FULL PAPER *Laboratory Animal Science*

Age-Related Changes in Bone Mineral Density, Cross-Sectional Area and the Strength of Long Bones in the Hind Limbs and First Lumbar Vertebra in Female Wistar Rats

Satoshi FUKUDA[1)] and Haruzo IIDA[1)]

[1)]*International Space Radiation Laboratory, National Institute of Radiological Sciences, 4–9–1, Anagawa, Inage-ku, Chiba-shi, Chiba 263–8555, Japan*

(Received 24 September 2003/Accepted 9 February 2004)

ABSTRACT. Age-related changes in bone mineral density (BMD) and cross-sectional area and bone strength index (SSI) of the femur, tibia, humerus, and first lumbar vertebra in female Wistar (WM/MsNrs) rats were examined by a quantitative computed tomography (pQCT) method. One hundred and sixteen virgin female Wistar (WM/MsNrs) rats aged 2–33 months were used. The data indicate that the to tal BMD values of metaphyses and diaphyses of long bones increased until 12 months, then decreased to a varying degree depending on the bone after 15–24 months, but the values of cortical and trabecular BMD with age were not always similar to the total BMD value. Nevetheless, the values for cross-sectional area and SSI in the long bones increased regardless of the total BMD decrease with age, indicating that this increase might have been due to a characteristic of the modeling pattern in rats. The total and cortical BMD values in the first lumbar vertebra decreased after 18 months, and SSI did after 15 months. The data obtained in this study were compared with those obtained from males in a previous study. In conclusion, it was indicated that in this strain the rats over 12 months with the highest total BMD values in the femur and tibia, and before the onset of various tumors, are useful as a model animal for osteoporosis experiments and observation of senile bone change.

KEY WORDS: bone mineral density, cross-sectional area, female Wistar rat (WM/MsNrs), pQCT, strength strain index.

J. Vet. Med. Sci. 66(7): 755–760, 2004

We have previously reported the age-related changes in bone mineral density (BMD), cross-sectional area, and strength strain index (SSI), an indicator of bone fragility, in male rats [17], as rats are widely used for experiments regarding bone disease and for pharmacological tests. Female rats are more frequently used than males, for example, as a model of postmenopausal osteoporosis after ovariectomy treatment. Nevertheless, the differences between rats and humans in bone metabolic and morphologic characteristics have been contested [2, 7, 8, 18, 25, 34]. Furthermore, although many studies on bones in the rat have carried out, the data have varied because of differences in the bones observed and the measurement methods [3, 9, 11,13,16, 22, 27, 32, 33].

We consider it important to clarify the age-related changes in bones throughout the life span of rats to determine whether it is worthwhile to use rats in studies related to bones. Our report regarding the life span and incidence of tumors in this strain is available to aid in our understanding of the changes in bones with age in rats as laboratory animals [10].

The measurement of BMD, cross-sectional area, and SSI in the present study was done by the QCT method because this method has merits with regard to simultaneously measuring BMD and morphologic features by separating trabecular and cortical bones, and because it can be carried out in live rats, the same as in clinical use for humans, as described previously [17].

The purpose of the present study was to clarify the characteristics of age-related changes in BMD, cross-sectional area, and SSI of long bones and first lumbar vertebra in female rats, and to compare these characteristics with those of males, as obtained in our previous study.

MATERIALS AND METHODS

A total of one hundred and sixteen female Wistar (WM/MsNrs) rats were selected from groups which were virgin, intact and without mammary tumors in a large breeding colony. Rats were sacrificed, ten each at the ages of 2, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 months and six at the age of 33 months. Rats were housed five each in a stainless steel cage (height: 20 cm, wide: 26.5 cm and length 44 cm) under conventional conditions. They were kept in an animal room controlled at a constant condition (temperature: 22 ± 2°C; relative humidity, 55 ± 5%; 12-hr light-dark cycle). They were freely fed a commercial diet (MB-1, Funabashi Farm Co., Ltd., Japan) containing 1.0% calcium, 1.2% phosphorus, 24.2% crude protein, 4.4% crude fat, 3.6% crude cellulose, 5.4% ash and 54.4% non-nitrogeous substance, that provided 4.26 Kilo-calorie per gramme of feed, and water.

After the body weight was measured under anesthesia induced by chloroform inhalation, the femur, tibia, humerus, and first lumbar vertebra were extracted, and the muscles and tendons were removed.

