FULL PAPER *Laboratory Animal Science*

Age-Related Changes of Bone Mineral Density and Microarchitecture in Miniature Pigs

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(Received 26 March 2003/Accepted 19 January 2004)

ABSTRACT. Bone mineral density (BMD), distribution of its density and bone histomorphometric parameters were evaluated in lumbar vertebra of normally growing miniature pigs. The fourth lumbar vertebra (L4) of the Göttingen miniature pig were used in this cross-sectional study *in vitro*. The BMD of the miniature pig was similar to that of humans in tendency of gender differences and some growth patterns during puberty. In these regards this animal appears useful as a model for human bone study. However, the trabecular and cortical BMDs of lumbar spine were extremely high value (399.43 ± 26.36 mg/cm^3 in female trabeculae; 973.06 ± 69.55 mg/cm^3 in female cortical bone; 419.04 ± 34.84 mg/cm^3 in male trabeculae; 1038.81 ± 125.72 mg/cm^3 in male cortical bone in pigs 30 months or more). Furthermore, histomorphometric analysis yielded values that were remarkably different from those found in humans. From these results, it was revealed that miniature pig had a higher bone mass and denser trabecular network than human, indicating that its bone is probably stronger. Therefore, care should be taken in choosing the miniature pig as a bone study model.

KEY WORDS: bone mineral density, histomorphometric analyses, lumbar spine, miniature pig.

J. Vet. Med. Sci. 66(6): 599–609, 2004

Metabolic bone diseases such as osteoporosis are becoming more important because of an aging society in human medicine. These diseases increase the risk of fragility fracture as bone mineral levels decrease, and also reduces their quality of life. Previous studies have reported that bone strength and fracture risk are closely related to bone mineral density (BMD) [6, 9, 27]. In contrast, several studies have suggested that changes in bone architecture increase fracture risk [29, 31, 33, 34, 43]. Therefore, it is necessary to study the changes in both BMD and architecture for more sensitive investigation of the bone disease.

Quantitative computed tomography (QCT) has been widely investigated and applied in recent years as a means of non-invasive quantification of BMD. It has been reported that QCT might be most sensitive to changes in bone density caused by rapid bone turnover, such as in menopausal immobilization or hyperthyroidism [9]. QCT can selectively measure both trabecular and cortical bone [9, 13, 22, 38], and the BMD is expressed in milligrams per cubic centimeter (volumetric density) [9]. Ebbesen *et al.* showed highly correlation between BMD by QCT and compressive strength in the lumbar vertebrae [9].

Several studies have reported that bone disorders such as osteoporosis lead to trabecular and cortical bone alterations in humans. These alterations are characterized not only by a reduction of bone mass but also by structural changes in microarchitecture which is measured by histomorphometry of bone [29, 31, 33]. Therefore, it has been considered that histomorphometric analysis have an important role as contributor to bone strength, in addition to BMD.

Recently, miniature pig has been noticed as for experimental animal of bone study. The pig is an excellent model of the human because it is omnivore and diurnal animal, and has a 21-day reproductive cycle that is similar to the human menstrual cycle [28, 37]. Previous study has presented that pig has trabecular bone with a lamellar structure and a remodeling sequence similar to that of human bone [26]. Moreover, the studies using pigs or miniature pigs have been performed recently [4, 23]. However, although the pig is an excellent model for human bone study, it grows to an excessively large adult body weight of approximately 150 kg. In contrast, miniature pig rarely exceeds 60 kg at maturity, requires less housing space, and does not require special handling equipment [28, 37]. Consequently, in the future, more investigators may turn to the miniature pig as model for bone research, but at present virtually nothing is known of bone change with age in this animal.

The aim of this study was to evaluate BMD, distribution of density by QCT and histomorphometric parameters in normally growing miniature pigs, to establish normative data and to relate these values to age and sex.

MATERIALS AND METHODS

Animals: The lumbar spine of Göttingen miniature pig (CSK miniature pig) was studied cross-sectionally *in vitro*. 112 normal miniature pigs (55 male, 57 female), aged 0–76 months age, were used for QCT analyses and 56 normal miniature pigs (28 male, 28 female), aged 0–76 months age

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were used for histomorphometric analyses.

Study 1: QCT image analyses

Sample processing: The forth lumbar vertebrae (L4) were cut off from the specimen because some studies were used this portion to evaluate BMD of the lumbar spine [2, 8, 15]. Samples for QCT were prepared by a modification of the method of Gluer *et al.* [17]. Briefly, the samples were placed in physiological saline and the pressure was lowered to about 100 mmHg for 15 min. Each sample was removed anaerobically in a cylindrical acrylic resinous container (35.0 cm in length and 24.5 cm in diameter, with a wall thickness of 0.6 cm) which was filled with physiological saline. The sample within the cylindrical acrylic resinous container was set up on the X-ray computed tomography (CT) system (RADIX-PRATICO®, Hitachi medical Co., Tokyo, Japan) with a bone mineral calibration phantom (B-MASS 200®, Chugai Pharmaceutical Co., Tokyo, Japan).

QCT image acquisition: From the scanogram (lateral digital radiograph), the gantry angle for CT scanning was determined from 4 coordinate points (dorsal and ventral end of vertebral front edge and vertebral back edge, respectively in each of the L4). After the gantry angle was determined, the center transversal slice (mid-vertebral slice; MVS) of the vertebra was decided from the distance between the front edge and the back edge of the vertebra in accordance with the method of Kalender *et al.* [20]. The anterior vertebral slice (AVS), which was 1/6 of the vertebral length anterior to MVS, was determined. QCT scanning was performed at the AVS with the bone mineral calibration phantom by using the following technical parameters: slice thickness, 3.0 mm; scan time, 1.0 s; voltage, 100 kVp; anode current, 100 mA; field of view, 380 mm.

Calculation of BMD: A bone mineral calibration phantom with 4 different concentrations (50, 100, 150, 200 mg/m*l*) of calcium hydroxyapatite ($Ca_{10}(Po_4)_6(OH)_2$) was used to convert raw CT numbers into mineral equivalent values. The mean CT numbers within the optional regions of interest (ROI) in the trabecular and cortical bone in each of slices were calculated [27, 42]. The measurements were performed twice and the average of these CT numbers was used for each value. The raw CT numbers were converted to BMD by means of linear regression in accordance with the values for the bone mineral calibration phantom.

