Age-related changes in bone morphometry, densitometry and osteoarthritis in macaques

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Ph.D. Thesis

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Abstract

Evaluation of the age-related skeletal changes in nonhuman primates is important for understanding the life history and mechanism of skeletal changes, thus will contribute to improve the understanding of the human evolution. I studied age-related changes in Japanese (*Macaca fuscata*) and cynomolgus macaques (*M. fascicularis*), in terms of morphometry, bone mass and osteoarthritis (OA). Using both dried skeletons and living subjects, I studied lumbar vertebrae and radius in the two species of macaques living with different living conditions. Effects of factors, that is, sex, body mass (BM), physical activity and reproductive aging on bone mineral density (BMD), bone mineral content (BMC); and for OA, disc space narrowing (DSN) and osteophytosis (OST) were examined. In Chapter 1, I examined age-related changes in odd-numbered lumbar vertebrae in both sexes of *M. fuscata*. The most common age-related pattern of morphometric changes was an increase during young adulthood until reaching the peak, and then, a decrease with age. Most of the peaks were in the age group of 15–20 years and 10–15 years in females and males, respectively. In both sexes, the age-related decrease in the vertebral body depth was greater than those in height and width. The ventro-dorsal height (VBHv/VBHd) ratio continuously decreased with age. The trabecular BMD (TbBMD) continuously decreased after young adulthood. OST clearly increased with age in both sexes. Osteometry, TbBMD and OST severity appeared moderately correlated with each other in all vertebrae. In Chapter 2, using radiographic assessment, I compared DSN and OST prevalence and severity between *M. fuscata* reared in individual cages (captive condition) with higher BM and those in free-ranging (natural condition) with lower BM. The free-ranging *M. fuscata* had significantly lower prevalence and severity of DSN and OST than captive ones, comparable to those in humans. BM and physical activity were considered as factors influencing the OA. In Chapter 3, I investigated age-related and reproductive aging-related bone changes, and relationship between bone mass and OA at different skeletal sites in female *M. fuscata* and *M. fascicularis*, which differ in BM and lifestyle. They had similar changes in bone mass and OA with age and reproductive aging. *M. fuscata* had greater bone mass, except cortical BMD, than *M. fascicularis*, representing the influence of BM. The two species showed both bone loss and OA development with age, and relationships between bone mass and DSN were observed in the two species. They did not show any acceleration in bone loss and OA development with estrogen depletion, which is found in women. The findings reveal that macaques showed similar age-related bone changes in morphometry, bone mass and OA to those in humans. Certain differences of bone changes between them observed were that macaques have dramatically higher prevalence and severity of OA than humans, which is caused by the smaller amount of physical activity in captivity, and free-ranging macaques showed OA comparable to humans; and the females of the two macaque species lack significantly greater bone loss with estrogen deficiency which is normally found in elderly or postmenopausal women, meaning that skeletally healthy macaques would live long in postmenopausal life or *vice versa*.

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General Introduction

Aging is a deterioration process which includes increased susceptibility to disease, adverse changes in physiology, and the loss of mobility and agility including decrease in reproductive ability (Goldsmith 2010). Reproductive aging, such as increase of interbirth intervals, decline of fertility (Caro et al. 1995) or cessation of ovulation or menopause (Walker and Herndon 2008), relates to evolution of longevity of postreproductive life in human females but not in other primates (Hawkes et al. 1997). However, nowadays nonhuman primates kept in captivity tend to have longer maximum life span than those in wild, so their postmenopausal life spans are also extend. For instance, average life span of macaques is about 25 years of age and the onset menopause starts few years before death; however, the maximum life span of macaques in captivity was reported around 40 years of age, that is, their postreproductive life is approximately 40 % of their maximum life span (Walker and Herndon 2008). The increasing longevity is unavoidably related to various age-related disorders such as bone loss or degenerative joint disorder. Changes in bones are considered to be representative of physical aging because they show age-related changes with smaller inter-individual variation than other organs universally found in nonhuman primates. Bones are crucial mechanical components of the musculoskeletal system that they act as the rigid levers for muscle operating to produce movement (White et al. 2012). Since adverse bone changes decrease the locomotive ability and increase the fracture risk, they are a determinant of the quality of life.

Osteoporosis (OP) and osteoarthritis (OA) are two common age-related bone disorders affecting older people worldwide (Stewart and Black 2000). OP is the state of low bone mass, evaluated by bone mineral density (BMD) and bone mineral content (BMC), represented by deterioration of microarchitecture of bone tissue, and finally causes fragility and higher fracture risk (Office of the Surgeon General (US) 2004). OP is a silent disorder until it is emerged by fractures, especially at the proximal femur, distal radius and vertebra (National Osteoporosis Foundation 2014). Osteoporotic fractures contribute to pain, limited physical ability and death. OP normally occurs in elderly men and women (Cerroni et al. 2000). There are several risk factors of OP such as age, menopause, physical activity and body mass (BM) (National Osteoporosis Foundation 2014).

OA is a joint disorder affecting articular cartilage, synovial membrane and subchondral bone (Tanamas et al. 2011), which is observed using radiographs, represented by

joint space narrowing, bone spur or osteophyte, and endplate sclerosis (Lane et al. 1993). The most common joints suffered from OA are the weight-bearing joints such as knee, hand, hip and vertebra (Rogers et al. 2004). OA has been found in prehistoric humans (Bridges 1994) and modern populations (Murphy and Helmick 2012). OA produces pain, stiffness and reduces movement of the affected joints, which limits quality of life in elders (Centers for Disease Control and Prevention and Arthritis Foundation 2010). OA is caused by genetic and systemic factors including mechanical stresses; however, the high risk factors of prevalence and severity of OA are aging, high BM, excessive stress and sedentary lifestyles (Centers for Disease Control and Prevention and Arthritis Foundation 2010).

Bone loss and OA development with advancing age have been observed in all human populations. Although age-related bone loss and OA development occur both in men and women, bone loss apparently begins earlier in women and proceeds more rapidly in postmenopausal women, while the development of OA tends to have higher prevalence and severity in men than in women below 45 years of age, after that it is more common in women (Williams and Spector 2006).

Trabecular bone has large surface area responding to biomechanical or physical stimuli (Parkinson and Fazzalari 2012), thus its mass changes with age or with estrogen depletion have been found at the proximal femur, distal radius and vertebral body (Cerroni et al. 2000). In the present study, I concentrated on changes at the distal radius and lumbar vertebral region. The distal radius is the focused site of several studies because it is the most common site of fracture in human arms (Jupiter 1991), and it is easy to be measured bone mass by noninvasive method such as peripheral quantitative computed tomography (pQCT). Age-related and reproductive aging-related bone loss is commonly observed at the distal radius; however, naturally occurring OA is not found at this site. In contrast the lumbar vertebra, particularly the vertebral body, is a clinically focused subject because it supports the body weight and absorbs shock (Ankel-Simons 2007), and suffers from OA. Changes in the morphometry, bone mass and OA are three major aspects of age-related changes in vertebrae.

Vertebral morphometric changes have been studied and used to establish a reference database for clinical and epidemiological evaluations of vertebral pathology in humans (Masharawi and Salame 2011). Kyphosis is a common final outcome of vertebral aging, partly caused by the decrease in overall vertebral body height or the relative ventral height. Various studies on the human lumbar vertebral body have found that the height of the lumbar vertebral body and the ventral height relative to the dorsal one decreased with age (Ericksen 1976, 1978a, b; Evans et al. 1993; Hermann et al. 1993; Diacinti et al. 1995: but see Hedlund

and Gallagher 1988; Minne et al. 1988). However, age-related changes in both body width and depth of vertebral body have not been thoroughly studied, but in aged female samples (Evan et al. 1993) or in few lumbar vertebrae (Rühli et al. 2005).

There are two popular non-invasive techniques to measure bone mass: dual energy X-ray absorptiometry (DEXA) and pQCT. Each of these has advantages and disadvantages. Although DEXA is often used to clinically measured bone mass, which is represented as standard, as it is based on the analysis of the projected image of a bone, it has difficulty in determining real volumetric density independent from bone size and shape (Rauch and Schonau 2005). On the contrary, pQCT can measure volumetric density independently from bone size and shape, and examine structure of the bone (Gatti et al. 1996; Neu et al. 2001; Rauch and Schonau 2005) though not applicable to lumbar vertebral region. Thus, the present study used pQCT to assess bone mass, that is, BMD and BMC.

Bone mass is the main parameter used for assessing bone loss (Riggs et al. 2008). Age-related changes in bone mass occur in two phases in adult humans: an increase to a peak in young adulthood and a subsequent decrease with advancing age (Colman and Binkley 2002). Nevertheless, definite age at which the maximal bone mass is attained has not been determined (Colman and Binkley 2002; Riggs et al. 2004) because it is influenced by several factors, such as lifestyle, genetics and nutrition (Yu et al. 1999). Cortical bone loss starts in the middle stage of life and is accelerated by estrogen deficiency (Colman and Binkley 2002; Riggs et al. 2004), while views differ regarding whether trabecular bone loss starts before (Riggs et al. 2004, 2008) or after (Genant et al. 1982) menopause.

