Original Article

Characteristics of Rat Lumbar Vertebral Body Bone Mineral Density and Differential Segmental Responses to Sex Hormone Deficiency: a Clinical Multidetector Computed Tomography Study*

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Abstract

Objective To investigate sex hormone deficiency related osteoporosis and efficacy of different therapies.

Methods Orchiectomized and ovariectomized rat models are used to investigate sex hormone deficiency related osteoporosis and efficacy of different therapies. A rat vertebral body can be longitudinally divided into central portion, which contain more trabecular bone, and para-endplate portions which contain more compact bone. In matured male and female Wistar and Sprague-Dawley rat lumbar spines, we investigated baseline bone mineral density (BMD) characteristics and the differential segmental responses in bone loss within the lumbar vertebral body post gonadal surgery with clinical multidetector computed tomography.

Results Para-endplate sections had a higher BMD than central sections. The cephalad para-endplate sections had a higher BMD than the caudad para-endplate sections. Eight weeks after gonadal removal, there was more bone loss in central sections than para-endplate sections. The relative difference of bone loss between para-endplate and central sections was more apparent in male rats than in female rats. There was more bone loss in caudad sections than cephalad sections; this lead to a further increase of BMD difference between caudad para-endplate sections and cephalad para-endplate sections post gonadal surgery.

Conclusion The approach described in this study provided a consistent way to study BMD change within predominantly compact bone portion and trabecular bone portion of the vertebral body.

Key words: Bone mineral density; Quantitative computed tomography; Rat; Vertebra; Orchiectomy; Ovariectomy; Cortical bone; Trabecular bone

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INTRODUCTION

Osteoporosis, characterized by a low bone mass and an increased risk of fragility fracture, is the most common metabolic disorder of bone[1]. Osteoporotic fractures are a common source of pain and morbidity and deemed to increase as the average population age rises. Measurement of bone mass by densitometry, either by dual-X ray absorptiometry (DXA) or

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quantitative computed tomography, is central to the diagnosis and treatment of osteoporosis.

Pre-clinical studies involving measurement of bone mineral density (BMD) are commonly undertaken in small animals such as rats. Experimentally, orchiectomized and ovariectomized rat models are used to investigate sex hormone deficiency related osteoporosis and efficacy of different kind of therapies[2-7]. Loss of estrogens or androgens alters the balance of bone remodeling by inhibiting osteoblastogenesis, promoting osteoblast apoptosis, and osteoclastogenesis and, inhibiting osteoclast apoptosis[2,8]. The most commonly used bone densitometry for rat osteoporosis model is small bore peripheral quantitative computed tomography (pQCT). Recently, it has been shown that clinical multidetector computed tomography (MDCT) can be used as a viable alternative to pQCT when measuring the BMD of rat vertebra[9]. MDCT densitometry of rat lumbar vertebrae is repeatable, accurate and responsive[9]. MDCT lumbar spine densitometry is much quicker than pQCT densitometry. The reduced examination time of MDCT densitometry can also help minimize animal stress from anesthesia. Faster acquisition time afforded by MDCT also allows other parts of the skeleton, such as the thoracic spine, pelvis and femora, to be included in the scan plane, while isotropic reconstruction should allow easier vertebral body numbering, easier detection of vertebral anomalies that could influence BMD, and better assessment of rat skeleton morphology. Clinical multidetector computed tomography (MDCT) systems are more widely available than pQCT machines. This is relevant for institutions embarking on animal-based bone densitometry studies where only a modest number of animal studies are performed, and the cost of a dedicated small animal pQCT is not justified.

A characteristic of bone remodeling is that the process is nonuniform. It differs from one bone to the other and between cortical and trabecular bone and, from one trabecular bone site to another. It is known that“postmenopausal” osteoporosis is different from “senile” osteoporosis. In sex hormone-deficient rats, osteoporosis predominantly occurs in trabecular bone[2]. For in-vivo densitometry studies, researchers tend to either examine the whole bone inclusive of cortical and trabecular structures, or concentrate on the trabecular structures within the central aspects of a bone[4,7,10-11]. A rat vertebral body can be longitudinally divided into central portion, which is predominantly composed of trabecular bone, and para-endplate portions which contain more compact bone. In this study, we investigated the characteristics of rat lumbar vertebral body BMD and their differential segmental responses to sex hormone deficiency in male and female Wistar and Sprague-Dawley rats with MDCT.