Measurements of the BMD and cross-sectional area were performed with a pQCT (XCT-960A, Norland & Stratec Co.). The strength-strain index (SSI) was also calculated by means of the software described in detail in a previous study [17]. Total (cortical + trabecular) BMD, cortical BMD, and

trabecular BMD as well as the cross-sectional area of the metaphyses, and the cortical BMD and cross-sectional area of the diaphyses of long bones were measured at 3 and 12 mm from the center of the growth-plate image in the distal epiphysis of the femur and the proximal epiphyses of the tibia and humerus, respectively. The measurement in the first lumbar vertebra was performed at the center of the transverse direction.

Statistical analysis was performed by a one-way ANOVA test and the Student's *t*-test. Significance was defined as the 0.05 level.

The laboratory animals used in this study were treated and/or handled according to the "Recommendations for the Handling of Laboratory Animals for Biomedical Research", compiled by the Committee on the Safety and Ethical Handling Regulations for Laboratory Animal Experiments at our institute.

RESULTS

Body weight (Fig. 1): The body weights of rats increased rapidly until 12 months of age, and thereafter continued to increase until 27 months, and then had decreased at 33 months.

Total, trabecular, and cortical BMD values in the metaphyses of long bones (Fig. 2): The total BMD was high from 9 to 15 months in the femur and tibia, and 12 to 21 (except 15) months in the humerus (Fig. 2a). The cortical BMD was high from 9 to 33 (except 24 and 30) months in the femur, 9 to 15 months in the tibia, and 6 to 27 (except 15) months in the humerus (Fig. 2b). The trabecular BMD was high from 6 to 27 (except 18 and 24) months in the femur, 6 to 33 months in the tibia, and 9 to 24 (except 12 and 21) months in the humerus (Fig. 2c).

Cortical BMD values in the diaphyses of long bones (Fig. 3): The cortical BMD of the diaphysis was high from 9 to 33 (except 24) months in the femur, from 9 to 30 months in the tibia, and from 6 to 12, 21, 24 and 33 months in the humerus.

BMD values in the first lumbar vertebra (Fig. 4): The total, cortical, and trabecular BMD values of the first lumbar

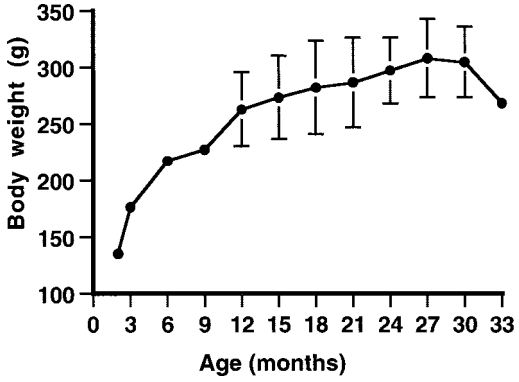

Fig. 1. Changes in body weight of rats. Values and bar are the mean ± SD.