In humans, the weight of the upper body acts on the lumbar region (Alini et al. 2008); therefore, this region is ideal for study OA which is found in load-bearing joints in human body. However, because of the differences in subject populations including confounding factors, and in the methods used to evaluate lumbar vertebral OA, the lumbar vertebral OA prevalence and severity with respect to age and sex (e.g. Duncan et al. 2012) are still not well understood.

Even though OP and OA are common disorders in the elders, several findings demonstrated that the two disorders are rarely found together (Dequeker et al. 1993). Although their inverse relationship is generally considered (Dai 1998; Roux et al. 2008), as the two disorders may result from different mechanisms that they may be independent with each other; and their relation is still in controversy (Sambrook and Naganathan 1997; Miyakoshi et al. 2003). Previous studies conducted on humans found correlation between

BMD at several sites of skeleton and OA (e.g. Ichchou et al. 2010), in contrast others found no association between BMD and OA (e.g. Muraki et al. 2004).

Studies on age-related bone disorders in humans are limited because of issues related to legality, ethics and practice (Colman and Binkley 2002). Moreover, to study etiology or progression of age-related bone disorders, particularly OP and OA, on humans is difficult because of several factors related with OP and OA such as BM, physical activity or lifestyle (Turner 2001; Little and Smith 2008). *In vivo* studies especially using animal models provide an excellent chance compared with *in vitro* studies. Although the latter provides a simple and basic model including defining specific mechanisms, there is the complex relationship between cells surrounded by large extracellular matrix and interaction of systemic and local factors from *in vivo* which is much different from *in vitro* (Alini et al. 2008; Little and Smith 2008). Using animal models, for example, progression of the disorders can be frequently monitored or confounding factors can be controlled (Pritzker 1994). Thus, to be a model of age-related bone disorders, the animals have to share important aspects with humans and also should provide knowledge about the mechanisms related to development of the disorders.

Nonhuman primates are widely used in bone research because they share similarities in reproductive functions and skeletal physiology with humans compared with other mammals. For instance, the antero-posterior diameter of vertebral body increases from cervical to lumbar region in humans and monkeys (Aiello and Dean 2002) but this dimensional change was not observed in large quadrupedal mammals (Wilke et al. 1997) which may pose differently load-bearing force to vertebrae. Moreover, increase in bone loss due to estrogen depletion (e.g. Hartke 1998) in the elderly is observed in humans and nonhuman primates. In humans, bone mass is known to attain its peak in young adulthood, then it decreases with both age and sex steroid hormone concentrations, especially estrogen. Similar age-related changes and disorders in the bone such as loss of bone mass (Black et al. 2001), decrease in stature (Roth et al. 2004) and increase in prevalence and severity of OA (Little and Smith 2008), are also found in nonhuman primates, particularly old world monkeys (Chen et al. 2000), which are second only to apes in genetic proximity to humans. Therefore, they are considered as suitable models for studies related to aging and maintenance of skeletal structure (Havill et al. 2003).

Macaques (genus *Macaca*) are considered as useful models. They have the widest geographical distribution (Napier and Napier 1985; Fleagle 1998) and variations among macaque species would be valuable for studies on the evolution of human aging. Macaque model offers several advantages against other animal models. Macaques share much genetic

similarity to humans which is about 95 % of genetic coding sequence identity to humans (Magness et al. 2005) and they are generally comparable to human biological system (Shively and Clarkson 2009). They have large size which is enough to be noninvasively studied such as pQCT or radiographic assessment. They are long-lived and they age at a rate of approximately 2.5 to 3.5 times faster than that human age (King et al. 1988; Tigges et al. 1988; Duncan et al. 2012). Macaques show bone loss with age (Black and Lane 2002) and they experience accelerated bone loss due to estrogen depletion from both naturally occurring (in rhesus macaques (*Macaca mulatta*), Champ et al. 1996; Colman et al. 1999a) or ovariectomy (in cynomolgus macaques (*M. fascicularis*), Jerome et al. 1995; Jerome et al. 1997), or gradual bone loss with advancing age in male rhesus macaques (Colman et al. 1999b) as those in humans. Although macaques are in general a quadruped, they frequently use upright sitting posture (Kramer et al. 2002) which is considered relating with the development of vertebral OA as in humans (Hadjipavlou et al. 1999) and they develop natural vertebral OA with advancing age (e.g. Duncan et al. 2012).

Macaques in particular from captive conditions show much higher prevalence and severity of vertebral OA than those of humans (Kramer et al. 2002; Duncan et al. 2012). Excess BM or obesity has been observed in individual-caged macaques (e.g. Hansen and Bodkin 1986) which may result from lacking of physical activity and overeating. In humans, high BM or high body mass index (BMI) and sedentary lifestyles are considerably correlated with OA (Centers for Disease Control and Prevention and Arthritis Foundation 2010). Therefore, low BM and optimal physical stress may be important to preserve of normal joint condition.

Japanese macaques (*M. fuscata*) and cynomolgus macaques are closely related with each other (Fooden 1976), though they showed various species specific characteristics mainly related to BM, which may influence on their bone mass and OA. Cynomolgus macaques have less than a half of the adult BM of Japanese macaques (Black and Lane 2002). The two macaque species also differ in lifestyle, that is, arboreal in cynomolgus macaques and terrestrial in Japanese macaques (Kikuchi 2004).

Although macaques experience bone loss (e.g. Cerroni et al. 2000) and an significantly increasing severity of OA (e.g. Duncan et al. 2012) with advancing age, there are neither studies on the relationship between the age-related or reproductive aging-related changes in the morphometry, densitometry and OA observation nor effects of BM and physical activity to similar species of macaques, especially between free-ranging and captive

individuals or to different species which are closely related with each other such as Japanese and cynomolgus macaques.

The present study aimed to examine more details of risk factors relating to bone conditions which can be controlled in macaques but not in human study, and age-related and reproductive aging-related bone changes in macaques compared with humans.

The aim of the first chapter was to examine the age-related changes in the lumbar vertebrae of Japanese macaques, in terms of morphometry, BMDs and osteophytosis (OST), and interrelationship between the three aspects. The sex differences in the age-related changes and the differences/similarities between humans and macaque species were discussed.

In the second chapter, I compared prevalence and severity of lumbar vertebral OA between Japanese macaques reared in individual cages (captive condition) and living in free-ranging (natural condition) using radiographic data, which are evaluated through disc space narrowing (DSN) and OST. Differences of BM and physical activity may affect prevalence and severity of OA between macaques from the two different living conditions.

Finally, I investigated age-related and reproductive aging-related bone changes in terms of bone mass (BMDs and BMCs), OA (DSN and OST) and body size (BM and BMI), and relation between radial bone mass and lumbar vertebral OA in female Japanese and cynomolgus macaques, and compared these parameters with humans and other macaque species.

Chapter 1

Age-related changes in osteometry, bone mineral density and osteophytosis of the lumbar vertebrae in Japanese macaques

Introduction

Aging is a deterioration process in animals, including humans, which includes increased susceptibility to disease, adverse changes in physiology, and the loss of mobility and agility (Goldsmith 2010). Changes in bones are considered to be representative of physical aging because they show age-related changes with smaller inter-individual variation than other organs. Since adverse bone changes decrease the locomotive ability and increase the fracture risk, they are a determinant of the quality of life.

The lumbar vertebra, particularly the vertebral body, is a clinically focused subject because its main purpose is to support the body weight and to absorb shock (Ankel-Simons 2007). Changes in the morphometry, bone mineral density (BMD), and osteophytosis (OST), defined by the presence of osteophytes around a marginal body, are three major aspects of age-related changes in vertebrae. Vertebral morphometric changes have been studied and used to establish a reference database for clinical and epidemiological evaluations of vertebral pathology in humans (Masharawi and Salame 2011). Kyphosis is a common final outcome of vertebral aging, partly caused by the decrease in overall body height or the relative ventral height, e.g., vertebral wedging. Several studies on the human lumbar vertebral body have shown that the height of the lumbar vertebral body and the ventral height decrease relative to dorsal one with age (Ericksen 1976, 1978a, b; Evans et al. 1993; Hermann et al. 1993; Diacinti et al. 1995: but see Hedlund and Gallagher 1988; Minne et al. 1988). However, age-related changes in both body width and depth of vertebral body lack thorough studies, but on aged female samples (Evan et al. 1993) or on few lumbar vertebrae (Rühli et al. 2005).

The BMD or bone mass is the main parameter used for assessing bone loss (Riggs et al. 2008). Age-related changes in the BMD occur in two phases in adult humans: an increase to a peak in young adulthood and a subsequent decrease with advancing age (Colman and Binkley 2002). Nevertheless, the age at which the maximal BMD is obtained has not been definitely determined (Colman and Binkley 2002; Riggs et al. 2004) because it is influenced by several factors, such as lifestyle, genetics and nutrition (Yu et al. 1999). Cortical bone loss starts in the middle stage of life and is accelerated by estrogen deficiency (Colman and

Binkley 2002; Riggs et al. 2004), whereas views differ regarding whether trabecular bone loss starts before (Riggs et al. 2004, 2008) or after (Genant et al. 1982) menopause.