Measurement of transverse diameter of the vertebral canal: From the obtained CT image, the transverse diameter of the vertebral canal was measured to evaluate of the skeletal growth with age. These measurements were performed twice, and the average of these values was recognized as the transverse diameter of vertebral canal.

Calculation of density distribution and fragmentation indices (FI): Coefficients of variation (CV) was calculated from the mean of the raw CT numbers (MN) and its standard deviation (SD) in the ROIs of the trabecular and cortical bone, respectively.

$$\text{Coefficients of variation (CV)} = SD/MN$$

Study 2: Histomorphometric analyses

Sample processing: L4 was cut vertically at the AVS and fixed in Lillie's solution. The samples were decalcified and were performed exsiccation by ethanol, dealcoholization by xylol, and paraffin impregnation. Seven μm-thick sections were cut with microtome (Yamato Kohki, Saitama, Japan), and stained with hematoxylin-eosin for analysis of parameters. The microscopic pictures of the samples were taken with a digital still camera (Cyber-shot, DSC-S85®, SONY, Tokyo, Japan).

Histomorphometry: The histomorphometric parameters were analyzed by computer software (KS400®, Carl Zeiss, Oberkochen, Germany). First, two measurable tissue areas (TAs; mm^2) of 4.52 mm^2 were chosen in the trabecular parts of the sections. In each TA, bone area (BA; mm^2) and bone perimeter (B.Pm; mm) were obtained directly. The measurements were performed at the two parts and the average of these values was used for histomorphometric parameter.

Parameters such as trabecular number (Tb.N), bone volume/tissue volume (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), bone surface/bone volume (BS/BV) and bone surface/tissue volume (BS/TV) were derived from these primary measures by using standard formule [16, 31]. Cortical width (Ct.Wi) was measured directly at the ventral corpus vertebrae at two parts symmetrically on each side. The average of these two parts was used for each value. Nomenclature and abbreviations followed the recommendations of the American Society for Bone and Mineral Research [32].

Statistic analyses: Statistical analyses were performed by the following methods to examine each analysis of QCT and histomorphometric results. Fitted curves were obtained from locally weighted scatterplot smoother by the least squares method (LOWESS) using commercial software (Stat-View 5.0, SAS Institute Inc., North Carolina, U.S.A.). The tension parameter of LOWESS was set at 66%. For comparing gender differences after the end of skeletal growth, the mean values of each parameters were obtained after the age of 30 months or more. When the F value was not significant, Student's *t*-test was used to identify significant gender differences. When the F value was significant, the Welch test was used for statistical evaluation. For each test, differences were considered significant if $p < 0.05$. These data were expressed as the mean ± standard deviation (SD).

RESULTS

Study 1: QCT image analysis

BMD: Up to about 35 months of age the female trabecular BMD tended to be higher than the male values on the scatter plot (Fig. 1a). On the scatter plot, the male trabecular BMD showed a gradual increase with age, but the female values showed a steeper increase until about 20 months. In females over 20 months the trabecular BMD stabilized.

In females, cortical BMD increased until about 30 months of age (Fig. 1b). In males, cortical BMD showed a steeper

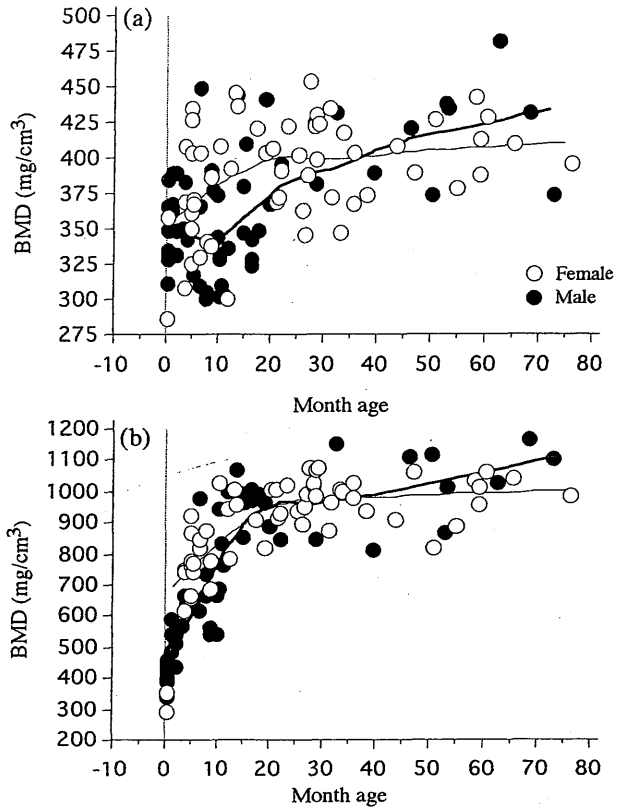

Fig. 1. Scatter plot of trabecular BMD (a) and cortical BMD (b). The explanatory note was described on (a). The tension parameter of LOWESS for fitted curves was set at 66%.

increase than in females until about 25 months, and after which time a gentle increase prevailed. Similar tendencies were observed in mean value of each month (Tables 1–2). The gender difference of BMD at age of 30 months or more was not significant (Table 5).

Transverse diameter of vertebral canal: Fitted curves of the transverse diameter of the vertebral canal showed a dramatic increase until about 20 months of age in males and about 25 months in females (Fig. 2a). After these times male and female values stabilized.

FI: A tendency for CV to decrease until about 30 months age in trabecular bone (Fig. 2b) and until about 15 months age in cortical bone (Fig. 2c) was observed in both males and females. After that, the CV almostly stabilized. Fitted curves showed a trend for a greater CV in males than in females in both trabecular and cortical bone. Similar tendencies were observed in mean value of each month (Tables 1–2). The gender difference of CV at age of 30 months or more was not significant (Table 5).

Study 2: Histomorphometric analysis

Trabecular bone: Fitted curves for the histomorphometric values of trabecular bone are shown in Fig. 3. The mean values are showed in Table 3.

The fitted curves of Tb.N decreased continuously with growth in both males and females in scatter plot (Fig. 3a). A trend toward greater values in females than in males was observed from 10 months of age. There was a significant difference between males and females at 30 months or more ($p<0.05$) (Table 5).

The fitted curves of BV/TV indicated a tendency to increase with growth in both males and females on the scatter plot (Fig. 3b).

The fitted curves of Tb.Th showed a continuous increase with growth in both males and females (Fig. 3c). A trend toward a greater value in males than in females was observed.

The fitted curves of Tb.Sp on the scatter plot decreased continuously with growth in females (Fig. 3d).