MATERIALS AND METHODS

The experimental protocol was approved by the Animal Experiment Ethics Committee of the Chinese University of Hong Kong. All the animal procedures were carried out according to the guidelines set out by the Laboratory Animal Services Centre of the Chinese University of Hong Kong. Animals included five Sprague-Dawley (SD) males, seven Wistar females, ten SD females, five Wistar females. All were 6.5 to 7-month-old when the study started. All animals were bred at the Laboratory Animal Services Centre of the Chinese University Of Hong Kong. Animals were housed 2-3 animals per stainless steel cage at 22 °C temperature and with a 12-h light and 12-h dark cycle, and received a standard rat chow (Prolab RMH 2500, PMI Nutrition International LLC, Brentwood, USA) and water *ad libitum*. For both CT densitometry examination and surgery, rats were anesthetized using a combination of Xylazine (10 mg/kg) and Ketamine (90 mg/kg).

As previously described[9], rat lumbar vertebrae BMD were measured using a 64-slice multi-detector CT scanner (LightSpeed VCT 64, General Electric, USA) employing continuous axial 0.625 mm slice thickness acquisitions in helical scan mode, with energy level of 120 KV and 150 mA, and a pitch of 0.53. QCT Torso Phantom (Image Analysis Inc, Columbia, USA) was used as the external reference. CT raw data were transferred to a dedicated workstation (Advantage Windows, GE Healthcare, USA) where true cross-sectional images of individual vertebrae of L1-L6 were selected based on multi-planar reconstruction and three-dimensional surface rendering techniques, and region of interests (ROI) drawn slice-by-slice manually along L1- L6 vertebral body excluding the posterior elements[9]. This ROI encompassed both compact and trabecular bone as it was difficult to consistently separate these two components on axial CT images. As the rat lumbar spine is straight, rather than lordotic, true axial images were obtainable directly through each vertebral body without the need for gantry tilting or

oblique image reformation. QCT 5000^{TM} Bone densitometry software (Image Analysis Inc.) was used for converting CT attenuation value to BMD value. Vertebral body BMD was calculated as the mean of the summated acquisitions for that vertebral body. Images encompassing all or part of the intervertebral disc were excluded from analysis. It has been reported that this described approach is accurate and reproducible[9].

In this study, the section (slice) number of individual vertebrae varied between eight to fourteen. As the four sections adjacent to the cephalad and caudad endplates contain relatively more compact bone, while those sections through the central parts of the vertebral body contain more trabecular bone, the following portions were defined and their BMD per section was measured: 1) cephalad para-endplate sections: the two sections near cephalad endplate; 2) caudad para-endplate sections: the two sections near caudad endplate, and para-endplate sections refers to the combination of cephalad para-endplate sections and caudad para-endplate sections; 3) central sections: the central sections included the central 2 slices when total slice number was 8 and 10, the central 3 slices when total slice number was 9 and 11, the central 4, or 5, or 6 slices when total slice number was 12, 13, or 14; 4) middle sections: the sections excluding cephalad para-endplate sections and caudad para-endplate sections, but including central sections. These definitions are explained in Figure 1.

