

## KEPADATAN TULANG METACARPAL SIMPANSE USIA 0 SAMPAI 44 TAHUN

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### ABSTRAK

Sifat fisik tulang merupakan indikator yang baik untuk studi pertumbuhan dan penuaan. Tulang adalah jaringan dinamis karena adanya proses modeling dan remodeling. Tulang berubah tidak hanya pada ukuran dan bentuknya, tetapi juga kepadatannya yang disebabkan karena perubahan kandungan mineralnya. Osteoporosis atau kekeroposan tulang merupakan salah satu tanda umum penuaan manusia. Osteoporosis di kalangan anthropoid masih belum diketahui. Dalam penelitian ini kami melakukan pengukuran kepadatan korteks tulang metacarpal simpanse (*Pan troglodytes*) usia 0 sampai 44 tahun berdasarkan radiografi. Pengukuran dilakukan dengan metode mikro-densitometri pada 68 simpanse betina dan 49 simpanse jantan. Kami menemukan bahwa kepadatan tulang meningkat pesat sampai usia sekitar 10 tahun. Pada simpanse jantan kepadatan tulangnya terus meningkat sampai usia 44 tahun, sedangkan pada simpanse betina kepadatan tulangnya menurun mulai usia 20 tahun. Penurunan kepadatan tulang simpanse betina dapat disebabkan karena kalsium tulang digunakan pada masa kehamilan dan menyusui. Namun demikian, simpanse betina diketahui tidak mengalami menopause. Jadi tidak seperti wanita, kejadian osteoporosis pada simpanse betina bukanlah akibat dari menopause. Kemungkinan hal ini berkaitan dengan berkurangnya kadar estrogen pada simpanse lanjut usia.

**Kata Kunci:** osteoporosis, kepadatan tulang, metacarpal, simpanse

### ABSTRACT

The physical properties of bone are good indicators of growth and aging. Bone is a dynamic tissue as its modeling and remodeling change not only the size and shape but also its density due to changes in mineral content. The incidences of bone loss in aging (osteoporosis) among anthropoids major interests for aging study. We assessed the age change of the density of second metacarpal bone in chimpanzees (*Pan troglodytes*) which was measured, by micro-densitometry on radiographs. We found that chimpanzee exhibited an initial increase in metacarpal density with age. It increased rapidly until around 10 years of age. It continued to grow slowly for several years and acquired maturation at around age 16 years. Sexual difference which was greater in males than in females, has been apparent since younger stage. In adulthood, male has a longer plateau than female. During elderly, the bone density of many females continued to decline since age 20 years but males continued its plateau phase or even slight increasing instead. The decline of bone mass in female chimpanzee elderly could not be explained as the result of menopause as female chimpanzees have no clear menopausal stage. It may be caused by the decline in fertility which occurs in parallel with decline in overall aging. This aging effect on fertility may include the diminishing estrogen.

**Keywords:** osteoporosis, bone density, metacarpal, chimpanzee.

### INTRODUCTION

The physical properties of bone are good indicators of growth and aging. Bone characterizes unique life history of human, that is, extended lifespan, presence of adolescent growth and long postmenopausal life, and in turn it relates to quality of life in elderly. As the storage of calcium, bone is a dynamic tissue that always changes itself by apposition and absorption of bone materials throughout life. Bone grows in length and width and yet maintains its general shape. Nevertheless, bone grows in more complicated way than simple elongation or enlargement. It involves modeling and remodeling which change not only the size and shape but also its density due to changes in mineral content. During growth, bone modeling results in diameter enlargement and cortical thickening while remodeling results in bone mineral content accrual to decrease diameter and cortical thickness (Buckwalter *et al.*, 1995; Robling *et al.*, 2006).

Bone mineral content has been determined by many methods including assessment using radiographs. The radiograph of human metacarpal bones is commonly used to provide a general index of skeletal maturity (Ives and Brickley, 2004). The metacarpal is assessed easily using standard anteroposterior radiographs of the hand with low and safe doses of radiation (Tothill, 1989; Ives and Brickley, 2004; Barker *et al.*, 2005). Their anatomical areas are considered as fair representatives of long tubular bones. They give precise information on status of bone resorption and apposition. A method to measure bone density based on radiography is photodensitometry (radiographic absorptiometry). It has been used extensively to estimate bone mass in the total skeleton and anatomical sites of interest (Wahner *et al.*, 1984). The principle of photodensitometry is quantitative measurement of optical density of an object captured on light-sensitive medium due to exposure to light. The greater the density of the object is, the lesser the amount of light is permitted. Under X-ray, the mineralized mass of bone is of greater density than surrounding tissues so it blocks the exposure of the light-sensitive film. Therefore the amount of the light reaching the film after



passing through the bone is a measure of the BMC of the whole bone (Seeman, 2001; Haidekker *et al.*, 2004; Symmons, 2004).

