	0.00	0.00	0.00
Maximal load to failure (N)							
OVX	90.2±11.3	91.3±8.8	89.6±7.7	86.9±7.8	86.5±9.8	85.1±8.7	83.1±9.0
sham	92.6±10.7	95.1±10.0	96.7±7.5	95.8±9.9	97.2±9.1	96.2±8.2	97.1±9.2
p (t)	0.63	0.38	0.05	0.04	0.02	0.01	0.00
Energy absorption($Nmx10^{-3}$)							
OVX	39.9±4.8	41.4±5.1	42.2±4.5	40.8±6.5	39.4±5.2	37.7±5.8	37.3±4.8
sham	41.2±5.7	44.5±4.7	47.4±5.3	46.6±4.9	48.8±5.9	45.6±6.1	47.4±5.9
p (t)	0.59	0.18	0.03	0.04	0.00	0.01	0.00

See Fig. 1 and Fig. 2 for details.

morphology between the right and left femur (data omitted).

Ovariectomy was associated with typical osteopenic changes in the femoral neck and proximal femur (site 1). Compared with the femoral neck BMD in sham rats, which remained relatively constant at 0.21-0.22 g/mm^2 after a slight increase during the first 6 weeks, the BMD in the OVX rats declined gradually (Table 1). This phenomenon also occurred in the maximal load to failure, the energy absorption of the femoral neck and the marrow cavity of the proximal femur (Table 1, Table 2). No difference was observed between sham and OVX rats in the cross-section area of the proximal femur (Table 2).

No significant difference was detected in the geometrical parameters in the upper one-third section of femoral diaphysis (site 2) (Table 2).

As far as osteopenic changes in the femoral neck and proximal femur are concerned, the BMD, biomechanical properties of the femur neck and geometrical parameters of the proximal femur did not change simultaneously (Table 1, Table 2). In the femoral neck, the BMD changed earlier than the biomechanical parameters. The BMD of OVX rats decreased significantly more than sham rats as early as 6 weeks after operation ($p<0.05$), while maximal failure (breakage) load and energy absorption of the OVX rats decreased significantly more than the sham rats at 9 weeks post-operation ($p<0.05$). The enlargement of the proximal femur marrow cavity came to statistical difference even later than the biomechanical changes in the femoral neck. These turned out to be significantly enlarged at 15 weeks post-operation ($p<0.05$).

Discussion and Conclusion

The proximal femur has been an attractive site for the investigation of osteoporosis and hip fracture in recent years (*Bagi et al., 1996; Bagi et al., 1997; Li et al., 1997*). Distinct from the previous studies on the osteoporotic change in the proximal femur, the

Table 2. Geometric parameters of femur shaft. (Mean±SD)

	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks	18 weeks	21 weeks
Femoral length (mm)							
OVX	38.3±0.7	39.9±1.0	40.6±0.8	40.8±0.8	41.4±0.8	40.9±0.9	41.5±1.2
sham	37.7±1.1	38.9±0.9	39.5±1.0	39.8±1.1	40.2±1.0	39.7±1.1	40.0±1.1
p (t)	0.140	0.059	0.021	0.026	0.007	0.019	0.008
Marrow area of site 1 (mm^2)							
OVX	3.63±0.13	3.70±0.17	3.76±0.14	3.81±0.13	3.83±0.12	3.89±0.11	3.91±0.12
sham	3.62±0.12	3.68±0.10	3.73±0.11	3.74±0.11	3.71±0.12	3.73±0.13	3.69±0.14
p (t)	0.87	0.79	0.55	0.20	0.04	0.01	0.00
Cross-section area of site 1 (mm^2)							
OVX	13.42±0.18	13.72±0.22	13.85±0.18	13.83±0.18	13.82±0.19	13.93±0.19	13.87±0.21
sham	13.48±0.18	13.64±0.17	13.78±0.15	13.92±0.15	13.90±0.15	13.86±0.15	13.90±0.16
p (t)	0.41	0.37	0.31	0.29	0.32	0.33	0.73
Marrow area of site 2 (mm^2)							
OVX	3.42±0.14	3.44±0.12	3.47±0.15	3.53±0.12	3.55±0.10	3.58±0.11	3.63±0.15
sham	3.41±0.13	3.47±0.11	3.50±0.11	3.49±0.12	3.49±0.12	3.50±0.17	3.51±0.20
p (t)	0.85	0.56	0.65	0.52	0.22	0.07	0.06
Cross-section area of site 2 (mm^2)							
OVX	9.19±0.07	9.42±0.12	9.65±0.10	9.72±0.12	9.62±0.10	9.72±0.11	9.75±0.10
sham	9.21±0.09	9.36±0.07	9.57±0.14	9.69±0.09	9.71±0.15	9.67±0.09	9.64±0.15
p (t)	0.50	0.17	0.36	0.51	0.15	0.27	0.09