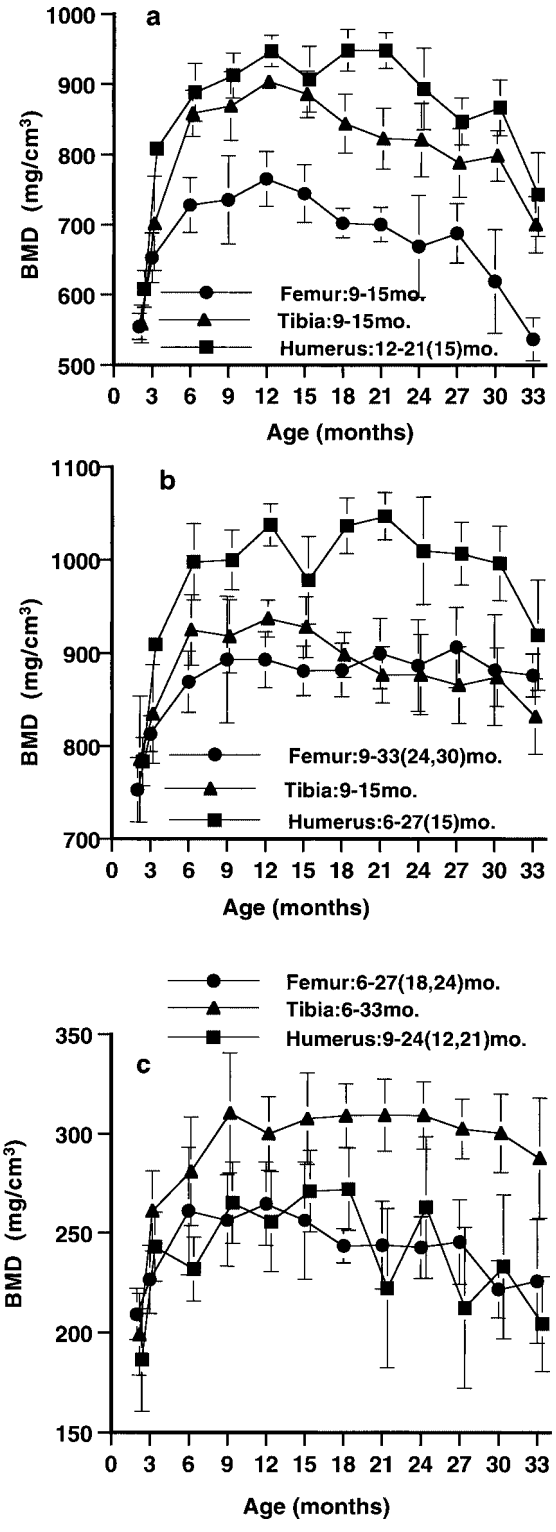

Fig. 2. Total (a), cortical (b) and trabecular (c) BMD values in the metaphyses of the femur, tibia and humerus. The ages with high values are shown in each figure. Values and bar are the mean ± SD.

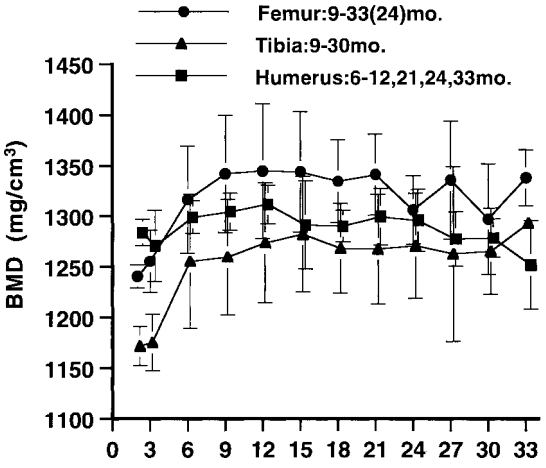

Fig. 3. Cortical BMD values in the diaphyses of the femur, tibia and humerus. Values are the mean ± SD. The ages with high values are shown in the figure.

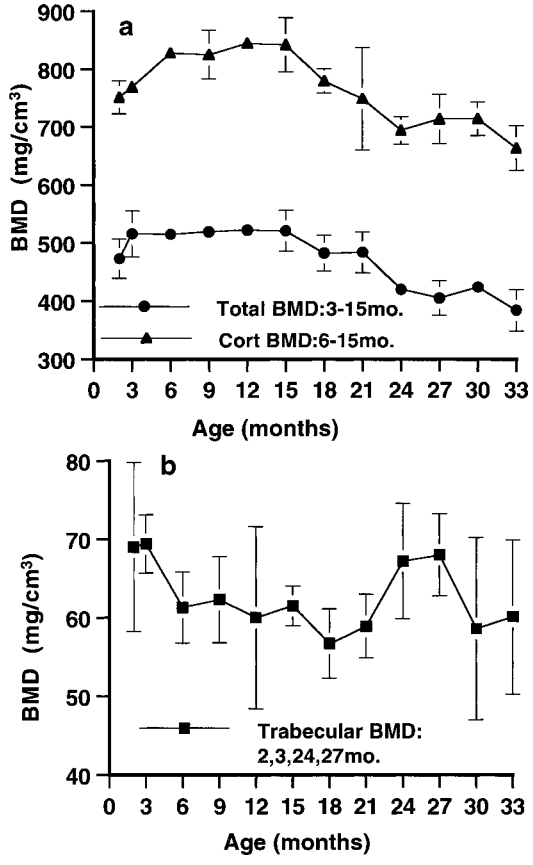

Fig. 4. Total and cortical (a), and trabecular (b) BMD values in the first lumbar vertebra. The ages with high values are shown in each figure. Values and bar are the mean ± SD.