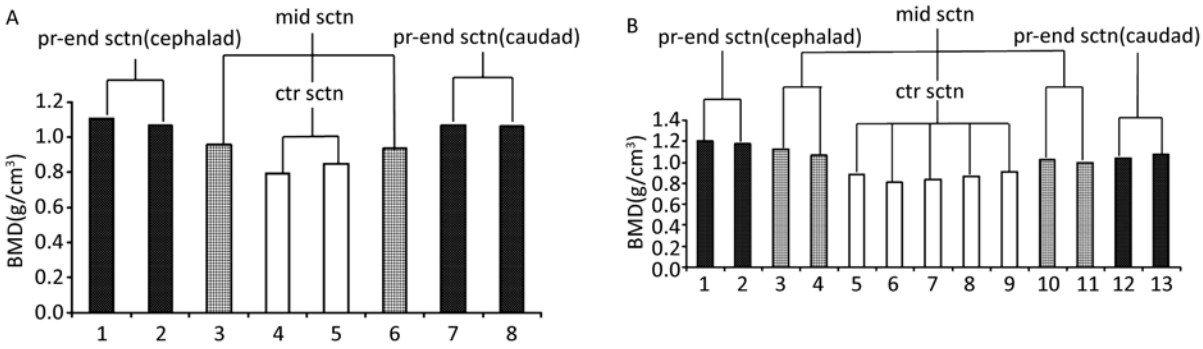

Figure 1. BMD of sequential axial acquisitions of lumbar vertebral bodies from two rats (A, slice number =8, and B, slice number =13). Y-axes represent BMD in g/cm^3 while the X-axes indicate sequential image number along the vertebral body from cephalad to caudad. For each vertebral body, sections at the cephalad and caudad portions with substantial compact bone component have higher BMD than the central sections dominated with trabecular bone. pr-end sctn: para-endplate sections; ctr sctn: central sections; mid sctn: middle sections.

After baseline CT densitometry examination, animals underwent bilateral orchiectomy or ovariectomy surgery. For orchiectomy, a small incision was made in the center of the scrotum exposing each testicle. The ductus deferens, main arteries and veins were isolated, ligated, and transected allowing the testicles and epididymes to be removed. The incision was then closed by suture. For ovariectomy, the surgery was carried out through flank incisions. Fur on both sides of the body was shaved from a proximal thigh to the lower chest. Bilateral ovariectomy was performed using an incision 1.5 cm inferior to the costal margin. Ovaries together with surrounding fat tissue were removed. The incision was closed using muscle and skin sutures. Success of ovariectomy was confirmed at necropsy through absence of ovarian tissue and atrophy of uterine horns. CT densitometry was repeated and analysed eight weeks post-orchiectomy or post-ovariectomy using an identical technique to the baseline examination.

162 vertebrae in total from four groups of 27 rats were included in the analysis. Data were expressed as mean±standard deviation. Paired and unpaired student *t*-tests, and one-way ANOVA were used for statistical analysis with a $P<0.05$ being considered statistically significant.

RESULTS

As it has been reported[9], the periosteal margin of the vertebral body compact bone could be

clearly demarcated from the paravertebral soft tissues on axial CT images, while the endosteal margin of the cortex between compact bone and trabecular bone could not be clearly separated (Figure 2). For all the four groups of rats, male rats had larger lumbar vertebrae than females had. Male vertebral bodies were longer and larger than those of female (Table 1). Prior to surgery, cross-sectional area was 9.6±2.1 mm^2 for male rats and 8.3±1.9 mm^2 for female rats (P<0.0001). With all the rats, vertebra L1 was the shortest, with a mean slice number of 9 (0.625 mm/section), followed by vertebra L6, with a mean slice number of 9.35 (Table 1). Vertebrae L4 and L5 were longest for all rats.

With the individual vertebrae, in all the four groups, vertebra L6 had a higher BMD than the rest vertebrae (L1-L5, P<0.05), while there is no significant difference between L1-L5 in overall BMD (P>0.05, Table 2). In all the four groups, para-endplate sections had a higher BMD than middle sections and central sections (Figure 3, P<0.0001). The cephalad para-endplate sections had a higher BMD than the caudad para-endplate sections for all the four group rats (P<0.0001, Figure 4).

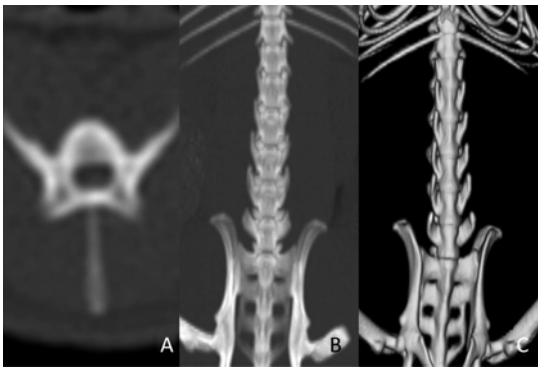

Figure 2. A: Multi-slice CT axial image of a female rat vertebra. B: multi-planar reconstruction image of a female rat lumbar spine. C: 3D rendering image of a female rat lumbar spine.