The fitted curves of BS/BV (Fig. 3e) and BS/TV (Fig. 3f) on the scatter plot decreased continuously with growth in both males and females.

Cortical bone: Age-related changes in the histomorphometric values of cortical bone are shown in Fig. 4. The mean values are showed in Table 4.

The fitted curves of Ct.Wi on the scatter plot increased continuously with growth in both male and females. A trend toward a greater value in males than in females was observed on the scatter plot, and there was a more pronounced tendency to increase in males than in females. The mean male value was significant higher than the female value at 30 months or more ($p<0.01$) (Table 5).

DISCUSSION

Miniature pigs are important animal as experimental model because of which have some characteristics similar to human. In fact, Boyce *et al.* suggested that calcium-restricted ovariectomized Sinclair minipigs may be a useful animal model of cancellous osteopenia and trabecular plate perforation, and be valuable in modeling some of the changes in cancellous bone remodeling, three dimensional architecture, and strength in response to estrogen deficiency [4]. Furthermore, Borah *et al.* used Sinclair S-1 miniature pigs in the study for trabecular architecture and bone strength [3]. Therefore, the importance of the miniature pigs in bone study will increase. However, the normative data of bone in miniature pigs is unknown. Therefore, in this study, we established normative data of growing Göttingen miniature pigs .

The BMD of female miniature pig vertebrae was higher than that of males until about 35 months of age in trabecular bone and about 15 months age in cortical bone. Generally, in humans, although the whole BMD of spine do not differ between females and males in infancy [2, 8, 39], the BMD of females is higher than that of males during puberty. In contrast, the whole BMD of spine in human males are greater than in females after puberty [8, 15, 25]. Gilsanz *et al.* reported that the BMD of vertebral bone during the final stages of puberty in African-American girls was substantially higher than in Caucasian American girls, although BMD did not differ between the two groups before the onset of puberty and during the early stages of puberty [14]. They

Table 1. BMD and CV values of trabecular bone in 55 male and 57 female miniature pigs

	n		Male		Female		Male		Female	
	Male	Female	BMD (mg/cm^3)	SD	BMD (mg/cm^3)	SD	CV	SD	CV	SD
0	8	2	341.42	23.26	322.01	51.29	0.24	0.04	0.27	0.03
1	3	–	373.20	14.17	–	–	0.27	0.01	–	–
2	2	–	356.14	29.25	–	–	0.31	0.05	–	–
3	3	3	358.67	21.27	361.44	50.53	0.26	0.05	0.28	0.03
4	1	–	341.54	–	–	–	0.28	–	–	–
5	1	8	317.10	–	379.95	38.27	0.27	–	0.26	0.02
6	3	2	374.48	70.06	366.00	51.29	0.27	0.03	0.28	0.01
7	–	1	–	–	341.36	–	–	–	0.28	–
8	4	2	333.77	41.36	361.67	33.39	0.29	0.01	0.26	0.04
9	3	–	364.79	18.40	–	–	0.28	0.03	–	–
10	3	1	320.37	16.24	407.04	–	0.29	0.03	0.21	–
11	3	–	315.95	18.23	–	–	0.31	0.05	–	–
12	–	2	–	–	346.45	65.34	–	–	0.27	0.04
13	1	2	443.09	–	440.60	6.87	0.18	–	0.22	0.02
14	–	–	–	–	–	–	–	–	–	–
15	3	–	378.51	31.47	–	–	0.23	0.02	–	–
16	3	–	331.10	10.01	–	–	0.29	0.01	–	–
17	1	1	349.19	–	420.08	–	0.29	–	0.27	–
18	–	–	–	–	–	–	–	–	–	–
19	2	1	403.85	50.98	402.63	–	0.24	0.05	0.22	–
20	–	1	–	–	406.63	–	–	–	0.22	–
21	–	3	–	–	377.36	11.16	–	–	0.22	0.01
22	1	–	394.87	–	–	–	0.24	–	–	–
23	–	1	–	–	421.97	–	–	–	0.22	–
24	–	–	–	–	–	–	–	–	–	–
25	–	1	–	–	401.51	–	–	–	0.21	–
26	–	2	–	–	353.94	11.12	–	–	0.25	0.04
27	–	2	–	–	420.37	45.47	–	–	0.21	0.02
28	1	4	381.81	–	418.49	14.04	0.20	–	0.22	0.01
29	–	1	–	–	423.57	–	–	–	0.22	–
30	–	–	–	–	–	–	–	–	–	–
31	–	2	–	–	402.65	44.24	–	–	0.20	0.03
32	1	–	431.95	–	–	–	0.23	–	–	–
33	–	2	–	–	382.12	49.49	–	–	0.21	0.03
34	–	–	–	–	–	–	–	–	–	–
35	–	–	–	–	–	–	–	–	–	–
36	–	2	–	–	385.49	25.05	–	–	0.23	0.02
37	–	–	–	–	–	–	–	–	–	–
38	–	1	–	–	373.82	–	–	–	0.19	–
39	1	–	388.59	–	–	–	0.21	–	–	–
40	–	–	–	–	–	–	–	–	–	–
41	–	–	–	–	–	–	–	–	–	–
42	–	–	–	–	–	–	–	–	–	–
43	–	1	–	–	407.65	–	–	–	0.21	–
44	–	–	–	–	–	–	–	–	–	–
45	–	–	–	–	–	–	–	–	–	–
46	1	–	420.55	–	–	–	0.20	–	–	–
47	–	1	–	–	389.59	–	–	–	0.21	–
48	–	–	–	–	–	–	–	–	–	–
49	–	–	–	–	–	–	–	–	–	–
50	1	1	374.05	–	426.75	–	0.23	–	0.21	–
51	–	–	–	–	–	–	–	–	–	–
52	–	–	–	–	–	–	–	–	–	–
53	2	–	435.58	2.15	–	–	0.22	0.01	–	–
54	–	1	–	–	378.24	–	–	–	0.22	–
55	–	–	–	–	–	–	–	–	–	–
56	–	–	–	–	–	–	–	–	–	–
57	–	–	–	–	–	–	–	–	–	–
58	–	1	–	–	441.83	–	–	–	0.21	–
59	–	2	–	–	399.99	18.63	–	–	0.22	0.01
60	–	1	–	–	427.55	–	–	–	0.21	–
61	–	–	–	–	–	–	–	–	–	–
62	1	–	480.50	–	–	–	0.25	–	–	–
63	–	–	–	–	–	–	–	–	–	–
64	–	–	–	–	–	–	–	–	–	–
65	–	1	–	–	409.85	–	–	–	0.21	–
66	–	–	–	–	–	–	–	–	–	–
67	–	–	–	–	–	–	–	–	–	–
68	1	–	431.51	–	–	–	0.22	–	–	–
69	–	–	–	–	–	–	–	–	–	–
70	–	–	–	–	–	–	–	–	–	–
71	–	–	–	–	–	–	–	–	–	–
72	1	–	373.11	–	–	–	0.26	–	–	–
73	–	–	–	–	–	–	–	–	–	–
74	–	–	–	–	–	–	–	–	–	–
75	–	–	–	–	–	–	–	–	–	–
76	–	1	–	–	394.61	–	–	–	0.25	–