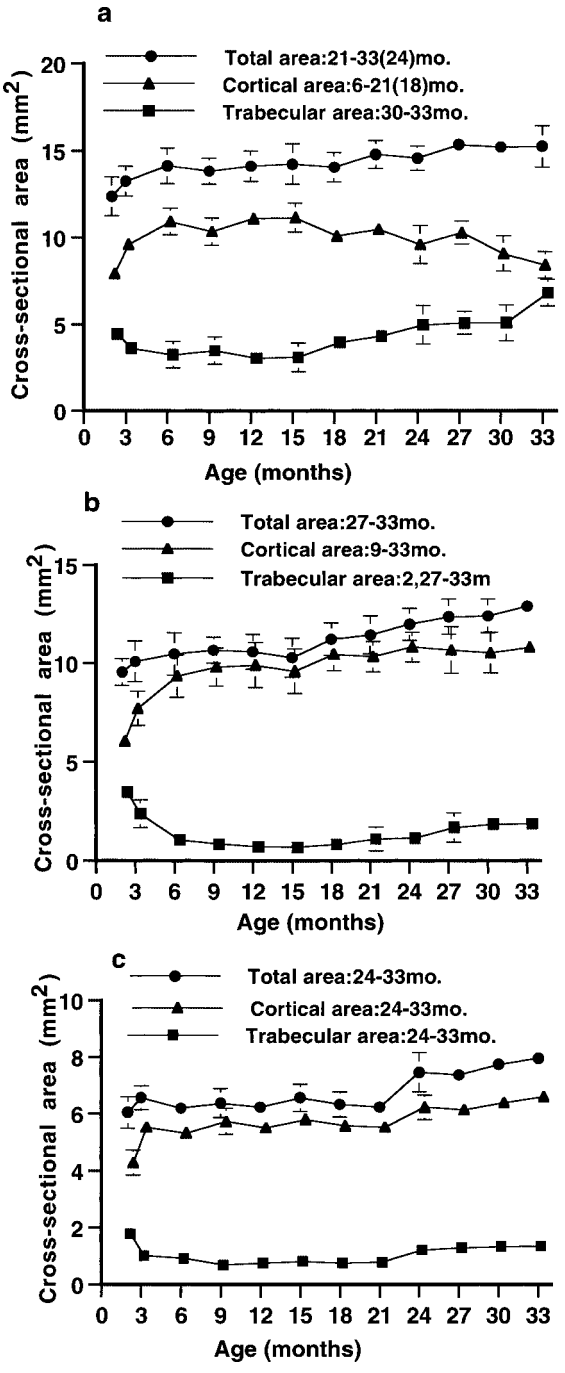

Fig. 5. Cross-sectional areas in the metaphyses of the femur (a), tibia (b) and humerus (c). Values are the mean ± SD. The ages with high values are shown in each figure. Values and bar are the mean ± SD.

vertebra were high from 3 to 15 months, 6 to 15 months (Fig. 4a) and 2, 3, 24 and 27 months (Fig. 4b), respectively.

Cross-sectional area of the metaphyses and diaphyses of long bones (Figs. 5 and 6): In the distal metaphysis of the

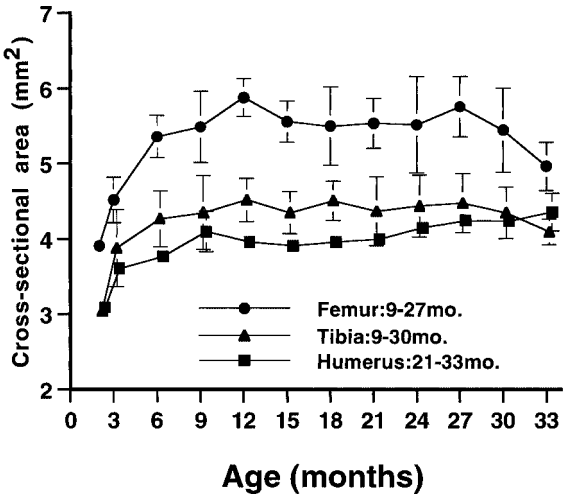

Fig. 6. Cross-sectional areas in the diaphyses of the femur, tibia and humerus. The ages with high values are shown in each figure. Values and bar are the mean ± SD.