Osteophytes are bony outgrowths of varying size and shape that arise around the margins of a joint. They are very common in any skeletal human population and their appearance considerably increases with age (Rogers and Waldron 1995). Although OST starts relatively later in life in macaques than in humans, it progresses more rapidly in macaques than humans (Duncan et al. 2012). However, because of the differences in subject populations and in the methods used to evaluate OST, the OST severity with respect to age (Beresford 1952; Duncan et al. 2012) and sex (Nathan 1962; Cvijetić et al. 2000; Duncan et al. 2012) are still not well understood.

Although age-related bone changes have been studied in humans (e.g. Mosekilde 2000), such studies are limited since human experiments are restricted because of issues related to legality, ethics and practice (Colman and Binkley 2002). Accordingly, other mammals and especially nonhuman primates have been used as surrogate models (Alini et al. 2008). Macaques (genus *Macaca*) are considered to be a good model for humans because they age at a rate of approximately 2.5 to 3.5 times faster than humans (King et al. 1988; Tigges et al. 1988; Duncan et al. 2012) and show similar age-related bone loss and natural development of OST as those in humans (Black and Lane 2002).

Macaques show vertebral bone loss (e.g. Cerroni et al. 2000) and an increasing severity of OST (e.g. Duncan et al. 2012) with advancing age. However, there are no studies on either the age-related changes in the vertebral morphometry in any macaque species or on the relationship between the age-related changes in the morphometry, densitometry and OST observation.

The aim of the present study is to examine the age-related changes in the lumbar vertebrae of Japanese macaques (*Macaca fuscata*), in terms of morphometry, BMD and OST, and interrelationship between the three aspects. Radiographic studies have examined the qualitative age-related bone changes in this species (Hamada and Yamamoto 2010), but previous studies have not examined quantitative age-related changes in bone in this species. The interrelationship between these three aspects was, therefore, examined in this study. The sex differences in the age-related changes and the differences/similarities between humans and macaque species are discussed.

Materials and Methods

Materials

I examined the first, third, fifth and seventh lumbar vertebrae (L1, L3, L5 and L7) (out of seven lumbar vertebrae) of 77 Japanese macaque skeletons (40 females, 37 males) housed at the Primate Research Institute, Kyoto University, Japan. As the majority of macaques have seven lumbar vertebrae (Schultz 1961), only individuals with all seven lumbar vertebrae were selected in this study. Specimens with any lumbar vertebrae fused together by the development of OST were not included in the analysis. All subjects were reared in corral cages (20 × 40 m) and were fed monkey chow and sweet potatoes. They were descendants of the animals that originated from Arashiyama, Takahama and Wakasa areas of Japan. They died when they were healthy and were without the effect of disorders in their bones. The exact ages at death were known from records (Matsubayashi et al. 2004), and are expressed as a decimal fraction of years in age. The subjects were divided into the four age groups: 7.0–9.9 years (14 females, 18 males), 10.0–14.9 years (12 females, 10 males), 15.0–19.9 years (6 females, 5 males), and 20.0 years or more (8 females, 4 males).

Vertebral body morphometry

Six parameters were measured on each vertebral body using a digital sliding caliper (Mitutoyo Absolute Coolant Proof IP67, Japan) to 0.01 mm: Height (cranio-caudal dimension measured on the midline of dorsal and ventral epiphyseal margin – VBHd and VBHv); Depth (ventro-dorsal dimension measured on the median plane of the epiphyseal margin on the cranial and caudal surfaces – VBDcra and VBDcau); and Width (maximum transverse diameter measured on the margins of the epiphyseal plates on the cranial and caudal surfaces – VBWcra and VBWcau) (Figure 1.1). Each parameter was measured three times and the average was used for analysis. Next, each value was standardized with respect to the body size surrogate that is the diameter of the left femoral head (average of the vertical and horizontal diameters).

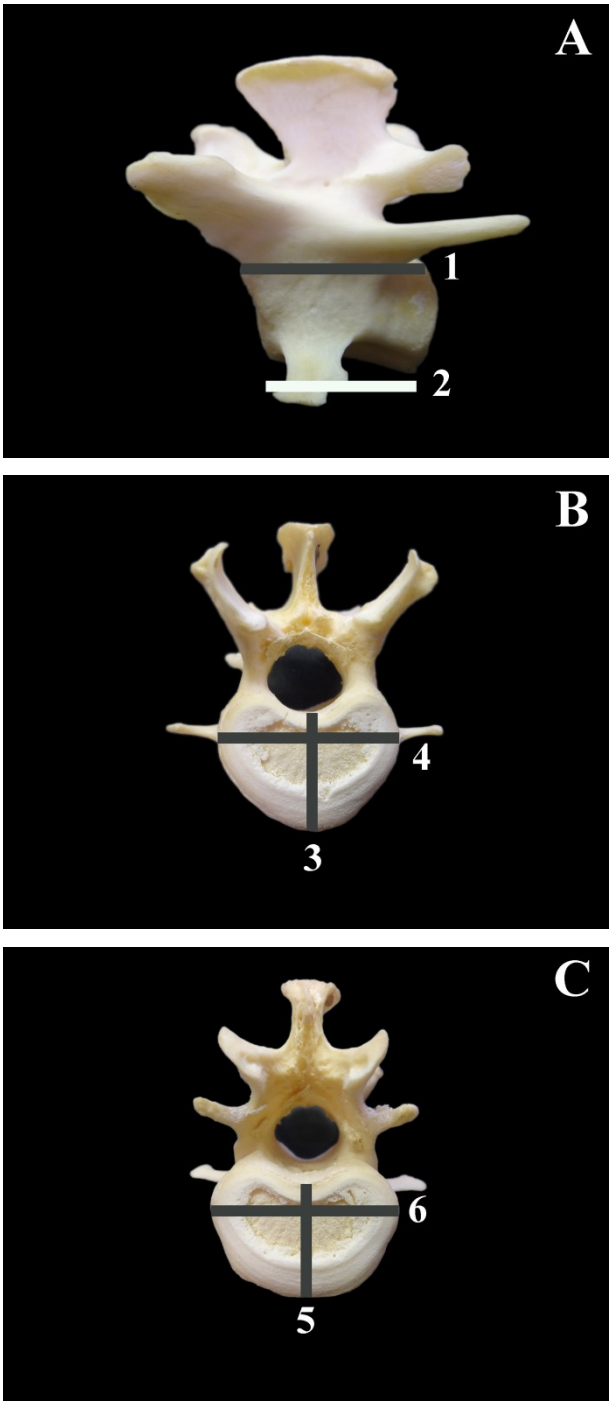

Figure 1.1 Illustrations and measurements of all morphometry in a lumbar vertebral body. **A**: (**1**) dorsal vertebral body height (VBHd); (**2**) ventral vertebral body height (VBHv). **B**: (**3**) cranial vertebral body depth (VBDcra); (**4**) cranial vertebral body width (VBWcra). **C**: (**5**) caudal vertebral body depth (VBDcau); (**6**) caudal vertebral body width (VBWcau)

Vertebral body mineral density

Peripheral quantitative computed tomography (pQCT) (XCT Research SA+; Norland Stratec Inc.) was used to examine the vertebral BMD. The caudal side of the vertebral body was fixed horizontally on a flat and sticky plate so that the scan plane became parallel to the sagittal plane. After the scout scan, the position of the middle of the vertebral body (the mid-sagittal section) was defined and then the CT scan was performed. Scan setting was as follows: tube voltage/current = 50.4 kV, 0.281 mA; collimation B, 0.25 x 0.9 mm for small animals and excised bones, 0.5 mm slice thickness, 1 block (160 projections), 0.1 mm voxel size and a 2 mm/s scan speed. The BMD at the manually defined region of interest was measured with the following parameters. A threshold of 160 mg/cm^3 at contour mode 1 was used to separate the bone areas from air. An inner threshold of 600 mg/cm^3 was set to separate the cortical areas from the trabecular areas, followed by an additional 5.0 % contraction of the endosteal borders to eliminate any possible cortical parts (peel mode 4 with concentric peel 5 %). Volumetric total and trabecular BMDs (vBMD and TbBMD, respectively) were obtained. Cortical BMD cannot be determined by pQCT in the thin-cortex structures of vertebrae because of partial volume artifacts (Augat et al. 1998).

Vertebral body osteophytosis

The presence and severity of osteophytes was graded using a score of zero to five based on observations in both the cranial and caudal vertebral body rims. The scoring system used in the present study was modified from Beresford (1952), DeRousseau (1988), and Agarwal (2001) to include a larger number of scales allowing characterization of subtle detail in OST in Japanese macaques. Grades zero to five were divided into eight scales (Table 1.1). The scores obtained from the cranial and caudal sites were averaged. Examples of grade 0 and 4 OST are shown (Figure 1.2).