Age-changes in human bone density have been well-documented. It increases with age during infant and juvenile stages until late adolescence and reaches a plateau during adulthood. An adolescent acceleration of increase (spurt) in bone density was found by Martin *et al.* (1997), Bass *et al.* (1999), and Braillon (2003). There are sexual differences in adult bone density which are almost always greater in males than in females. Bone loss (osteoporosis) is most apparent during elderly. Women experience a greater decline in bone mass during aging than men do. It is well-known that menopause had a major effect to the evidence of bone loss in many older women (Schneider *et al.*, 1997; Osei-Hyiaman *et al.*, 1998; Ahlborg *et al.*, 2003). With demographic shifting into older cohort in population pyramid, bone porosity raises medical concern.

Bone loss is a well established characteristic of human aging. It is found in all human populations. Thus, it is often considered to be a universal aspect of human ontogeny, although there are important sex and population differences in the rates at which bone loss occurs. Comparative investigation on age change of bone density in non-human primates is therefore necessary for the evolutionary consideration of growth (ontogeny) and aging and to find suitable model to study bone loss. The majority of reports of age-related bone density changes in nonhuman primates were made on macaques (*Macaca* spp.); DeRousseau, 1985; Pope *et al.*, 1989; Jayo *et al.*, 1994; Champ *et al.*, 1996; Colman *et al.*, 1999a; b; Hotckiss, 1999; Cerroni *et al.*, 2000; Black *et al.*, 2001). Few reports dealt with baboons (*Papio* spp.); Kammerer *et al.*, 1995; Havill *et al.*, 2003; Carlson and Pickering, 2004) and green monkeys (*Chlorocebus* sp.); Hiyaoka *et al.*, 1996). Although chimpanzee (*Pan troglodytes* and *Pan paniscus*) is the closest species to human within extant non-human primates (Perelman *et al.*, 2011), studies on the changes in bone density across the lifespan have not been well described, excepted some reports on the bone density states in adulthood (Sumner *et al.*, 1989; Kikuchi, 2003; Kikuchi *et al.*, 2003) and in elderly (Gunji *et al.*, 2003). The paucity of chimpanzee studies was due to the fact that the population of chimpanzee accessible for the study is small, as they are large animals with long lifespan and growth periods.

The objective of the present study is to delineate the cross-sectional and longitudinal age-related changes in the MCI and cortical density at the mid-shaft of the second metacarpal of chimpanzee aged 0 to 43.6 years using radiographs. Our study comprised a large sample of individuals to cover the long lifespan so that present study provides a new coherent data.

## MATERIALS AND METHODS

A total of 167 radiographs of chimpanzee's proximodistal left hands, collected by Primate Research Institute of Kyoto University, were used for the study. They were taken from 46 females and 35 males aged 0 to 43.62 years old. The chimpanzees belong to the Primate Research Institute of Kyoto University, Japan Monkey Center and the Kumamoto Primates Research Park of the Sanwa Kagaku Kenkyusho Co. Ltd. in Japan. We measured bone density of the second metacarpal cortex from the radiographs. The radiographs were transferred to the digital data before analysis. Bitmap images were captured from the radiographs using a **Fujifilm FinePix F100fd** digital camera. We applied an image analysis system, Scion Image Release Alpha 4.0.3.2 software for Windows (Scion Corporation, 2005) to measure linear dimensions and gray scale value of the pixels. Bone density of second metacarpal cortex was assessed from radiographs by applying photodensitometry. It is expressed as the thickness of an aluminum equivalent (mmAl) showing corresponding X-ray absorption (Seeman, 2001; Haidekker *et al.*, 2004; Symmons, 2004).

All data were presented as an age-change curve. We drew age-change curves for cross-sectional data by applying generalized additive models for location, scale and shape (GAMLSS) (Rigby and Stasinopoulos, 2005) with 9 levels of percentiles (3%, 5%, 10%, 25%, 50%, 75%, 90%, 95%, and 97%). Age-changes in cortical density were analyzed in the different life stages by the application of ANOVA model and followed by the use of Tukey HSD (honest significant difference) *post hoc* test for multiple comparisons. All statistical procedures were performed using the R software version 2.9.1 (R Development Core Team, 2009).