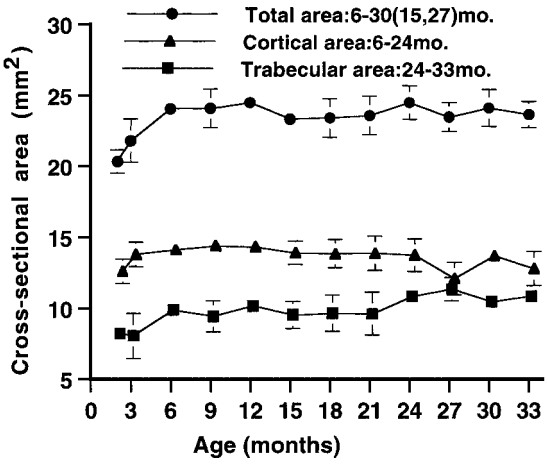

Fig. 7. Cross-sectional area of the first lumbar vertebra. The ages with high values are shown in the figure. Values and bar are the mean ± SD.

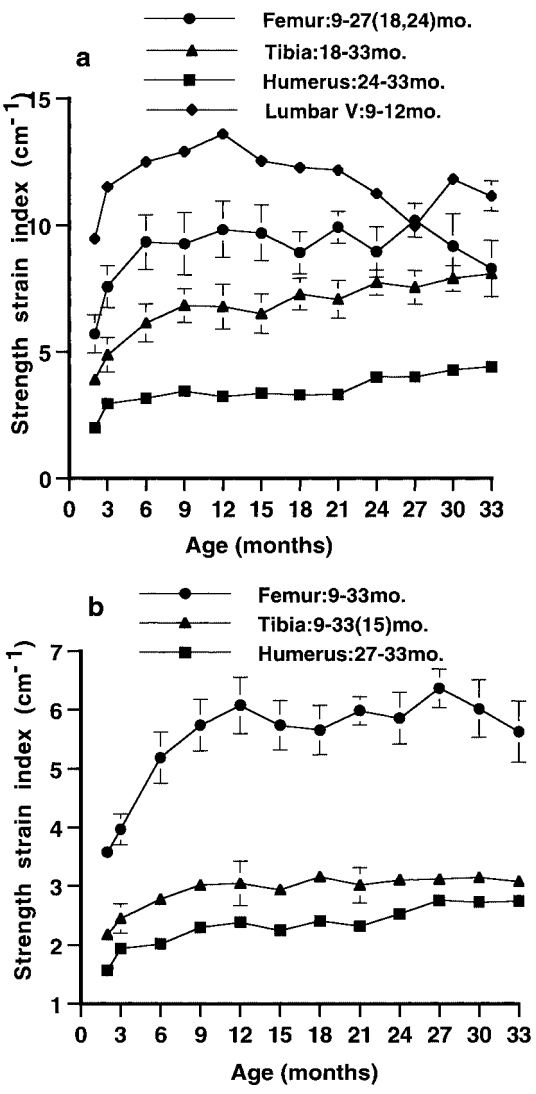

Fig. 8. SSI of the metaphyses of the femur, tibia and humerus and central cross-section of the first lumbar vertebra (a), and diaphyses of the femur, tibia and humerus (b). The ages with high values are shown in each figure. Values and bar are the mean ± SD.

femur, the total area increased with age and was high from 21 to 33 (except 24) months; the cortical area was high from 6 to 21 (except 18) months, and the trabecular area was from 30 to 33 months (Fig. 5a). In the proximal metaphysis of the tibia, the total and cortical areas increased with age and were high from 27 to 33 months and 9 to 33 months, and the trabecular area was high from 2 and 27 to 33 months (Fig. 5b). In the proximal metaphysis of the humerus, the total, cortical and trabecular BMD values were high from 24 to 33 months, respectively (Fig. 5c). The cortical area of the diaphyses of long bones was high from 9 to 27 months in the femur, 9 to 30 months in the tibia, and 21 to 33 months in the humerus, respectively (Fig. 6).

Cross-sectional area of the center of the first lumbar vertebra (Fig. 7): The total area was high from 6 to 30 (except 15 and 27) months, and the cortical area was high from 6 to 24 months. The trabecular area was high from 24 to 33 months.

SSI of the long bones and first lumbar vertebra (Fig. 8): The SSI of the metaphysis was high from 9 to 27 (except 18 and 24) months in the femur, and from 18 to 33 months in the tibia, and from 24 to 33 months in the humerus, and from 9 to 12 months in the first lumbar vertebra (Fig. 8a). The SSI of the diaphysis was high from 9 to 33 months in the femur, 9 to 33 (except 15) months in the tibia, and 27 to 33 months in the humerus (Fig. 8b), respectively.