Table 2. BMD and CV values of cortical bone in 55 male and 57 female miniature pigs

	n		Male		Female		Male		Female	
	Male	Female	BMD (mg/cm³)	SD	BMD (mg/cm³)	SD	CV	SD	CV	SD
0	8	2	414.54	38.63	320.77	41.61	0.22	0.05	0.22	0.01
1	3	–	536.33	52.46	–	–	0.20	0.04	–	–
2	3	–	496.41	52.89	–	–	0.22	0.02	–	–
3	3	3	623.27	51.62	702.73	74.92	0.15	0.02	0.16	0.04
4	1	–	665.41	–	–	–	0.15	–	–	–
5	1	8	642.52	–	770.48	90.73	0.19	–	0.15	0.03
6	3	2	736.98	208.73	833.41	21.25	0.18	0.05	0.16	0.02
7	–	1	–	–	871.43	–	–	–	0.12	–
8	4	2	623.15	90.91	732.23	64.43	0.19	0.06	0.17	0.02
9	3	–	658.90	113.71	–	–	0.15	0.02	–	–
10	3	1	770.75	147.80	1026.49	–	0.15	0.02	0.15	–
11	3	–	863.96	121.40	–	–	0.15	0.02	–	–
12	–	2	–	–	864.68	111.31	–	–	0.15	0.03
13	1	2	1068.54	–	980.50	34.84	0.14	–	0.13	0.01
14	–	–	–	–	–	–	–	–	–	–
15	3	–	935.09	74.94	–	–	0.15	0.02	–	–
16	3	–	987.32	20.07	–	–	0.13	0.01	–	–
17	1	1	993.29	–	905.76	–	0.16	–	0.14	–
18	–	–	–	–	–	–	–	–	–	–
19	2	1	926.41	56.89	815.43	–	0.15	0.01	0.11	–
20	–	1	–	–	1007.85	–	–	–	0.16	–
21	–	3	–	–	949.08	47.72	–	–	0.14	0.00
22	1	–	845.22	–	–	–	0.13	–	–	–
23	–	1	–	–	1020.06	–	–	–	0.15	–
24	–	–	–	–	–	–	–	–	–	–
25	–	1	–	–	937.62	–	–	–	0.11	–
26	–	2	–	–	921.06	37.23	–	–	0.13	0.02
27	–	2	–	–	1033.13	61.55	–	–	0.15	0.02
28	1	4	847.09	–	1024.07	36.16	0.17	–	0.12	0.01
29	–	1	–	–	1073.35	–	–	–	0.13	–
30	–	–	–	–	–	–	–	–	–	–
31	–	2	–	–	919.25	64.76	–	–	0.15	0.01
32	1	–	1149.63	–	–	–	0.11	–	–	–
33	–	2	–	–	1001.68	7.46	–	–	0.17	0.03
34	–	–	–	–	–	–	–	–	–	–
35	–	–	–	–	–	–	–	–	–	–
36	–	2	–	–	1001.82	29.96	–	–	0.14	0.01
37	–	–	–	–	–	–	–	–	–	–
38	–	1	–	–	933.48	–	–	–	0.12	–
39	1	–	808.09	–	–	–	0.15	–	–	–
40	–	–	–	–	–	–	–	–	–	–
41	–	–	–	–	–	–	–	–	–	–
42	–	–	–	–	–	–	–	–	–	–
43	–	1	–	–	907.21	–	–	–	0.13	–
44	–	–	–	–	–	–	–	–	–	–
45	–	–	–	–	–	–	–	–	–	–
46	1	–	1108.44	–	–	–	0.14	–	–	–
47	–	1	–	–	1059.24	–	–	–	0.14	–
48	–	–	–	–	–	–	–	–	–	–
49	–	–	–	–	–	–	–	–	–	–
50	1	1	1115.47	–	815.55	–	0.12	–	0.16	–
51	–	–	–	–	–	–	–	–	–	–
52	–	–	–	–	–	–	–	–	–	–
53	2	–	939.97	105.75	–	–	0.14	0.02	–	–
54	–	1	–	–	887.74	–	–	–	0.15	–
55	–	–	–	–	–	–	–	–	–	–
56	–	–	–	–	–	–	–	–	–	–
57	–	–	–	–	–	–	–	–	–	–
58	–	1	–	–	1034.38	–	–	–	0.13	–
59	–	2	–	–	984.59	34.87	–	–	0.13	0.00
60	–	1	–	–	1060.82	–	–	–	0.12	–
61	–	–	–	–	–	–	–	–	–	–
62	1	–	1024.07	–	–	–	0.19	–	–	–
63	–	–	–	–	–	–	–	–	–	–
64	–	–	–	–	–	–	–	–	–	–
65	–	1	–	–	1040.73	–	–	–	0.16	–
66	–	–	–	–	–	–	–	–	–	–
67	–	–	–	–	–	–	–	–	–	–
68	1	–	1163.21	–	–	–	0.13	–	–	–
69	–	–	–	–	–	–	–	–	–	–
70	–	–	–	–	–	–	–	–	–	–
71	–	–	–	–	–	–	–	–	–	–
72	1	–	1100.52	–	–	–	0.14	–	–	–
73	–	–	–	–	–	–	–	–	–	–
74	–	–	–	–	–	–	–	–	–	–
75	–	–	–	–	–	–	–	–	–	–
76	–	1	–	–	988.18	–	–	–	0.14	–