Table 1.1 Osteophytosis (OST) scoring

Grade	Osteophytosis development
0	No osteophyte
1	Isolated bone spur
2	Osteophytes continue around a marginal body but not prominence. A pitting or cracking of the articular surface may be present. Small pits can be noticed at the base of a bone spur.
2.5	Osteophytes continue around a marginal body prominently. Bone can be seen deposited on a body. A pitting or cracking of the articular surface can be observed.
3	Osteophytes discontinue. Wedged shaped vertebra can be seen. A ventro-lateral bone may form. Bone deposits on a body. Centrum begins as a splayed-out shape. Pitting or cracking of the articular surface can be observed.
3.5	Claw osteophytes can be observed. Osteophytes curve to the side of an intervertebral disc. Wedged shaped vertebra appear. Ventro-lateral bone may form. Bone deposits on a body. Centrum develops as a splayed-out shape. Pitting or cracking of the articular surface can be observed.
4	Claw osteophytes are prominent. Wedged shaped vertebra appear. Ventro-lateral bone form severely. Centrum fully developed in a splayed-out shape. Pitting at the centrum is prevalent.
5	Fusion of osteophyte

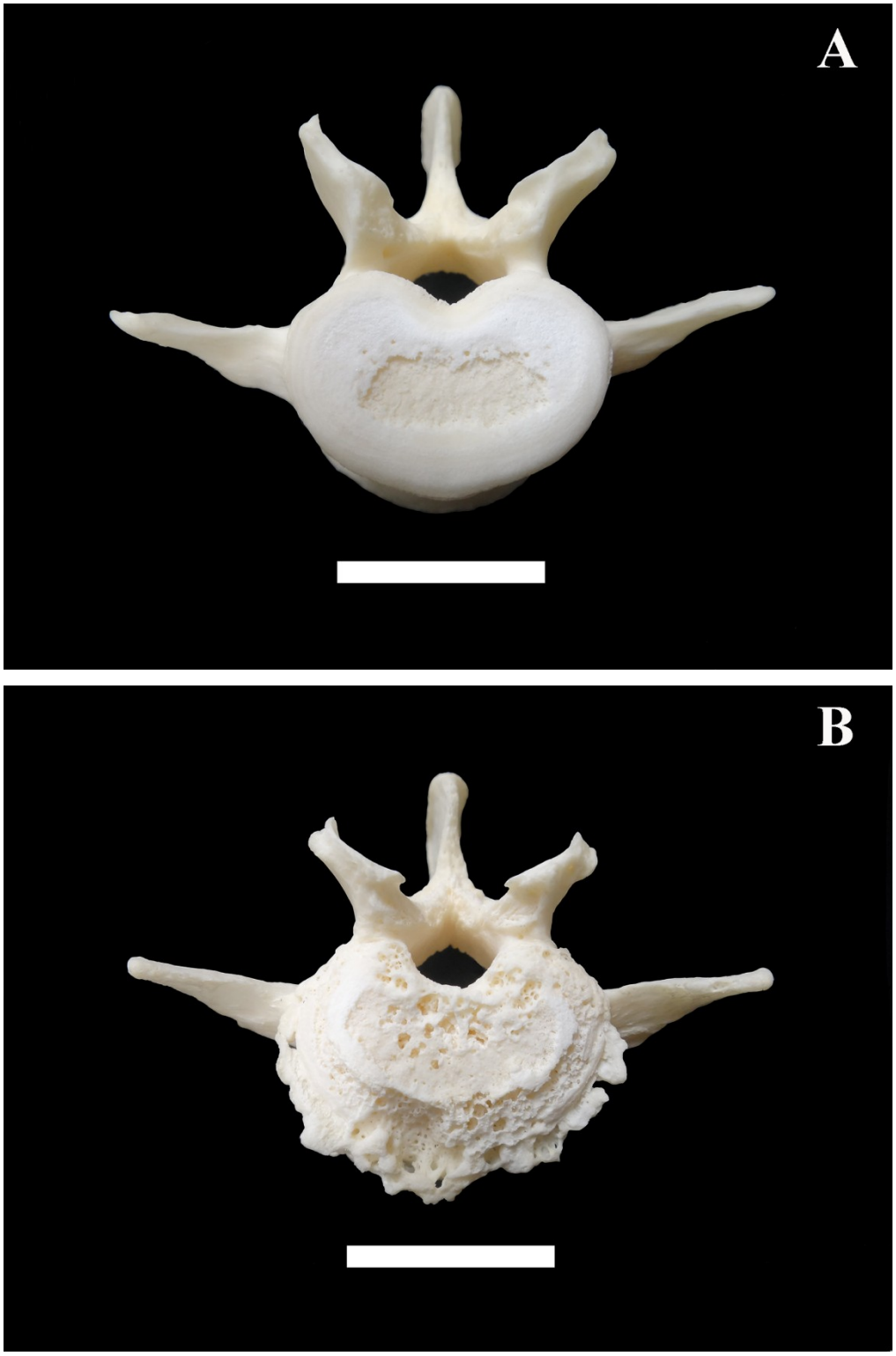

Figure 1.2 Vertebral osteophytosis: (**A**) grade 0 (11.1 years) and (**B**) grade 4 (30.2 years). Scale bar: 2 cm

Statistical analysis

Since the OST was found to clearly interfere with the BMD evaluation by increasing the values (Agarwal 2001; present study). Therefore, the vertebral BMD was analyzed by excluding six subjects with severe OST, that is, five females of 23.3–30.3 years of age and one male of 27.1 years of age. All of these subjects had OST scores greater than 3.5, except for one female that had scores of three or more in all vertebrae.

All statistical analyses were performed using SPSS (windows XP, version 16.0) or Excel (Microsoft Co. Ltd., version 2007). Both parametric and nonparametric tests were applied, depending on the normal distributions of the data. The relationships of all parameters according to age group in both sexes were examined. The relationships between the three aspects of age-related bone changes (morphometry, BMD and OST) were analyzed using the partial correlation (r_p) by controlling for the influence of age. Statistical significance was accepted at the $P < 0.05$ level.

Results

Vertebral body morphometry

The common pattern of age-related change in the vertebral body dimensions was an increase during young adulthood (7–10 year group) until reaching the peak (15–20 year group and 10–15 year group in females and males, respectively) and then subsequent decrease with age (Table 1.2). For example, the VBHd of L3 in females changed from 1.35 in young adulthood to 1.48 at peak and to 1.39 in the oldest age group (>20 years), and in males from 1.26 in young adulthood to 1.36 at peak and to 1.31 in the oldest age group (>20 years). Ages at peak in L7 varied somewhat from those in L1–L5 in both sexes.

A significant decrease (0.81 to 0.78) after the VBDcau peak was found in the L1 of females, whilst in males, significant decreases were found in the VBDcra (0.79 to 0.69), VBWcra (1.22 to 1.14) and VBWcau of L1 (1.30 to 1.20) and the VBDcra of L3 (0.85 to 0.70).

As for sex differences, females had significantly higher means than males for the VBHd of L1 to L7 (age group 7–10 years for L1 to L7 and age group 15–20 years for L3 and L5), VBHv of L3 to L7 (age group 7–10 years) and VBDcra of L7 (age group >20 years). On the other hand, males had significantly higher means than females for the VBDcra of L3 to L7 (age group 7–10 years for L3 to L7 and age group 10–15 years for L3) and the VBDcau of L3 (age groups 7–10 and 10–15 years).

Table 1.2 Age changes in the standardized (to the femur head diameters) vertebral body height (dorsal and ventral; VBHd and VBHv), depth (cranial and caudal; VBDcra and VBDcau), and width (cranial and caudal; VBWcra and VBWcau)