DISCUSSION

Although the general patterns of age-related changes in total, trabecular and cortical BMD values, cross-sectional area, and SSI in long bones were similar to those of males [17], sex differences were seen in the age when BMD reached its highest values: the highest values of total and cortical BMDs in the metaphyses of the femur and tibia in females were 9 months later than in males (6 months), whereas the trabecular BMD highest value was 6 months earlier than in males (9 and 12 months). The cortical BMD highest values for the diaphyses of the femur and tibia were 9 months, earlier than in males (12 months). Large age differences between males and females in the BMD highest value were also observed in the first lumbar vertebra; the ages in females and males were 3 months and 6 months for total BMD, 6 months and 15 months for cortical BMD, and 2 months and 15 months for trabecular BMD, respectively.

It seems that the BMD value is affected by many factors such as body weight and hormones [6, 14, 19, 20, 29, 35]. The increase curve of body weight in females was different from that of males; the body weight in males increased until 12 months and then reached a plateau (about 400 g), whereas that in females continued to increase to about 300 g (Fig. 1), but our data showed that the BMD values in females were also higher than those in males, as observed in Charles River CD rats aged 23~150 days after birth by Saville [28]. Wronski *et al.* [35] have reported that bone loss is induced regardless of the increase in body weight after ovariectomy, although the tendency to slightly increase bone volume is observed partly in the metaphyses of long bones. Although the burden ratio of body weight shared by the fore and hind limbs is 40:60% when a rat stands, the BMD values for the humerus, where the body burden is less than that on the hind limbs, are higher than those for the femur and tibia in females (Fig. 2a and b). Therefore, the BMD level might not be greatly affected by the body weight burden in rats, except for the growing period when the body weight and BMD increase.

Schapira *et al.* [29] have observed that the sex hormones in female Wistar (Charles River Farm, U.K.) reaches a peak at 12 months, and that the estrus cycles ceased at 15 months. The data obtained in the present study suggest that the total BMD values for the femur and tibia decrease after 15 months; but no clear point of inflection at which cortical and trabecular BMD values begin to decrease as observed in the ovariectomized rats and menopausal women, has been found [1, 4, 9, 12, 15, 21]. The trabecular bone values, which might have a greater response to sex hormone loss than cortical bone in the tibia, did not decrease after 15 months in this strain (Fig. 2c). Schot *et al.* have reported that bone mineral loss is not induced only by changes in the concentrations of sex hormones in the blood [29]. Therefore, the rapid decrease in BMD, as seen in postmenopausal women, might not be induced in normal rats. Probably the moderate decrease in BMD is due to a change in organs such as that occurring with changes in renal function, and/or calcium transport in the intestines related to changes in calcium and bone metabolism with increasing age [14, 26, 31].

The total, cortical, and trabecular areas in long bones tended to increase with age, except for the cortical area of the femur, as in males. Sontag [32] has also observed that the diameter of the mid-shaft of the femur enlarges in male and female Heiligenberg rats at ages from 62 to 840 days. This enlargement might be due to the dominance of periosteal formation rather than endosteal resorption, a characteristic of the modeling pattern in rats.

The SSI values in the metaphysis and cortical area in the diaphyses of long bones and the first lumbar vertebra in females were found to be lower than those in males. The SSI values in the metaphysis and diaphysis of long bones increased with age along with increasing cross-sectional area, despite the decreasing or unchanging BMD. The SSI is calculated from the BMD value based on the geometric values for the bone, if it is presented simply. The SSI value is used as an indicator to assess bone fragility as well as BMD. The BMD values in females were found to be higher than those in males, whereas the SSI values in females were found to be lower than in males. Therefore, the sex difference in the SSI of the long bones is strongly affected by the cross-sectional area of bone in rat, different from that in the bones of humans. On the other hand, in the first lumbar vertebra, the SSI decreased (Fig. 8a) with decreases in the total and cortical BMD values after 18 months (Fig. 4a). Therefore, the age changes in the first lumbar vertebra are similar to those in the bones of humans.