Table 3. Histomorphometric parameters (trabecular bone) in 28 male and 28 female miniature pigs

	n		Male		Female		Male		Females		Male		Female		Male		Female		Male		Female		Male		Female	
Month	Male	Female	Tb.N (/mm)	SD	Tb.N (/mm)	SD	BV/TV (%)	SD	BV/TV (%)	SD	Tb.Th (mm)	SD	Tb.Th (mm)	SD	Tb.Sp (mm)	SD	Tb.Sp (mm)	SD	BS/BV	SD	BS/BV	SD	BS/TV	SD	BS/TV	SD
0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1	2	–	6.33	0.13	–	–	51.27	7.12	–	–	0.16	0.02	–	–	0.08	0.01	–	–	25.06	2.99	–	–	12.66	0.26	–	–
2	1	–	4.56	–	–	–	49.78	–	–	–	0.22	–	–	–	0.11	–	–	–	18.29	–	–	–	9.11	–	–	–
3	2	2	4.43	0.72	4.07	0.09	43.86	0.70	39.05	3.91	0.20	0.03	0.19	0.02	0.13	0.02	0.15	0.01	20.23	3.02	20.92	1.68	8.86	1.44	8.13	0.17
4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5	–	3	–	–	4.29	0.19	–	–	41.89	5.60	–	–	0.20	0.03	–	–	0.14	0.02	–	–	20.71	2.90	–	–	8.58	0.38
6	–	1	–	–	4.50	–	–	–	39.38	–	–	–	0.17	–	–	–	0.14	–	–	–	22.95	–	–	–	9.00	–
7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8	2	2	4.37	0.61	3.97	0.06	39.60	6.88	42.37	7.51	0.18	0.01	0.21	0.03	0.14	0.04	0.15	0.02	22.25	0.93	19.23	2.78	8.75	1.21	7.94	0.12
9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10	2	–	3.74	0.26	–	–	40.65	3.36	–	–	0.22	0.00	–	–	0.16	0.02	–	–	18.39	0.21	–	–	7.47	0.53	–	–
11	2	–	4.02	0.46	–	–	38.94	9.70	–	–	0.19	0.03	–	–	0.15	0.04	–	–	20.99	2.89	–	–	8.03	0.91	–	–
12	–	1	–	–	4.27	–	–	–	57.19	–	–	–	0.27	–	–	–	0.10	–	–	–	14.92	–	–	–	8.54	–
13	1	–	3.85	–	–	–	48.67	–	–	–	0.25	–	–	–	0.13	–	–	–	15.91	–	–	–	7.69	–	–	–
14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
15	2	–	3.53	0.54	–	–	45.63	1.49	–	–	0.26	0.05	–	–	0.16	0.02	–	–	15.53	2.85	–	–	7.06	1.08	–	–
16	2	–	3.87	0.12	–	–	52.77	17.68	–	–	0.27	0.08	–	–	0.12	0.05	–	–	15.44	4.70	–	–	7.73	0.25	–	–
17	1	1	3.72	–	4.01	–	44.25	–	45.46	–	0.24	–	0.23	–	0.15	–	0.14	–	17.19	–	17.76	–	7.44	–	8.02	–
18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
19	2	–	3.42	0.07	–	–	57.02	4.93	–	–	0.33	0.04	–	–	0.13	0.01	–	–	12.04	1.27	–	–	6.83	0.13	–	–
20	–	1	–	–	3.67	–	–	–	47.68	–	–	–	0.26	–	–	–	0.14	–	–	–	15.68	–	–	–	7.34	–
21	–	2	–	–	4.32	0.21	–	–	50.44	5.94	–	–	0.23	0.02	–	–	0.12	0.02	–	–	17.22	1.15	–	–	8.64	0.42
22	1	–	4.37	–	–	–	46.35	–	–	–	0.21	–	–	–	0.12	–	–	–	18.84	–	–	–	8.74	–	–	–
23	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
24	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
26	–	1	–	–	3.59	–	–	–	42.81	–	–	–	0.24	–	–	–	0.16	–	–	–	16.77	–	–	–	7.18	–
27	–	1	–	–	3.56	–	–	–	42.70	–	–	–	0.24	–	–	–	0.16	–	–	–	16.69	–	–	–	7.13	–
28	–	2	–	–	3.82	0.16	–	–	45.69	1.88	–	–	0.24	0.00	–	–	0.14	0.01	–	–	16.74	0.01	–	–	7.65	0.33
29	–	1	–	–	3.64	–	–	–	43.03	–	–	–	0.24	–	–	–	0.16	–	–	–	16.89	–	–	–	7.27	–
30	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
31	–	1	–	–	3.68	–	–	–	61.06	–	–	–	0.33	–	–	–	0.11	–	–	–	12.07	–	–	–	7.36	–
32	1	–	3.49	–	–	–	47.79	–	–	–	0.27	–	–	–	0.15	–	–	–	14.65	–	–	–	6.99	–	–	–
33	–	1	–	–	4.18	–	–	–	54.98	–	–	–	0.26	–	–	–	0.11	–	–	–	15.19	–	–	–	8.35	–
34	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
35	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
36	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
37	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
38	–	1	–	–	3.78	–	–	–	50.88	–	–	–	0.27	–	–	–	0.13	–	–	–	14.90	–	–	–	7.56	–
39	1	–	3.47	–	–	–	47.35	–	–	–	0.27	–	–	–	0.15	–	–	–	14.71	–	–	–	6.93	–	–	–
40	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
41	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
43	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
44	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
45	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
46	1	–	3.60	–	–	–	49.12	–	–	–	0.27	–	–	–	0.14	–	–	–	14.67	–	–	–	7.20	–	–	–
47	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
48	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
49	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
50	1	1	3.11	–	3.30	–	60.84	–	49.56	–	0.39	–	0.30	–	0.13	–	0.15	–	10.23	–	13.32	–	6.22	–	6.60	–
51	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
52	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
53	1	–	3.45	–	–	–	57.63	–	–	–	0.33	–	–	–	0.12	–	–	–	11.99	–	–	–	6.90	–	–	–
54	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
55	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
56	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
57	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
58	–	1	–	–	3.98	–	–	–	62.61	–	–	–	0.32	–	–	–	0.09	–	–	–	12.70	–	–	–	7.95	–
59	–	2	–	–	3.71	0.21	–	–	52.16	0.86	–	–	0.28	0.02	–	–	0.13	0.00	–	–	14.24	1.05	–	–	7.42	0.43
60	–	1	–	–	3.95	–	–	–	54.54	–	–	–	0.28	–	–	–	0.12	–	–	–	14.50	–	–	–	7.90	–
61	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
62	1	–	3.42	–	–	–	52.88	–	–	–	0.31	–	–	–	–	–	–	–	13.09	–	–	–	6.85	–	–	–
63	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
64	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
65	–	1	–	–	3.39	–	–	–	53.21	–	–	–	0.32	–	–	–	0.14	–	–	–	12.89	–	–	–	6.77	–
66	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
67	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
68	1	–	3.14	–	–	–	60.40	–	–	–	0.39	–	–	–	0.13	–	–	–	10.39	–	–	–	6.28	–	–	–
69	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
70	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
71	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
72	1	–	3.57	–	–	–	60.18	–	–	–	0.34	–	–	–	0.11	–	–	–	11.88	–	–	–	7.13	–	–	–
73	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
74	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
75	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
76	–	1	–	–	3.79	–	–	–	52.88	–	–	–	0.28	–	–	–	0.12	–	–	–	14.34	–	–	–	7.57	–