## RESULT

We defined the bone age stage into five phases for tracing the change in bone density. It is a combination of life stages reported by Hamada *et al.* (1996) for captives and Sumner *et al.* (1989) for wild chimpanzees. Hamada *et al.* (1996) defined the growth period of captive chimpanzee into four phases from the viewpoint of somatic growth. They are infancy (from birth to 1 year of age), juvenile (from 1 to 6 years of age in females or from 1 to 7 years of age in males), adolescence (from 6 to 8 years of age in females or from 7 to 9 year of age in males), and the late growth phase (from 8 to 13 years of age in females or from 9 to 15 years of age in males). Sumner *et al.* (1989) classified wild chimpanzee into five life stages: infancy (from birth to 5 years of age), childhood (from 5 to 7 years of age), adolescence (from 8 to 15 years of age), maturity (from 16 to 33 years of age), and old age (>33 years of age). Phases of Hamada *et al.* covered age 0 to 15 years only while our data extended into >40 years of age. We therefore incorporated it to the Sumner's *et al.* phases although life stages of the wild chimpanzee were rather different to that of captive. Growth stage of wild chimpanzee is one to four years longer in each stage.

We examined mineral density age-change of the second metacarpal cortex. A comparison between female and male is shown in Figure 1, in which data on cortical density was plotted against age. It can be seen there is a significant sex difference in cortical density ( $p < 0.05$ , ANOVA), that female had a lower cortical density. Cortical density increased steadily for first 9 years of life, from about 7 mmAl/cm into 10.5 mmAl/cm in female and from about 9 mmAl/cm into 11.5 mmAl/cm in male. Increment of the female cortical density therefore was higher during these early stages than that of male. However, because of wide variation among individuals, there were not significant increases ( $p > 0.05$ , Tukey HSD) from infancy to juvenile and from adolescence to adult in both sexes. At about age 13 years, female attained full maturation of bone density, while male did approximately at the age of 12 years. The density at maturation does not differ between sexes, ca. 12mmAl/cm. Female had a short plateau and decreased soon since 21 years of age through elderly, although the decrease was statistically insignificant ( $p > 0.05$ , Tukey HSD). In contrast, male had a longer plateau stage during adulthood. Cortical density consistently grew with low rates instead through elderly phase in males, even though their increments were also insignificant ( $p > 0.05$ , Tukey HSD). The variation among individuals was very wide in both sexes e.g., 19 years in females, the density ranged from 7 to 15 mmAl (about 2 times), and in males, ranges from 8.5 to 17 mmAl (about 2 times).

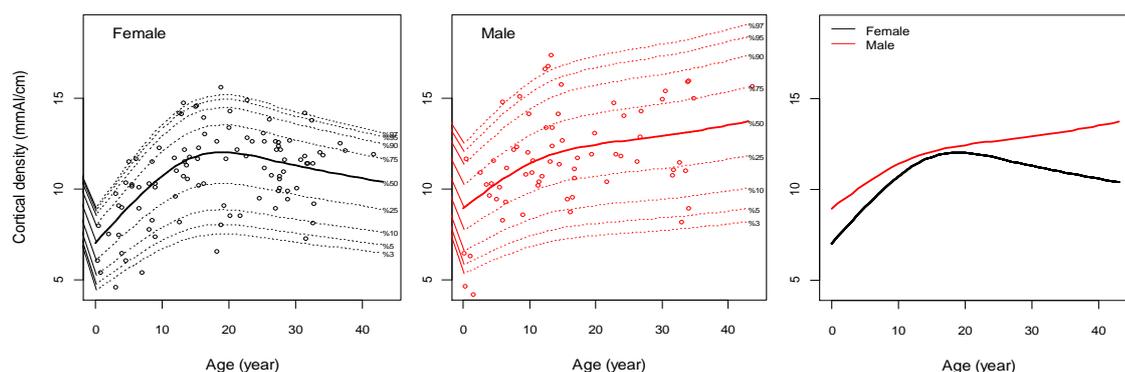


Figure 1 Curves of age-change of the cortical density in the second metacarpal in female (left) and male (center) chimpanzees from the cross-sectional study. Solid line for 50<sup>th</sup> percentile and dotted-lines for 3<sup>rd</sup>, 5<sup>th</sup>, 10<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup>, 90<sup>th</sup>, 95<sup>th</sup>, and 97<sup>th</sup> percentiles (bottom to top). Combined of both sexes curves (right) were derived from 50<sup>th</sup> percentile.

## DISCUSSION

The size, shape and density of bone are good indicators of both growth and aging, not only intraspecific but also interspecific assessment, e.g., skeletal maturation. According to the calcium demands, modeling and remodeling processes occur in bone in humans, which are shared by non-human primates, but not by such experimental animals as rats. Representing the growth and aging processes, the state of bone would be beneficial to study the evolution of unique life-history of humans, which is characterized by the long life-span, long growth period with adolescent growth spurt (acceleration), and long postmenopausal life. Within extant nonhuman primates the chimpanzee (*Pan troglodytes*) is the closest species to humans (Perelman *et al.*, 2011). Knowledge of growth and aging of bone, especially the bone mineral density, in



chimpanzee at present is scarce because its measurement is difficult and the population accessible for study is small, as they are large animals with long lifespan and growth periods.