The age at which the decrease in BMD starts is an important indicator of the time when bone fragility begins in humans. Similarly, the age in rats is interesting to determine when planning for the use of female rats in osteoporosis experiments. Our results indicate that the ages at which total BMD decreases began were different: 15 months in the femur and tibia, 24 months in the humerus and 18 months in the first lumbar vertebra, respectively. We observed that the average life span of females of this strain was 813 ± 214 days (n=907), and mammary tumors began to appear after 18–24 months, with the final incidence being 20.1% [10]. Namely, the age when the BMD in long bones and first lumbar vertebra begins to decrease may overlap in time with or be close to the onset of tumors. Nevertheless, this strain has the merit that the incidences of mammary and other tumors were lower than in other strains such as the Sprague-Dawley and Donryu [5, 23, 24].

In conclusion, it was indicated that in this strain, the rats over 12 months with the highest values for the femur and tibia and before the onset of various tumors are useful for osteoporosis experiments and observation of senile bone changes.

REFERENCES

1. Aitken, J.M., Armstrong, E. and Anderson, J.B. 1972. Osteoporosis after oophorectomy in the mature female rat and the effect of oestrogen and/or progestogen replacement therapy in its prevention. *J. Endocrinol.* **55**: 79–87.

2. Bagi, C.M., Wilkie, D., Georgelos, K., Williams, D. and Bertolini, D. 1997. Morphological and structural characteristics of the proximal femur in human and rat. *Bone* **21**: 261–267.
3. Baldock, P.A.J., Moore, R.J., Durbridge, T.C. and Morris, H.A. 1999. Comparison of three methods for estimation of bone resorption following ovariectomy in the distal femur and the proximal tibia of the rat. *Bone* **24**: 597–602.
4. Durbridge, T.C., Morris, H.A., Michelle Parsons, A.M., Parkinson, I.H., Moore, R.J., Porter, S., Need, A.G., Nordin, B.E. C. and Vernon-roberts, B. 1990. Progressive cancellous bone loss in rats after adrenalectomy and oophorectomy. *Calcif. Tissue Int.* **47**: 383–387.
5. Durbin, P.W., Williams, M.H., Jeung, N. and Arnold, J.S. 1966. Development of spontaneous mammary tumors over the life-span of the female Charles River (Sprague-Dawley) rat: the influence of ovariectomy, thyroidectomy, and adrenalectomy-ovariectomy. *Cancer Res.* **26**: 400–411
6. Epstein, S., Bryce, G., Hinman, J.W., Miller, O.N., Riggs, B.L., Hui, S.L. and Johnston, C.C. 1986. The influence of age on bone mineral regulating hormones. *Bone* **7**: 421–425.
7. Frost, H.M. 1973. pp. 28–53. *In*: Bone Remodeling and Its Relationship to Metabolic Bone Disease. Charles C Thomas, Springfield.
8. Frost, H.M. and Jee, W.S.S. 1992. On the rat model of human osteopenias and osteoporoses. *Bone Mineral* **18**: 227–236.
9. Fukuda, S. and Iida, H. 1991. Age and sex-related changes in bone metabolism in normal rats and the effects in ovariectomized and orchidectomized rats at different age. *J. Jpn. Soc. Bone Morphom.* **1**: 89–94.
10. Fukuda, S. and Iida, H. 2003. Life span and spontaneous tumors incidence of the Wistar Mishima (WM/MsNrs) rat. *Exp. Anim. (Tokyo)* **52**: 173–178.
11. Gasser, J.A. 1995. Assessing bone quantity by pQCT. *Bone* **17**: 145S-154S.
12. Gaumet, N., Seibel, M.J., Braillon, P., Giry, J., Lebecque, P., Davicco, M.J., Coxam, V., Rouffet, J., Delmas, P.D. and Barlet, J.P. 1996. Influence of ovariectomy on bone metabolism in very old rats. *Calcif. Tissue Int.* **58**: 256–262.
13. Geusens, P., Dequeker, J., Nijs, J. and Bramm, E. 1980. Effect of ovariectomy and prednisolone on bone mineral content in rats: evaluation by single photon absorptiometry and radiogrammetry. *Calcif. Tissue Int.* **47**: 243–250.