Table 4. Histomorphometric parameters (cortical bone) in 28 male and 28 female miniature pigs

Month	n		Male		Female	
	Male	Female	Ct.Wi (mm)	SD	Ct.Wi (mm)	SD
0	–	–	–	–	–	–
1	2	–	0.38	0.03	–	–
2	1	–	0.44	–	–	–
3	2	2	0.34	0.07	0.34	0.07
4	–	–	–	–	–	–
5	–	3	–	–	0.36	0.04
6	–	1	–	–	0.41	–
7	–	–	–	–	–	–
8	2	2	0.44	0.10	0.31	*
9	–	–	–	–	–	–
10	2	–	0.44	0.01	–	–
11	2	–	0.52	0.22	–	–
12	–	1	–	–	0.44	–
13	1	–	0.57	–	–	–
14	–	–	–	–	–	–
15	2	–	0.57	0.07	–	–
16	2	–	0.58	0.02	–	–
17	1	1	0.56	–	0.49	–
18	–	–	–	–	–	–
19	2	–	0.79	*	–	–
20	–	1	–	–	0.45	–
21	–	2	–	–	0.43	0.04
22	1	–	0.71	–	–	–
23	–	–	–	–	–	–
24	–	–	–	–	–	–
25	–	–	–	–	–	–
26	–	1	–	–	0.41	–
27	–	1	–	–	0.43	–
28	–	2	–	–	0.43	0.0035
29	–	1	–	–	0.56	–
30	–	–	–	–	–	–
31	–	1	–	–	0.39	–
32	1	–	0.95	–	–	–
33	–	1	–	–	0.61	–
34	–	–	–	–	–	–
35	–	–	–	–	–	–
36	–	–	–	–	–	–
37	–	–	–	–	–	–
38	–	1	–	–	0.49	–
39	1	–	0.80	–	–	–
40	–	–	–	–	–	–
41	–	–	–	–	–	–
42	–	–	–	–	–	–
43	–	–	–	–	–	–
44	–	–	–	–	–	–
45	–	–	–	–	–	–
46	1	–	0.79	–	–	–
47	–	–	–	–	–	–
48	–	–	–	–	–	–
49	–	–	–	–	–	–
50	1	1	0.75	–	0.44	–
51	–	–	–	–	–	–
52	–	–	–	–	–	–
53	1	–	0.82	–	–	–
54	–	–	–	–	–	–
55	–	–	–	–	–	–
56	–	–	–	–	–	–
57	–	–	–	–	–	–
58	–	1	–	–	0.57	–
59	–	2	–	–	0.51	0.00
60	–	1	–	–	0.59	–
61	–	–	–	–	–	–
62	1	–	0.95	–	–	–
63	–	–	–	–	–	–
64	–	–	–	–	–	–
65	–	1	–	–	0.57	–
66	–	–	–	–	–	–
67	–	–	–	–	–	–
68	1	–	0.93	–	–	–
69	–	–	–	–	–	–
70	–	–	–	–	–	–
71	–	–	–	–	–	–
72	1	–	–	–	–	–
73	–	–	–	–	–	–
74	–	–	–	–	–	–
75	–	–	–	–	–	–
76	–	1	–	–	0.52	–

* It was not able to measure in one of specimen

suggested that racial differences could account for numerous metabolic and hormonal changes during puberty, including increases in the production of growth hormone, gonadotropins, and sex steroid hormones [14]. Moreover, McCormick *et al.* reported that early onset of puberty in females was related to differences in BMD values between the sexes during puberty [25]. It was recently shown that sex steroids have receptors and exert biologic effects on both epiphyseal cartilage cells [1, 7] and bone cells [5, 21]. In these studies, it was suggested that these hormones play an important role in skeletal growth and mineralization. In the miniature pig, sexual maturity occurs at about 5 months in females and about 7 months in males. Notwithstanding these different times of sexual maturity, in the miniature pig we observed tendency of gender differences in BMD similar to those occurring in humans during puberty and adolescence.

In humans, BMD increases continuously during adolescence until peak bone mass is reached [8, 24]. A slow rate of increase of BMD has been observed during adolescence in humans after the steeper increase during puberty [8]. Szulc *et al.* suggested by dual x-ray absorptiometry (DXA) in men that peak bone mass of the lumbar spine was achieved at the age of 29 years, although the end of height growth was observed at 19–25 years [40].

In Caucasian women, it has been reported that height growth finishes at the age of about 17–18 years [2], but maximum BMD of L3, as assessed by DXA, is reported to occur at 24 years [24]. These studies suggest that in both sexes the whole BMD of the lumbar spine continues to increase after the end of skeletal growth. In this study, the peak bone mass of male in both of trabecular and cortical bone were not observed, and the BMD continued to increase. In contrast, in females peak bone mass was achieved at about 20 months in trabecular bone and at about 30 months of age in cortical bone; afterward, the BMD of both types of bone stabilized. Moreover, the present study indicated that the end of skeletal growth was about 20 months age in male and about 25 months age in female as the result of measuring the vertebral diameter. From these results, it was considered that BMD in male miniature pigs continued to increase after the end of skeletal growth in a manner similar to that occurring in humans. However, in female of miniature pig, the cortical BMD continuously increased until 30 months age when the skeletal growth was already finished, although the trabecular BMD finished increase before the end of skeletal growth. Afterward, the BMD of both bone types attained certain value. From these results, it was expected that the whole BMD of spinal bone continuously increased after the end of skeletal growth. As described above, some studies in humans have reported that the whole BMD of the spine, as assessed by DXA, continues to increase after the end of skeletal growth. Thus, the pattern of increase of BMD in female miniature pigs might be similar to that in human females.