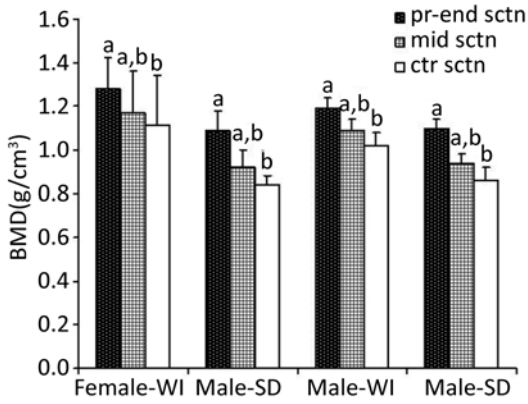

Figure 3. Baseline BMD of para-endplate sections, middle sections and central sections. Para-endplate sections have higher BMD than middle sections, and middle sections have higher BMD than central sections. a: comparison between para-endplate sections and middle sections P<0.0001; b: comparison between middle sections and central sections P<0.0001.

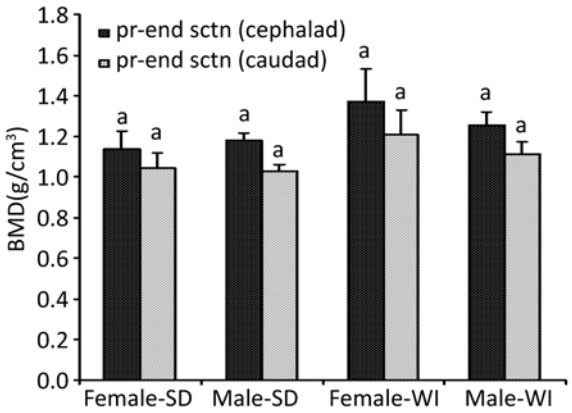

Figure 4. The cephalad para-endplate sections have a higher baseline BMD than the caudad para-endplate sections. a: comparison between BMD of cephalad para-endplate sections and caudad para-endplate sections, P<0.0001.

Table 1. Slice number of Individual Vertebral Body for Male and Female SD and Wistar Rats

	L1	L2	L3	L4	L5	L6	Mean±SD
Female-SD	8.50±0.71	8.70±0.48	8.90±0.74	9.30±1.25	9.10±0.74	8.50±0.53	8.83±0.57
Male-SD	10.67±0.58	12.00±0.00	12.33±1.53	12.33±0.58	12.33±0.58	11.33±0.58	11.83±0.17
Female-WI	9.00±0.71	9.60±0.55	10.20±0.45	10.20±0.84	10.00±0.71	9.40±0.89	9.73±0.52
Male-WI	9.29±0.49	10.29±0.49	11.00±0.00	11.00±0.00	11.00±0.00	10.57±0.53	10.52±0.22
Mean±SD	9.00±0.97	9.55±1.23	9.95±1.47	10.15±1.46	10.09±1.33	9.35±1.23	

Note. WI=Wistar.

Table 2. Baseline BMD of Individual Vertebral Body for Male and Female SD and Wistar Rats

	L1	L2	L3	L4	L5	L6	Mean±SD
Female-SD	0.99±0.06	0.97±0.05	0.98±0.05	0.97±0.05	0.98±0.05	1.11±0.08	1.00±0.05
Male-SD	0.99±0.03	1.00±0.04	0.98±0.01	0.98±0.03	0.98±0.01	1.08±0.02	1.00±0.04
Female-WI	1.17±0.14	1.18±0.15	1.20±0.19	1.21±0.20	1.22±0.20	1.33±0.16	1.22±0.06
Male-WI	1.10±0.05	1.13±0.04	1.11±0.04	1.15±0.04	1.14±0.03	1.16±0.04	1.13±0.02
Mean±SD	1.06±0.09	1.06±0.12	1.06±0.13	1.07±0.14	1.07±0.13	1.16±0.12	

Note. unit: g/cm^3, WI=Wistar. In all four groups of rats, vertebra L6 has a higher BMD than the rest vertebrae (P<0.05, L1-L5) while there is no significant difference between L1-L5 in BMD (P>0.05).