We delineated a general pattern of age change in cortical density of the second metacarpal bone in chimpanzees. Cortical density estimates absolute bone mass in the cortical sites (Wahner *et al.*, 1984). Based on cross-sectional analysis, chimpanzee exhibited an initial increase in bone density with age. It increased for a longer period, until around 10 years of age. Sexual difference in bone density, which was greater in males than in females, has been apparent since the early ages. It is earlier than the onset of sexual difference on body length and weight, both of which were reported approximately at the age of 8 years (Leigh, 1992; Hamada and Udono, 2002). Bone density continued to increase slowly for several years and acquired maturation at around age 16 years. Maturation age in bone density was much later than reproductive maturity which is observed at the age of 7 years in females (menarche) and 8 years in males (development of testicular size; Hamada and Udono, 2002). Bone density decreases in females from about 20 years of age, though not in males. We found, thus, that the pattern of change in density of cortical bone of chimpanzee's second metacarpal was quite similar to the human growth pattern. Nevertheless there were differences due to difference in age of each stage which reflects the life span distinction between chimpanzee and human (Bogin and Smith, 1996; Wallis, 1997, Thompson *et al.*, 2007).

Bone aging in humans is characterized by gradual decrease in adulthood and rapid loss in postmenopausal women, which is another point of interest of age change pattern of bone density in chimpanzees. In adulthood through elderly, male chimpanzees show a longer plateau than female chimpanzee, that is, bone density is maintained. Some female chimpanzees experienced bone loss since approximately age 20 years, as it was reported by Sumner *et al.* (1989) and Gunji *et al.* (2003). However, in both of female and male age-related BMC changes during adult through elderly were statistically insignificant. Individual variation is rather wide, especially in cortical density. Despite these, the sequential decline of bone mass in female may be said to be genuine.

Some evidence supports the aging of bone in female chimpanzees, for instance Gunji *et al.* (2003) reported extraordinary low bone density in wild elderly (estimated about 40 years at death) female chimpanzee. The low bone mineral content in adult female might be caused by the demands of pregnancy and lactation (Sumner *et al.* 1989). Female chimpanzees have high fertility rate during age 15 years until 30 years (Thompson *et al.*, 2007). Several studies in human reported that maternal bone acts as a calcium storage depot for the fetal and neonatal mineral nutrient (Drinkwater and Chesnut, 1991; Sowers *et al.*, 1993; More *et al.*, 2001). Adult bone loss due to pregnancy and lactation was also reported in rhesus macaques (deRousseau, 1985), green monkeys (Hiyaoka *et al.*, 1996) and cynomolgus macaques (Lees *et al.*, 1998). These phenomena might also be true for chimpanzee. So we may conclude that female chimpanzees experience sequential decline in bone mass as it is observed in human.

For our study, estrogen deficiency cannot be dismissed out of hand as a factor causing the loss of bone density since diminished ovarian estrogen levels has been observed in old captive chimpanzees, after the age of 40 years (Gould *et al.*, 1981; Videan *et al.*, 2006). In human, a normal level of estrogen inhibits bone resorption by directly inducing apoptosis of the bone-resorbing osteoclasts (Kameda *et al.*, 1997; Frost, 1999; Riggs *et al.*, 2002). Nevertheless the low bone density in elderly female chimpanzee could not be explained by the postmenopausal hormonal changes as it had been thought to occur in human. Menopause is not a typical characteristic of chimpanzee life histories (Thompson *et al.*, 2007; Lacreuse *et al.*, 2008). According to the study on wild chimpanzee, females continue to reproduce in their life-span although the inter-birth interval is rather long in elderly. On the other hand their survival rate is constantly decreasing from birth. Therefore their fertility decline occurs in parallel with declines in overall aging (Thompson *et al.*, 2007). This aging effect on fertility decline may include the diminishing of estrogen which might be responsible for the decline of bone mass.

## CONCLUSION

Chimpanzee exhibited an increase of bone density until adulthood. It increased until around 10 years of age. Sexual difference, which was greater in males than in females, has been apparent since the early ages. Bone density continued to increase more slowly for several years and acquired maturation at around age 16 years. In adulthood, male has a longer plateau phase than female. Some female chimpanzees



experienced bone loss approximately since age 20 years. During aging, the bone density of many females declined but male's continued its plateau phase or increase instead. Despite there was a statistical insignificant in age-related changes in aging, the decline of bone mass in female may be said to be genuine.

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## DISKUSI –