14. Ghishan, F.K., Jenkins, J T. and Younoszai, M.K. 1980. Maturation of calcium transport in the rat small and large intestine. *J. Nutr.* **110**: 1622–1628.
15. Hui, S.L., Slemenda, C.W., Johnston, C.C. and Appledorn, C.R. 1987. Effects of age and menopause on vertebral bone density. *Bone Mineral* **2**: 141–146.
16. Iida, H. and Fukuda, S. 1993. Age-related changes in bone weights and their components in rats. *Exp. Anim. (Tokyo)* **42**: 349–356.
17. Iida, H. and Fukuda, S. 2002. Age-related changes in bone mineral density, cross-sectional area and strength at different sites in male rats. *J. Vet. Med. Sci.* **64**: 29–34.
18. Jee, W.S.S., Li, X.J. and Ke, H.Z. 1991. The skeletal adaptation to mechanical usage in the rat. *Cells Mater. (Suppl.)* **1**: 131–142.
19. Kalu, D.N., Hardin, R.H., Cockerham, R. and Yu, B.P. 1984. Aging and dietary modulation of rat skeleton and parathyroid hormone. *Endcrinology* **115**: 1239–1247.
20. Kalu, D.N., Liu, C.C., Hardin, R.R. and Hollis, B.W. 1989. The aged rat model of ovarian hormone deficiency bone loss. *Endocrinology* **124**: 7–16.
21. Kalu, D.N. 1991. The ovariectomized rat model of postmenopausal bone loss. *Bone and Mineral* **15**: 175–192.
22. Li, X.J., Jee, W.S.S., Chow, S. and Woodbury, D.M. 1990. Adaptation of cancellous bone to aging and immobilization in the rat: a single photon absorptiometry and histomorphometry study. *Anat. Rec.* **227**: 12–24.
23. Maekawa, A., Onodera, H., Tanigawa, H., Furuta, K., Matsuoka, C., Kanno, J., Ogiu, T. and Hayashi, Y. 1986. Spontaneous neoplastic and non-neoplastic lesions in aging donryu rats. *Jpn. J. Cancer Res.* **77**: 882–890.
24. Mandybur, T.I., Ormsby, I., Samuels, S. and Mancardi, G.L. 1985. Neural, pituitary, and mammary timors in Sprague-Dawly rats treated with X iiradiation to the head and N-Ethyl-N-nitrosoures (ENU) during the early postnatal period: a statistical sudy of tumor incidence and survival. *Radiat. Res.* **101**: 460–472.
25. Miller, S.C., Bowman, B.M. and Jee, W.S.S. 1995. Available animal models of osteopenia-small and large. *Bone* **17**: 117S–123S.
26. Rudman, D. 1988. Kidney senescence. A model for aging. *Nutrition Rev.* **46**: 209–214.
27. Safadi, M., Shapira, D., Leichter, I., Reznick, A. and Silbermann, M. 1988. Ability of different techniques of measuring bone mass to determine vertebral bone loss in aging female rats. *Calcif. Tissue Int.* **42**: 375–382.
28. Saville, P.D. 1969. Changes in skeltal mass and fragility with castration in the rat: a model of osteoporosis. *J. Am. Geriatr. Soc.* **17**: 155–164.
29. Schapira, D., Lotan-Miller, R., Barzilai, D., and Silbermann, M. 1991. The rat as a model for studies of the aging skeleton. *Cells Mater. (Suppl.)* **1**: 181–188.
30. Schot, L.P. and Schurers, A.H.W. 1990. Patho-physiology of bone loss in castrated animals. *J. Steroid Biochem. Mol. Biol.* **37**: 461–465.
31. Silbermann, M., Safadi, M., Schapira, D., Leichter, I. and Steinberg, R. 1989. Structural and compositional changes in aging bone: osteopenia in lumbar vertebrae of Wistar female rats. *Scanning Microsc.* **3**: 945–952.
32. Sontag, W. 1986. Quantitative measurements of periosteal and cortical-endosteal bone formation and resorption in the midshaft of female rat femur. *Bone* **7**: 55–62.
33. Sontag, W. 1992. Age-dependent morphometric alterations in the distal femora of male and female rats. *Bone* **13**: 297–310.
34. Thompson, D.D., Simmons, H.A., Pirie, C.M. and Ke, H.Z. 1995. FDA guidelines and animal models for osteoporosis. *Bone* **17**: 125S–133S.
35. Wronski, T.J., Schenck, P.A., Cintron, M. and Walsh, C.C. 1987. Effect of body weight on osteopenia in ovariectomized rats. *Calcif. Tissue Int.* **40**: 155–159.