Eight weeks post gonadal surgery, lumbar vertebral body BMD decreased in all the four groups of rats. The segmental differences of BMD decrease is shown in Figure 5. For all the four groups of rats, there was more bone loss, i.e. BMD decrease, in central sections than in the para-endplate sections, and the degree of bone loss of middle sections lied in between (Figure 5). The relative difference of bone loss between para-endplate sections and central sections was more apparent in male rats than in female rats (Figure 5, 13.3%±5.4% *vs* 4.3%+/-5.1% for SD rats, 7.8%+/-5.3% *vs* 3.8%+/-5.4% for Wistar rats). In all the four groups of rats, there was more bone loss in caudad sections than in the cephalad sections (Figure 6), this leads to a further increase of BMD difference between the caudad para-endplate sections and the cephalad para-endplate sections (Figure 7).

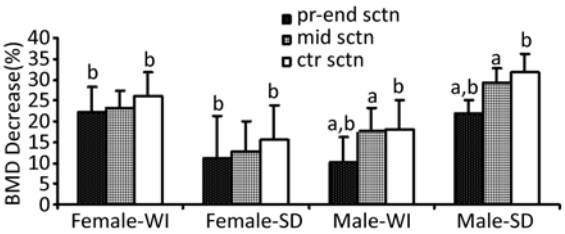

Figure 5. Percentage of BMD decrease 8 weeks post gonadal surgery. For all four groups of rats, there is more bone loss in central sections than para-endplate sections, and the degree of bone loss of middle sections lies in between. a: comparison of para-endplate sections and middle sections. P<0.001 for male rats. b: comparison of para-endplate sections and central sections, P<0.0001.

There was a trend of increase of vertebral body length between 6.5~7 months age and 8.5~9 month age for all the four groups of rats, as reflected by the increase of MDCT slice number per vertebral body (Figure 8). Vertebral body length increase of female SD rats reached statistical significance in this study (P<0.001).

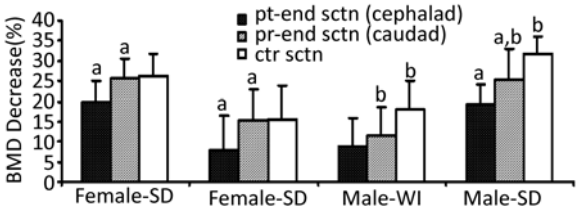

Figure 6. BMD decrease post gonadal surgery in central sections, cephalad para-endplate sections, and caudad para-endplate sections. a: comparison between cephalad para-endplate sections and caudad para-endplate sections P<0.01, b: comparison between caudad para-endplate sections and central sections P<0.01.

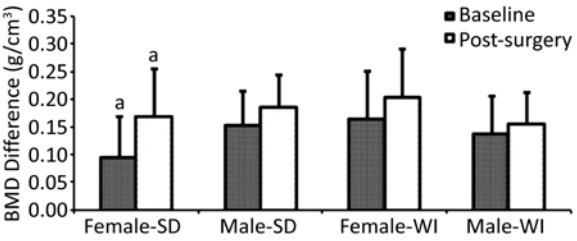

Figure 7. The BMD difference between cephalad para-endplate sections and caudad para-endplate sections. At baseline, BMD of cephalad para-endplate sections is higher than caudad para-endplate sections. The bone loss post gonadal surgery is greater in caudad para-endplate sections than cephalad para-endplate sections. This lead to further increase in cephalad and caudad para-endplate sections BMD difference. This further increase of BMD difference reached statistical significance in female SD rats. a: P<0.0001.

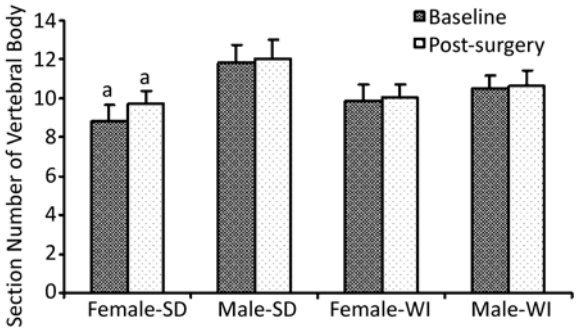

Figure 8. Mean slice number of total lumbar vertebral bodies (L1-L6) between 6.5-7 month old and 8.5-9 month old, a: $P<0.001$.