	Sex	Age group (years)	VBHd				VBHv				VBDcra				VBDcau				VBWcra				VBWcau			
			Mean	SD	Age diff.	Sex diff.	Mean	SD	Age diff.	Sex diff.	Mean	SD	Age diff.	Sex diff.	Mean	SD	Age diff.	Sex diff.	Mean	SD	Age diff.	Sex diff.	Mean	SD	Age diff.	Sex diff.
L1	Females	7–10	1.16	0.08		+	0.94	0.07			0.74	0.03			0.76	0.04	b**		1.18	0.07			1.25	0.05		
		10–15	1.17	0.07			0.94	0.08			0.76	0.06			0.79	0.06			1.20	0.06			1.26	0.06		
		15–20	1.24	0.06			0.97	0.02			0.74	0.05			0.81	0.01	a*, b**		1.21	0.05			1.27	0.06		
		>20	1.19	0.07			0.93	0.05			0.71	0.03			0.78	0.02	a*		1.17	0.06			1.26	0.05		
	Males	7–10	1.10	0.06		+	0.89	0.08			0.75	0.05			0.78	0.05			1.20	0.05			1.24	0.05		
		10–15	1.19	0.08			0.94	0.09			0.79	0.05	e*		0.83	0.05			1.22\	0.03	e*		1.30	0.07	d*,e*	
		15–20	1.14	0.15			0.87	0.11			0.75	0.05			0.79	0.05			1.18	0.05			1.21	0.05	d*	
		>20	1.12	0.06			0.88	0.10			0.69	0.03	e*		0.75	0.02			1.14	0.03	e*		1.20	0.05	e*	
L3	Females	7–10	1.35	0.08	b**	++	1.18	0.08		+++	0.76	0.03		++	0.78	0.04	b**	+	1.34	0.07			1.34	0.07		
		10–15	1.36	0.06	d*		1.15	0.09			0.79	0.05		+	0.81	0.04		+	1.33	0.05			1.37	0.06		
		15–20	1.48	0.08	d*,b**	+	1.24	0.06			0.79	0.03			0.85	0.06	b**		1.34	0.08			1.38	0.08		
		>20	1.39	0.08			1.17	0.11			0.74	0.03			0.80	0.06			1.29	0.05			1.30	0.06		
	Males	7–10	1.26	0.07	a*	++	1.07	0.09		+++	0.81	0.06	c*	++	0.83	0.06		+	1.32	0.05			1.34	0.06		
		10–15	1.36	0.08	a*		1.15	0.12			0.85	0.06	e**	+	0.87	0.05		+	1.34	0.07			1.37	0.06		
		15–20	1.31	0.11		+	1.05	0.19			0.79	0.07			0.83	0.05			1.28	0.06			1.33	0.05		
		>20	1.31	0.04			1.08	0.05			0.70	0.05	c*,e**		0.78	0.03			1.26	0.04			1.32	0.03		
L5	Females	7–10	1.41	0.06		+++	1.25	0.09		+++	0.82	0.04	b*	++	0.87	0.04	b*,c*		1.40	0.09			1.42	0.07		
		10–15	1.45	0.09			1.26	0.11			0.87	0.05			0.89	0.06			1.41	0.07			1.45	0.07		
		15–20	1.51	0.08		+	1.30	0.06			0.89	0.04	b*		0.94	0.04	b*		1.38	0.06			1.46	0.06		
		>20	1.43	0.09			1.20	0.15			0.86	0.05			0.94	0.06	c*		1.35	0.04			1.48	0.07		
	Males	7–10	1.31	0.08	a**	+++	1.13	0.11		+++	0.88	0.07		++	0.92	0.08			1.39	0.04			1.42	0.05		
		10–15	1.44	0.09	a**		1.24	0.15			0.92	0.06			0.94	0.08			1.42	0.06			1.45	0.04		
		15–20	1.37	0.09		+	1.15	0.15			0.89	0.05			0.93	0.05			1.39	0.06			1.42	0.06		
		>20	1.42	0.03			1.14	0.07			0.81	0.07			0.85	0.04			1.34	0.05			1.38	0.07		
L7	Females	7–10	1.15	0.08		+++	1.03	0.10		++	0.82	0.04	c***	+	0.81	0.05	c*		1.42	0.09			1.48	0.09		
		10–15	1.12	0.12			1.00	0.14			0.85	0.04	e*		0.82	0.05	e*		1.43	0.07			1.50	0.11		
		15–20	1.18	0.11			1.03	0.14			0.87	0.04			0.81	0.05	f*		1.42	0.07			1.39	0.11		
		>20	1.16	0.13			0.99	0.12			0.92	0.05	c***,e*	+	0.91	0.08	c*,e*,f*		1.40	0.03			1.55	0.20		
	Males	7–10	1.05	0.08	a*,b*,c*	+++	0.90	0.12	a*	++	0.88	0.08		+	0.85	0.07			1.46	0.06			1.50	0.08		
		10–15	1.16	0.14	a*		1.10	0.16	a*		0.89	0.06			0.85	0.05			1.46	0.08			1.52	0.10		
		15–20	1.16	0.14	b*		1.01	0.22			0.87	0.07			0.82	0.06			1.40	0.04			1.39	0.13		
		>20	1.18	0.03	c*		0.96	0.10			0.83	0.06		+	0.84	0.07			1.39	0.10			1.40	0.13		

[a-f] Statistically significant differences between age groups (ANOVA) at the [*] P <0.05, [**] P <0.01 and [***] P <0.001 level for [a] 7–10 vs. 10–15 years, [b] 7–10 vs. 15–20 years, [c] 7–10 vs. >20 years, [d] 10–15 vs. 15–20 years, [e] 10–15 vs. >20 years and [f] 15–20 vs. >20 years; [+-+++] statistically significant differences between sexes of the same age group (Independent *t* test) at the [+] P <0.05, [++] P <0.01 and [+++] P <0.001 level. *Note* because of severe OST development in age group >20 years (N = 8 and 4 for females and males, respectively), some vertebrae cannot be measured: for females; N = 7 in VBHv for L1, VBWcra for L1, VBHd for L3, VBWcra for L3 and VBWcau for L5; N = 6 in VBHv for L3, VBDcra for L3, VBHv for L5, VBDcra and VBDcau for L5, VBWcra for L5, VBDcra for L7 and VBWcra for L7; N = 5 in VBHv for L7 and VBDcau for L7; N = 4 in VBWcau for L7; for males; N = 3 in VBHv for L1, VBDcau for L1, VBDcra for L5 and VBWcau for L7

The average ventro-dorsal height (VBHv/VBHd) ratio of the vertebral body decreased, but only slightly, with age in adult life in general (Table 1.3), such as from 0.82 to 0.79 in females and from 0.81 to 0.78 in males for the L1. In females, L3 and L5 showed statistically significant decreases (P <0.05) from the age group of 7–10 years to the older age groups (>15 years). In males, age group 10–15 years displayed a significantly higher VBHv/VBHd ratio for L7 (0.95) than that in the oldest age group (0.82; P <0.05). No statistical differences in the VBHv were found between the sexes.

Tentatively, the threshold of an abnormality (kyphosis) was determined to be 0.7, which was obtained as the average minus two standard deviations of all subjects in both sexes. Three females and three males were diagnosed as abnormal kyphosis by this criterion.

Table 1.3 Age-related changes in the ventro-dorsal height ratio of the vertebral body (VBHv/VBHd) in Japanese macaques

	Sex	Age group (years)	VBHv/VBHd ratio			
			Mean	SD	Age diff.	Sex diff.
L1	Females	7–10	0.82	0.04		
		10–15	0.80	0.03		
		15–20	0.78	0.03		
		>20	0.79	0.03		
		All	0.80	0.04		
	Males	7–10	0.81	0.06		
		10–15	0.79	0.03		
		15–20	0.77	0.02		
		>20	0.78	0.04		
		All	0.80	0.05		
L3	Females	7–10	0.88	0.03	a*,b*	
		10–15	0.85	0.03	a*	
		15–20	0.84	0.04	b*	
		>20	0.85	0.05		
		All	0.86	0.04		
	Males	7–10	0.85	0.06		
		10–15	0.84	0.04		
		15–20	0.79	0.08		
		>20	0.82	0.02		
		All	0.84	0.06		
L5	Females	7–10	0.89	0.05	c*	
		10–15	0.87	0.04		
		15–20	0.86	0.05		
		>20	0.83	0.10	c*	
		All	0.87	0.06		
	Males	7–10	0.86	0.07		
		10–15	0.86	0.07		
		15–20	0.84	0.06		
		>20	0.80	0.04		
		All	0.85	0.07		
L7	Females	7–10	0.90	0.09		
		10–15	0.88	0.06		
		15–20	0.87	0.13		
		>20	0.85	0.08		
		All	0.88	0.09		
	Males	7–10	0.86	0.11	a*	
		10–15	0.95	0.09	a*,e*	
		15–20	0.87	0.11		
		>20	0.82	0.07	e*	
		All	0.88	0.10		

[a-f] Statistically significant differences between age groups (ANOVA) at the [*]P <0.05 level for [a] 7–10 vs. 10–15 years, [b] 7–10 vs. 15–20 years, [c] 7–10 vs. >20 years, [d] 10–15 vs. 15–20 years, [e] 10–15 vs. >20 years and [f] 15–20 vs. >20 years. *Note* because of severe OST development in age group >20 years (N = 8 and 4 for females and males, respectively), some vertebrae cannot be measured: for females; N = 7 for L1; N =6 for L5; N =5 for L3 and L7; for males; N = 3 for L1

Vertebral body mineral density

The inter-individual variation found was great within each age group as shown by the wide range in each vertebra and in every age group in both sexes (Table 1.4). The level of variation within each age group increased with increasing age. For example, in female L1 the % coefficients of variation increased from 25.19 in young adulthood up to 96.46 in the oldest age group.