Faulkner *et al.* reported that a model that depended only on bone density was not likely to be a highly accurate reflec-

Table 5. The gender differences of parameters at 30 months or more

Parameter		Sex	
Trabecular bone	BMD (mg/cm^3)	Male	419.04 ± 34.84
		Female	399.43 ± 26.36
	CV	Male	0.22 ± 0.01
		Female	0.21 ± 0.01
Cortical bone	BMD (mg/cm^3)	Male	1038.81 ± 125.72
		Female	973.06 ± 69.55
	CV	Male	0.13 ± 0.02
		Female	0.14 ± 0.01
Vertebral canal diameter	(mm)	Male	14.53 ± 1.16
		Female	14.80 ± 1.30
Histomorphometric analysis	Tb.N (/mm)	Male	3.39 ± 0.18*
		Female	3.74 ± 0.27
	BV/TV (%)	Male	55.48 ± 5.91
		Female	54.4 ± 4.24
	Tb.Th (mm)	Male	0.32 ± 0.04
		Female	0.29 ± 0.02
	Tb.Sp (mm)	Male	0.13 ± 0.01
		Female	0.12 ± 0.01
	BS/BV	Male	12.42 ± 1.86
		Female	13.83 ± 1.08
	BS/TV	Male	6.78 ± 0.36
		Female	7.49 ± 0.54
	Ct.Wi (mm)	Male	0.83 ± 0.08†
		Female	0.51 ± 0.06

The data expressed as the mean ± SD. * Significant different value between sexes ($p<0.05$). † Significant different value between sexes ($p<0.01$).

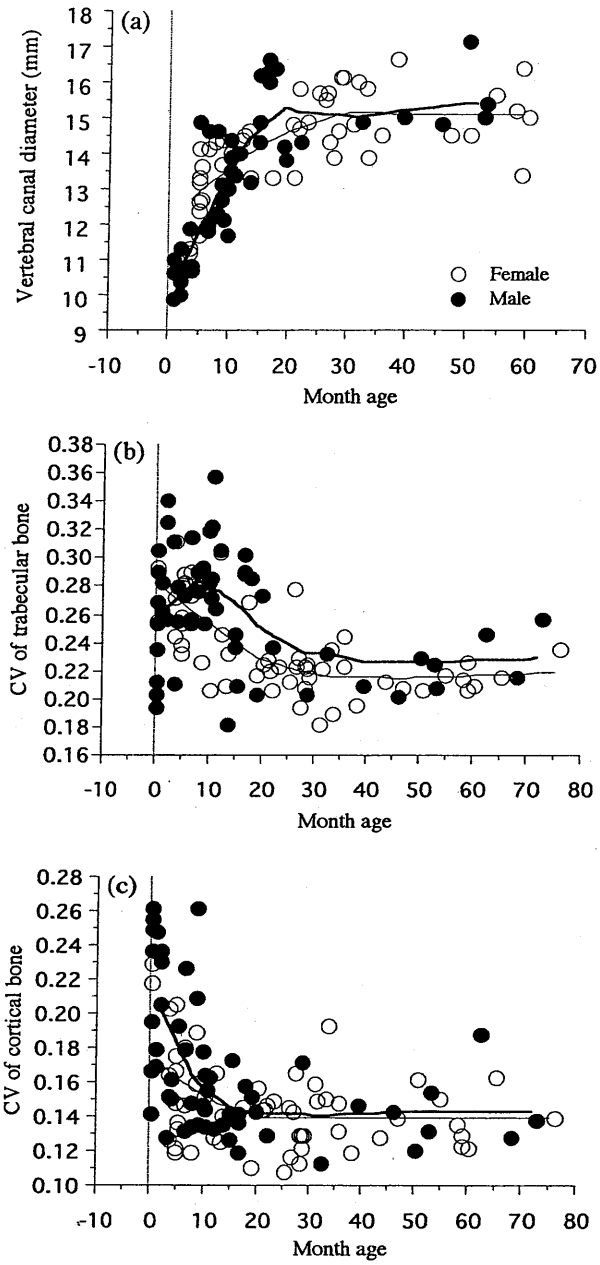

Fig. 2. Scatter plot of diameter of vertebral canal (a), trabecular CV (b) and cortical CV (c). The explanatory note was described on (a). The tension parameter of LOWESS for fitted curves was set at 66%.

tion of the strength of bone *in vitro*, although it was currently the best available model as judged by noninvasive measurements of bone mineral density [12]. Moreover, Engelke *et al.* suggested the possibility which CV might be better than BMD to improve the discriminatory capabilities for separation of osteoporotic from nonosteoporotic subjects [11].

The results in this study presented that the CV indicated lower value in female than in male during puberty. This suggests that in females the distribution of density was more uniform than in males in both trabecular and cortical bone during puberty. However, as there were no gender differences in CV in miniature pigs at 30 months or older, it was considered that there were no gender differences of density distribution during adolescence.

In humans, a number of detailed histomorphometric normative data related to the mature and aging skeleton have been studied during the last two decades [16, 30, 35]. Hildebrand *et al.* reported adult human histomorphometric values for L4 as follows: Tb.N: 1.16 ± 0.18 /mm, Tb.Th: 0.14 ± 0.03 mm, and Tb.Sp: 0.85 ± 0.14 mm [18]. The results in L4 of our miniature pigs were: Tb.N: 3.39 ± 0.18 /mm in males, 3.74 ± 0.27 /mm in females, Tb.Th: 0.32 ± 0.04 mm in males, 0.29 ± 0.02 mm in females and Tb.Sp: 0.13 ± 0.01 mm in males, 0.12 ± 0.01 mm in females at 30 months or more. Therefore, L4 in miniature pigs beyond adolescence has notably higher values of Tb.N and Tb.Th and lower values of Tb.Sp than in humans. In addition, Ito *et al.* reported that the BMD of lumber spine of Japanese aged 21–25 were 194.3 ± 27.1 mg/cm^3 in female trabeculae, 327.3 ± 46.3 mg/cm^3 in female cortical bone, 197.8 ± 21.9 mg/cm^3 in male trabeculae, and 398.0 ± 45.6 mg/cm^3 in male cortical bone [19]. The BMD of the miniature pig was more than 250 mg/