DISCUSSION

Peripheral quantitative CT (pQCT) densitometry, one of the most commonly used methods of densitometry in small animals, cannot reliably separate the compact bone and trabecular bone components of small animal vertebral bodies. Histomorphological methods have been therefore applied to separate the compact bone and trabecular bone components in small animals[12-13]. Micro-CT, which possesses a spatial resolution down to 20 μm pixel size, is capable of separating compact bone and trabecular bone. However, micro-CT is available only in limited specialist institutions and most scanners can only hold specimens of small size (field of view at maximum direction equals 3.6-7.6 cm depending scanner's versions). High radiation dosage is also commonly associated with micro-CT in vivo studies, which can lead to radiation burn to animals in many cases. Radiation exposure, which increases as a cubic function compared to a linear increase in resolution, is the principal factor limiting further improvements in resolution in living animals[14]. On the other hand, multi-detector CT scanner is widely available nowadays, and can be used for bone densitometry studies for small animals. Radiologists are increasingly engaged in osteoporosis research, both clinical studies with human subjects, and pre-clinical studies involving animals[10,15-17]. It is relevant to know the characteristics of rat lumbar vertebral body BMD derived from clinical MDCT.

Some results in study are as expected, including 1) male rat lumbar vertebrae are larger than those of female rat[18-19], 2) para-endplate sections have a higher BMD than that of central sections, 3) post gonadal surgery, central sections lose more bone than the para-endplate sections. The approach described in this study provides a consistent way to study BMD change within predominantly trabecular portions of the vertebral body. Depending on the aims of studies, the central sections or middle sections can be selected for assessing bone loss post gonadal surgery. This portion of vertebral body will be more sensitive to sex hormone deficiency, while the para-endplate sections can be less responsive.

At the meantime, a number of new results are observed. It is noted that vertebra L6 had a higher BMD than those of the rest lumbar vertebrae. A shorter vertebra may contain less trabecular bone and include relatively more compact bone per vertebral body dimension than a longer vertebra[9]. However, L1 had even shorter vertebral body than L6 (table 1). Our study showed the cephalad para-endplate sections had a higher BMD than the caudad para-endplate sections in all four group of rats. Interestingly, it was also recently reported that in human subjects, as measured by micro-CT on cadaver specimen, the endplates cranial to intervertebral disks were also thicker and had higher BMD than the corresponding caudal endplates[20]. In the post gonadal surgery, there was more bone loss in caudad sections than cephalad sections. This can be part of the reported site specificity of the sex hormone deficiency response[2]. Additionally, this study demonstrated that in the post gonadal surgery, the bone loss difference between para-endplate sections and central sections was greater in the male rats than female rats, both for Wistar rats and SD rats (Figure 5).

In our study, the BMD loss rate of the four groups of animals eight weeks after the post gonadal surgery varied (Figure 5). In literature, a wide range of bone loss rates in post gonadal surgery have been reported[6,21-26]. Rats from different batches may response differently in bone loss post gonadal surgery. This further stresses the importance of having a batch marched controls during designing and executing these experiments.

Rats of 6-7 months old have been commonly used to investigate sex hormone deficiency induced bone loss[14,27-30]. Our study suggested rat vertebrae continued to grow, though very mildly, between the ages of 6.5-7 months to 8.5-9 months (Figure 8). Jee et al. recommended the evaluation of treatments in the 9-month-old ovariectomized female rat[2]. The age selection of rat for osteoporosis study shall depend on the balance of the primary aim of the study and the availability and cost of the animals.

One limitation of the current study is that the number of animals used in this study is small. However, consistent trends were seen across vertebral bodies (*n*=6 per rat), and also across genders and strains. The authors believe that the increase of animal number will not change the conclusion of this study. Instead, some statistically non-significant observances would be significant if more animals were used.

In conclusion, this is the first observational study to demonstrate the characteristics of rat lumbar vertebral body BMD and differential segmental responses to sex hormone deficiency using clinical MDCT. As radiologists and radiology departments are increasingly engaged experimental research on osteoporosis using imaging tools, the knowledge obtained from the current study can help plan the animal based studies properly in order to ensure the appropriate areas are being tested and analysed.

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