Most vBMDs and TbBMDs decreased with age from that in young adulthood with some variations (Table 1.4). Decreases in the TbBMD with age were marked in both sexes. In young adulthood (7–10 years), females had significantly greater vBMDs and TbBMDs than males in all four examined vertebrae (except for the vBMD of L7). The female TbBMDs significantly decreased through the age group >20 years in all lumbar vertebrae, such as from about 137 to 71 mg/cm^3 for L1. The decreases in TbBMD were also numerically large in males, but they were not statistically significant in any vertebrae. The greatest decreases were found from age groups of 15–20 years to >20 year (Table 1.4), except for L5 in females, where the maximum decrease was found from age group of 10–15 years to 15–20 years.

Table 1.4 Age-related changes in the volumetric total bone mineral density (vBMD) and trabecular bone mineral density (TbBMD) in Japanese macaques

	Sex	Age group (years)	vBMD (mg/cm^3)						TbBMD (mg/cm^3)					
			Mean	SD	Range	% CV	Age diff.	Sex diff.	Mean	SD	Range	% CV	Age diff.	Sex diff.
L1	Females	7–10	174.19	35.21	97.1-234.9	20.22		+	136.78	34.45	67.4-197.6	25.19	c*	+
		10–15	165.96	54.10	71.4-227.4	32.60			125.37	48.40	42.0-186.9	38.61		
		15–20	164.37	49.43	83.9-216.8	30.07			102.32	44.17	29.4-155.1	43.17		
		>20	127.97	68.06	60.4-196.5	53.18			70.90	68.39	13.1-146.4	96.46	c*	
	Males	7–10	147.47	29.69	93.0-185.4	20.13		+	112.16	26.82	63.5-153.3	23.91		+
		10–15	138.90	43.97	75.2-210.6	31.66			92.20	38.04	34.9-152.4	41.26		
		15–20	153.76	45.44	101.8-225.4	29.55			97.88	40.33	45.8-159.1	41.2		
		>20	158.37	41.11	117.9-200.1	25.96			76.40	40.88	50.2-123.5	53.5		
L3	Females	7–10	173.59	37.40	93.2-223.5	21.54		+	138.70	36.53	66.2-185.0	26.34	c**	+
		10–15	159.28	44.71	71.1-213.4	28.07			118.91	38.64	45.2-169.5	32.50	e*	
		15–20	165.07	56.68	83.8-245.9	34.34			106.42	45.86	33.0-146.1	43.10		
		>20	97.33	74.47	52.7-183.3	76.51			62.90	72.57	15.1-146.4	115.37	c**,e*	
	Males	7–10	148.19	30.36	87.1-191.1	20.49		+	114.37	27.16	63.9-155.9	23.75		+
		10–15	134.19	37.98	82.8-194.7	28.3			90.90	30.51	45.2-140.2	33.57		
		15–20	151.62	61.52	93.3-246.0	40.57			99.48	55.68	31.6-175.1	55.97		
		>20	147.80	42.64	114.3-195.8	28.85			67.73	43.85	33.0-117.0	64.73		
L5	Females	7–10	181.21	43.65	87.0-284.5	24.09		+	140.43	35.22	55.8-210.2	25.08	b*,c*	+
		10–15	179.78	51.92	105.6-268.5	28.88			133.66	41.58	60.2-198.8	31.11		
		15–20	148.12	58.18	74.9-244.6	39.28			96.38	45.86	26.7-143.8	47.58	b*	
		>20	132.90	54.66	70.3-171.2	41.13			83.30	50.45	32.7-133.6	60.57	c*	
	Males	7–10	146.07	37.01	81.2-202.6	25.34		+	110.95	32.2	55.3-156.5	29.02		+
		10–15	149.83	42.53	85.0-217.0	28.38			100.08	34.11	34.4-150.5	34.09		
		15–20	147.72	63.14	70.6-226.7	42.74			96.48	57.5	18.5-158.5	59.6		
		>20	118.97	44.33	80.2-167.3	37.26			60.93	43.95	18.8-106.5	72.13		
L7	Females	7–10	165.04	29.47	102.7-212.1	17.86	c*		132.13	28.35	88.3-184.7	21.46	c**	+
		10–15	171.48	35.20	99.6-214.5	20.53	e**		126.64	33.64	56.6-157.7	26.56	e*	
		15–20	151.47	41.56	88.4-180.9	27.44			96.37	47.83	32.7-141.7	49.64		
		>20	98.37	80.23	26.8-185.1	81.56	c*,e**		94.85	66.54	47.8-141.9	70.15	c**,e*	
	Males	7–10	138.45	46.79	66.0-200.9	33.8			103.66	47.93	29.6-167.4	46.24		+
		10–15	154.93	26.84	118.7-209.5	17.32			104.59	21.04	72.6-144.1	20.12		
		15–20	187.38	108.48	69.4-357.1	57.9			121.08	86.13	17.1-247.7	71.13		
		>20	160.80	23.12	134.7-178.7	14.38			101.07	32.29	80.8-138.3	31.95		

[a-f] Statistically significant differences between age groups (ANOVA) at the [*] P <0.05, [**] P <0.01 and [***] P <0.001 level for [a] 7–10 vs. 10–15 years, [b] 7–10 vs. 15–20 years, [c] 7–10 vs. >20 years, [d] 10–15 vs. 15–20 years, [e] 10–15 vs. >20 years and [f] 15–20 vs. >20 years; [+] statistically significant difference between sexes of the same age group (Independent t test) at the P <0.05 level

For the TbBMD, the threshold value to determine if the subject had an abnormally low TbBMD was obtained using the data from the young adulthood age group (7–10 years) by using the average minus two standard deviations. However, the calculated value for L7 in males was extraordinarily low and so was excluded. Rounding up the average of all the obtained values (excluding that of L7) gave 70 mg/cm^3 as the threshold. There were seven females and 13 males that had smaller TbBMDs than this threshold.

Vertebral body osteophytosis

The OST scores in the odd-numbered lumbar vertebrae of all examined subjects are summarized in Table 1.5. The first detection of OST was earlier in males than in females, except for in L7. The onset age of OST in males was 8.5 years in L3–L7 and 9.4 years in L1, whereas in females it was 11.1 years for L1–L5 and 8.1 years for L7. The severity of OST significantly increased with increasing age in both sexes (Table 1.6), and the L1–L5 vertebrae exhibited a similar pattern of OST changes in both sexes. Although the OST scores of females were lower than those of males in the younger age groups (<15 years), they dramatically increased with age and surpassed those of males in females after 15 years, and accelerated in the oldest group. The OST scores of L7 were greater in females than in males, except for the age group 7–10 years. Thus, females had greater total average OST scores than males in all vertebrae with average scores of all data, ranging from 0.89 (L5) to 1.07 (L1) compared to from 0.45 (L7) to 0.83 (L1) in males. The L1 vertebrae tended to have the most severe OST at any age in both sexes, followed by the L3, then L5/L7 (Table 1.5).

Table 1.5 Osteophytosis (OST) scores in the L1–L7 odd vertebrae of all examined subjects, shown as the average, range, earliest stage observed and age observed, and the minimum age after which all subjects had OST

Sex	Vertebra	Average	Min stage	Max stage (age)	Earliest stage (age first observed)	Min, age[#]
Female	L1	1.07	0	3.75 (29.6 and 30.8)	1.5 (11.1)	16.6
	L3	0.97	0	4 (30.3)	1 (11.1)	16.6
	L5	0.89	0	4 (29.6, 30.2 and 30.3)	1 (11.1)	16.6
	L7	0.95	0	4 (23.3, 29.6, 30.2 and 30.3)	0.5 (8.1)	18.2
Male	L1	0.83	0	4.25 (27.1)	1 (9.4)	16.5
	L3	0.77	0	3 (27.1)	0.5 (8.5)	15.0
	L5	0.66	0	3 (20.8)	1 (8.5)	16.5
	L7	0.45	0	4.25 (27.1)	1 (8.5)	23.7

Min = minimum, Max = maximum. Values in brackets indicate age (years); minimum is 6.6 and 7.0 years of age for females and males, respectively; [#] age (years) from which all subjects older than this have OST

Table 1.6 Age-related changes in the osteophytosis (OST) in Japanese macaques; mean (SEM, range)