See Fig. 1 and Fig. 2 for details.

present study emphasised the temporal relationship between the changes of biomechanical changes in the femur neck and morphology of the proximal femur shaft following withdrawal of estrogen. Our results revealed that, in the osteoporotic process, the expansion of the marrow cavity in the proximal femur occurred after the reduction of femur neck strength in OVX rats.

Several points need to be made on the design of the present study, as follow. First, though the morphology of the proximal femoral marrow cavity area has an inherent manner of alternation due to its physiological state (e.g. ageing and bone loss etc.), it will also change to adapt to the presence of an implant (*Crowninshield et al., 2004*). Because the marrow cavity area will also be influenced by the presence of the implant, this will disturb the impact of osteoporosis, as an independent factor, on the effects of the marrow cavity. For this reason, no implant was inserted in the femur in order to explore the temporal relationship between marrow cavity expansion in the proximal femur and the reduction of femur neck strength. Second, osteoporotic hip fracture, as a clinical phenomenon, is multi-factorial. Although efforts have been made to establish effective animal models for fragile fracture, no rat model for spontaneous osteoporotic fracture has ever been developed successfully. In the current study, the impairment of biomechanical strength in femoral neck was assumed to be analogous to the occurrence of femoral neck fracture.

Prosthesis replacement has been the recommended method for the treatment of osteoporotic femoral neck fractures, especially those, which were dis-

placed (*Tidermark et al., 2003*). It had been proved that endosteal bone lysis, resulting in excessive medullar cavity expansion, was associated with increased stem prostheses subsidence (*Collis et al., 1998; Engh et al., 2001*), which had been identified as a marker for the future failure of hip prostheses. It had been reported in human and animal investigations that ageing and osteoporosis are associated with an enhanced marrow cavity expansion (*Hofmann et al., 1989; Robinson et al., 1994; Ahlborg et al., 2004; Takeuchi et al., 1998*), while no study has ever identified osteoporosis as an explanatory factor predicting implant failure after THA in the clinic. Ahlborg et al. stated that age-related medullary expansion can have implications for the long-term "fixation" (hence success) of hip prostheses (*Ahlborg et al., 2004*). However, they merely monitored the changes of the marrow cavity with ageing, and had no idea whether the age-related expansion of the medullary cavity takes place after hip fracture or has ever enlarged before it. It is important to clarify impact of osteoporosis on the clinical problem of the integration of a hip prosthesis in osteoporotic patients, because the conclusion that marrow expansion would have implications for prosthesis loosening can be drawn only on the assumption that the enlargement of the marrow cavity occurred after deterioration of femur neck strength. In present study, we delighted to have found that the expansion of the endocortical envelope area of the proximal femur became evident later than the deterioration of the biomechanical strength of femoral neck.

In conclusion, the current study showed, in the osteopenic process of the rat, that the marrow cavity in the proximal femur shaft will continue expanding after the deterioration of the femoral neck strength. If the temporal pattern of osteopenic changes in the proximal femur is analogous between OVX rats and postmenopausal women, this would strengthen the implication that menopause-related medullary expansion could be a risk factor for the longevity of hip prosthesis.

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