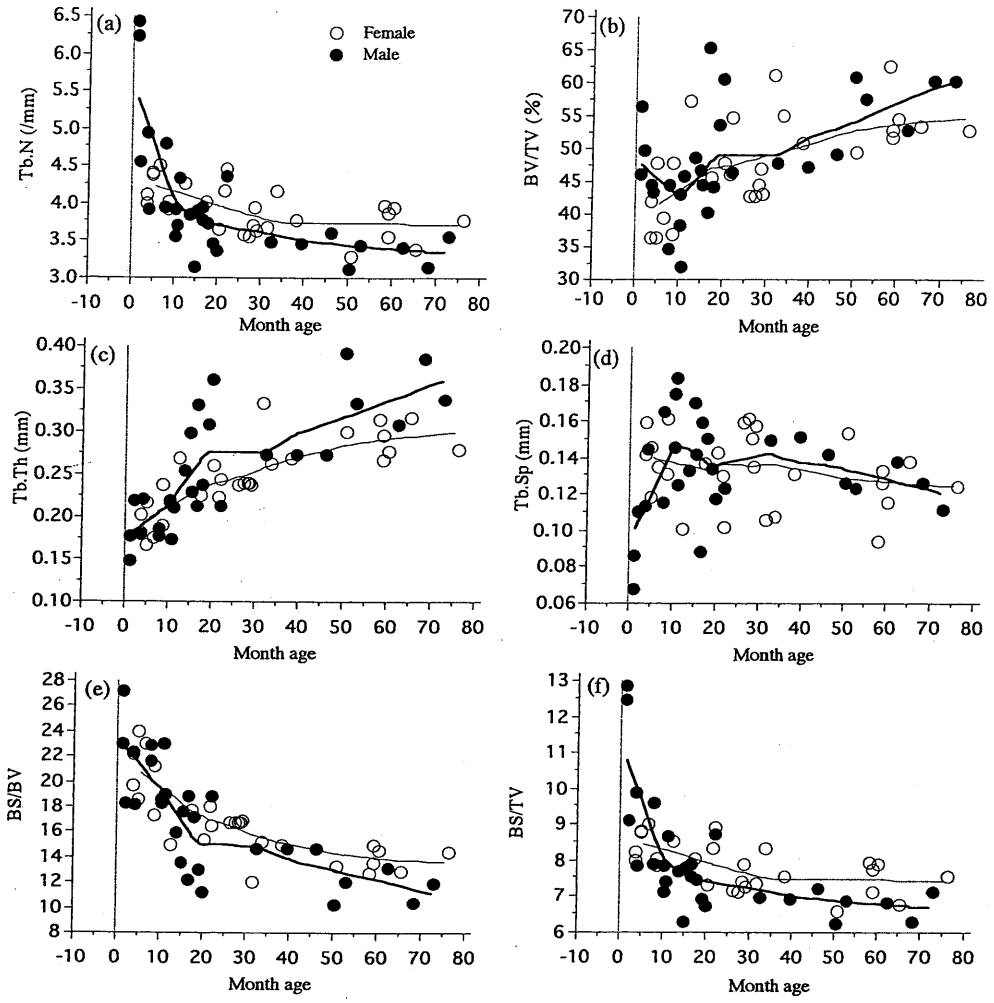

Fig. 3. Scatter plot of trabecular Tb.N (a), BV/TV (b), Tb.Th (c), Tb.Sp (d), BS/BV (e) and BS/TV (f). The explanatory note was described on (a). The tension parameter of LOWESS for fitted curves was set at 66%.

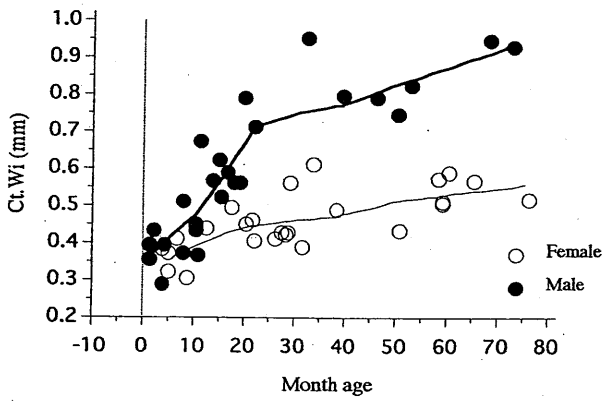

Fig. 4. Scatter plot of Ct.Wi. The tension parameter of LOWESS for fitted curves was set at 66%.

cm^3 in both trabecular and cortical bone, even immediately after birth, and in pigs aged 30 months or more it was 399.43 ± 26.36 mg/cm^3 in female trabeculae, 973.06 ± 69.55 mg/cm^3 in female cortical bone, 419.04 ± 34.84 mg/cm^3 in male trabeculae, and 1038.81 ± 125.72 mg/cm^3 in male cortical bone. There are thus conclusive differences in BMD and microarchitecture between humans and miniature pigs. These results suggest that the higher trabecular BMD of the miniature pig is caused by its high Tb.Th and low Tb.Sp. And then, it was considered that the miniature pig in this study had high strength in both of trabecular and cortical bone when compared with human.

Several studies have not been able to detect significant gender-dependent differences in histomorphometric analyses in humans [30, 41]. However, we found significant gender differences in Tb.N in miniature pigs 30 months or older. These results suggest that the miniature pig has gender difference in trabecular bone that are not recognized in humans during adolescence.

In contrast, previous studies have suggested that cortical thickness decreases in both men and women with age [36]. Vesterby *et al.* reported that Ct.Wi supports a more significant portion of the compressive spinal load and that elderly women become more dependent on Ct.Wi for the maintenance of vertebral body strength [43]. Edwards *et al.* presented that in humans the mean Ct.Wi of lumbar vertebrae was 0.68 ± 0.32 mm [10] and our study in miniature pig at more than 30 months age presented 0.83 ± 0.08 mm in male and 0.51 ± 0.06 mm in female. From these results, we suggest that unlike the case with Tb.N, Tb.Th, and Tb.Sp, there is no remarkable difference between the Ct.Wi values of humans and miniature pigs. However, in miniature pigs the pattern of growth in Ct.Wi was different between the sexes, and males had significantly higher values. In humans, several studies have found no significant differences in Ct.Wi between men and women [10, 16]. Our results suggest that the male strength of vertebral bone depended on Ct.Wi was higher than that of female in miniature pig during adolescence.

In conclusion, our findings suggest that there are similarities of BMD between humans and miniature pigs in tendency of gender differences and some growth patterns. In these regards the miniature pig may be useful as an experimental animal for bone study. However, marked differences in other BMD and histomorphometric values between humans and miniature pigs indicate that miniature pig bone is stronger than human bone. Because of these difference, researchers must be careful in choosing the miniature pig as a model for human bone studies.

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