Sex	Age group (years)	L1			L3			L5			L7		
		Mean (SEM, range)	Age diff.	Sex diff.	Mean (SEM, range)	Age diff.	Sex diff.	Mean (SEM, range)	Age diff.	Sex diff.	Mean (SEM, range)	Age diff.	Sex diff.
Females	7–10	0	a*,b***,c***		0	a*,b***,c***		0	b***,c***		0.04 (0.04, 0–0.5)	b*,c***	
	10–15	0.44 (0.21, 0–2)	a*,d*,e***		0.25 (0.12, 0–1)	a*,d*,e***	+	0.21 (0.11, 0–1.5)	d*,e***	+	0.42 (0.20, 0–2)	e***	
	15–20	2.04 (0.50, 0–3.25)	b***,d*,f*		1.75 (0.57, 0–3)	b***,d*,f*		1.42 (0.52, 0–2.75)	b***,d*,f*		1.25 (0.56, 0–2.75)	b*,f*	
	>20	3.16 (0.24, 1.75–3.75)	c***,e***,f*		3.16 (0.24, 1.75–4)	c***,e***,f*	+	3.06 (0.36, 1.25–4)	c***,e***,f*		3.13 (0.43, 1–4)	c***,e***,f*	
Males	7–10	0.08 (0.06, 0–1)	a**,b***,c***		0.11 (0.07, 0–1)	a**,b***,c***		0.11 (0.08, 0–1)	a**,b***,c***		0.08 (0.06, 0–1)	a*,b***,c**	
	10–15	1.00 (0.31, 0–2.25)	a**,e*		1.03 (0.28, 0–2.25)	a**	+	0.88 (0.25, 0–2)	a**	+	0.35 (0.13, 0–1)	a*,d*	
	15–20	1.80 (0.46, 0–2.5)	b***		1.75 (0.27, 1–2.5)	b***		1.15 (0.47, 0–2.75)	b***		0.90 (0.23, 0–1.25)	b***,d*	
	>20	2.56 (0.78, 0.5–4.25)	c***,e*		1.88 (0.55, 0.5–3)	c***	+	1.94 (0.52, 0.5–3)	c***		1.81 (0.98, 0–4.25)	c**	

[a-f] Statistically significant differences between age groups (Kruskal-Wallis) at the [*] P <0.05, [**] P <0.01 and [***] P <0.001 level for [a] 7–10 vs. 10–15 years, [b] 7–10 vs. 15–20 years, [c] 7–10 vs. >20 years, [d] 10–15 vs. 15–20 years, [e] 10–15 vs. >20 years and [f] 15–20 vs. >20 years; [+] statistically significant difference between sexes of the same age group (Mann-Whitney U) at the P <0.05 level

Interrelationship between vertebral body morphometry, density, and osteophytosis

A correlation was found between the morphometry, density, and OST in all examined vertebrae, but not all were statistically significant after controlling for the influence of age. This may in part be due to either wide inter-individual variation in each of the three aspects, the difference in the patterns of the age-related changes between aspects, or a non-linear interrelationship. In all vertebrae of females, the negative relationship was achieved between the TbBMD and OST (r_p = –0.167 to –0.344, P <0.05 to 0.5) and the positive one was between VBWcau and vBMD (r_p = 0.163 to 0.233, P <0.2 to 0.5). On the other hand, in all vertebrae of males, the negative relationship was achieved between VBWcau and TbBMD (r_p = –0.148 to –0.443, P <0.01 to 0.5) and the positive ones were between VBDcra and OST (r_p = 0.154 to 0.425, P <0.01 to 0.5), and between VBDcau and OST (r_p = 0.213 to 0.409, P <0.02 to 0.5).

No strong or significant relationship was found between the TbBMD and the VBHv/VBHd ratio (Figure 1.3) indicating that age change and inter-individual variation in the TbBMD was great. Only six subjects (three females and male) had a smaller VBHv/VBHd ratio (<0.7) in their one or more vertebrae. Because of severe OST, the TbBMD was not measured in some vertebrae belonging to these individuals. The TbBMD value of L7 in female and L3 in male was 105.8 and 112.9mg/cm^3, respectively (Figure 1.3). Thus, the abnormal kyphosis would occur with a TbBMD of 110 mg/cm^3 or smaller.

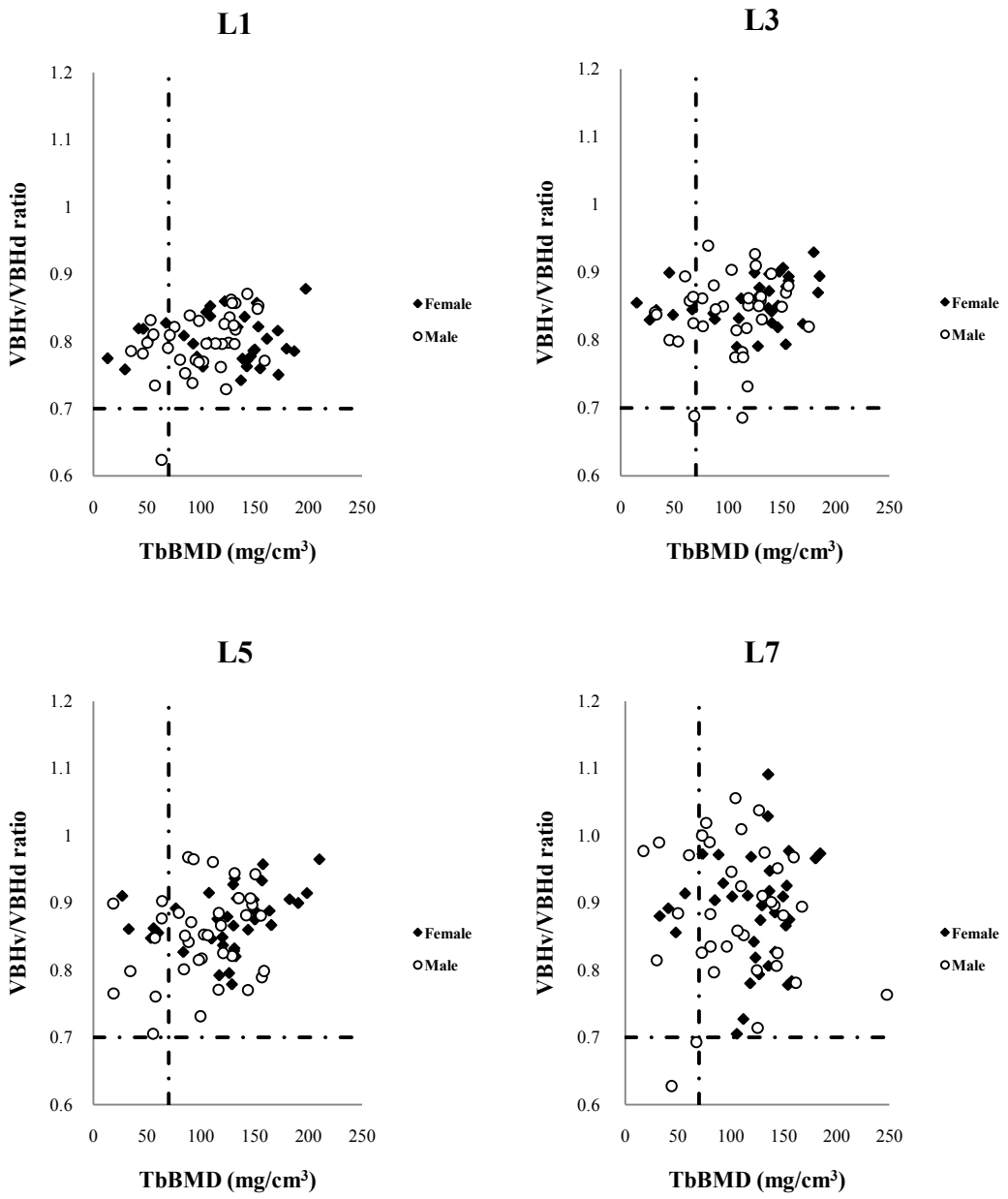

Figure 1.3 Relationship between the trabecular bone mineral density (TbBMD) and the ventro-dorsal height (VBHv/VBHd) ratio. Broken lines refer to normal thresholds of TbBMD (70 mg/cm^3) and VBHv/VBHd ratio (0.7). *Filled rectangular* = female, *open circle* = male

The relationship between the TbBMD and OST score was also vague (Figure 1.4), partly because the severe OST hampers to measure the TbBMD properly. Almost all vertebrae with a TbBMD of <50 mg/cm^3 showed an OST of >0. OST appeared in vertebrae with a TbBMD of 150 mg/cm^3 or less.

The subjects were divided into four TbBMD classes of <50, 50–99.9, 100–149.9 and ≥150 mg/cm^3, and into four categories of OST scores: 0< OST <1.0, 1.0≤ OST <1.5, 1.5≤ OST <2.0 and OST ≥2.0 (Figure 1.5). In both sexes, the <50 mg/cm^3 TbBMD class showed a higher frequency of nearly all OST criteria than the other higher TbBMD classes. In females and males, the <50 mg/cm^3 TbBMD class showed the highest frequency of OST ≥2.0, ranging from 60 % and 42 % from L1 to L7 in females and males, respectively.

No correlation was found between the OST score and the VBHv/VBHd ratio (data not shown). OST develops in vertebrae without abnormal kyphosis that appear intact in the VBHv/VBHd ratio (>0.7). Four subjects (two females and two males) showed both a smaller VBHv/VBHd ratio (equal or smaller than 0.7) and OST.

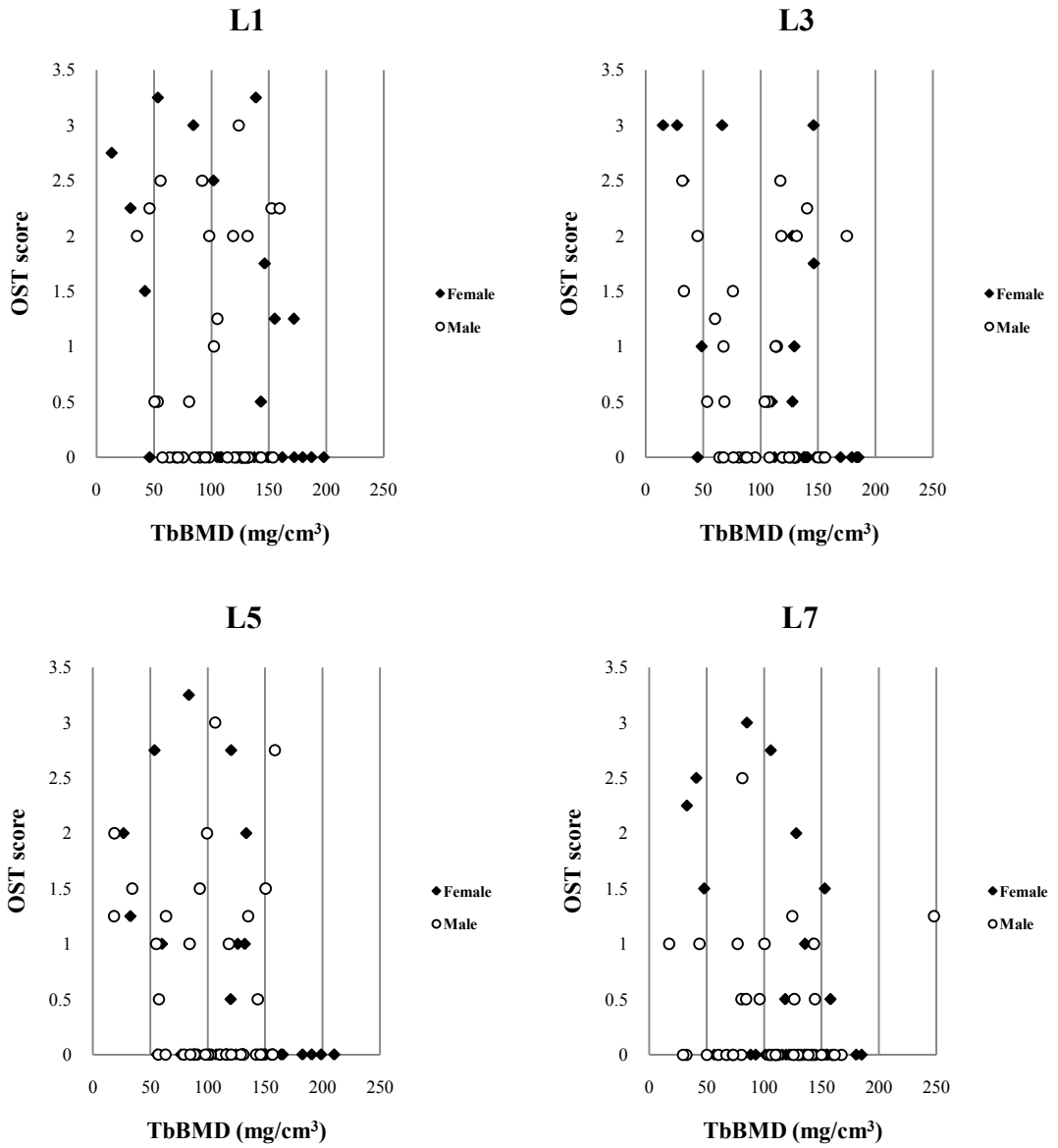

Figure 1.4 Relationship between the trabecular bone mineral density (TbBMD) and the osteophytosis (OST) score. *Filled rectangular* = female, *open circle* = male

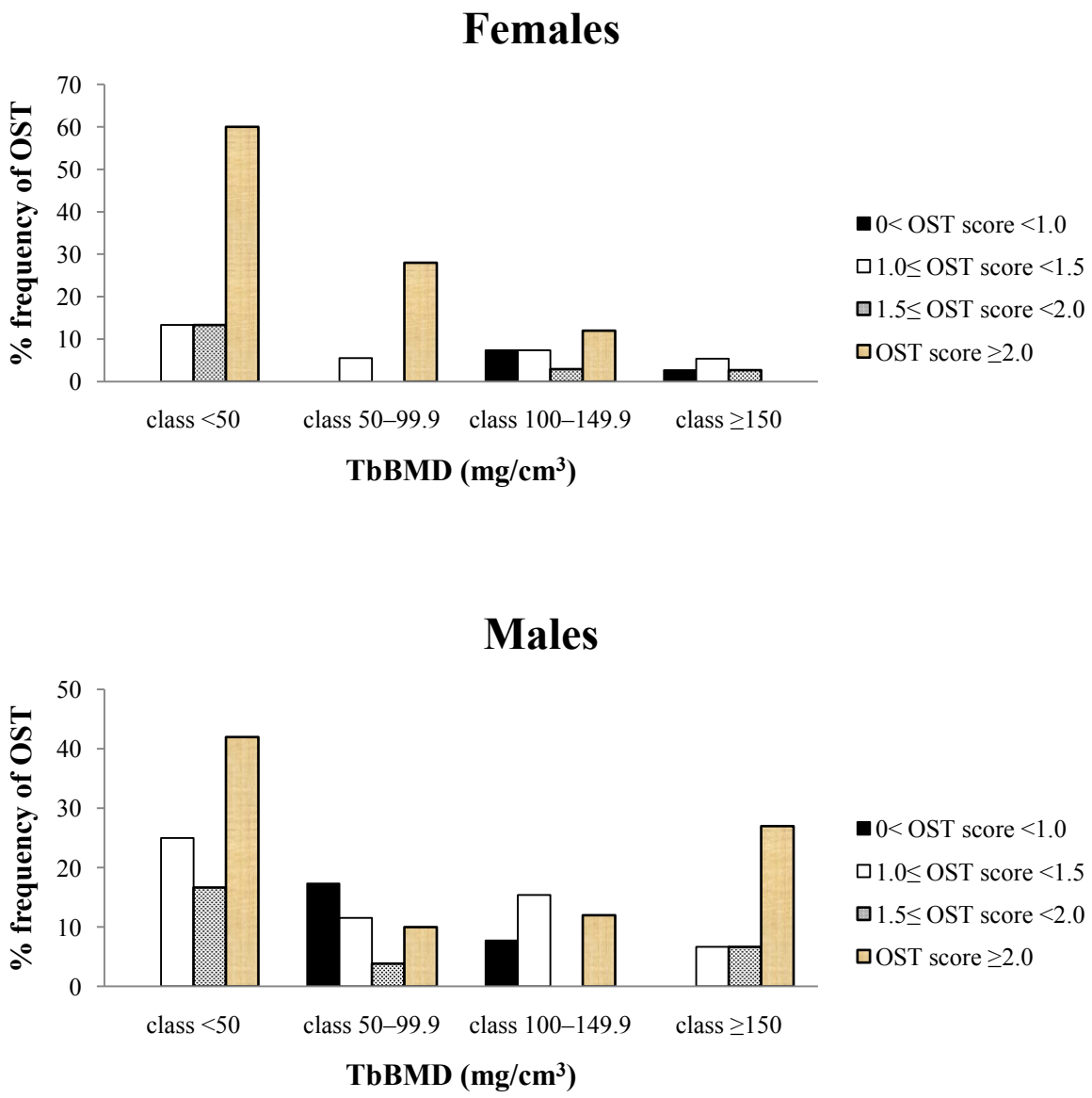

Figure 1.5 Relationship between the 4 classes of trabecular bone mineral density (TbBMD) and the % frequency of subjects having osteophytosis (OST)

Discussion

Evaluating the age-related skeleton changes in nonhuman primate is important for understanding the life history and mechanism of skeletal changes, since this will contribute to improve understanding of the evolution of humans. Changes in the bone dimensions, density and OST are influenced by a combination of genetic and environmental factors. In the present study, the habitat (corral cage) and food (monkey chow and potato supplements) were controlled. Taking these conditions into consideration, I discuss the age-related changes in the lumbar vertebrae of Japanese macaques.

Vertebral body morphometry

The major pattern of the age-related changes in the vertebral body dimensions was an increase from young adulthood to the peak and then a subsequent decrease with age. Males reached this peak earlier (age group 10–15 years) than females (age group 15–20 years) in every measured dimension of the vertebra. The depths and widths of the vertebral body exhibited this pattern of age-related changes in all the vertebrae of Japanese macaques, with the exception of L7 in females. Males revealed a greater magnitude of change in the depths and widths with age compared to females. However, the equivalent dimensions appear to increase with age in humans, as was demonstrated clearly in both men and women between 20 and 90 years of age in some studies (Ericksen 1976, 1978a, b), and clearly in women between the 55 to 59 age group and the 75 to 79 age group (Evan et al. 1993). Furthermore, significant alterations were observed in association with aging in men (Rühli et al. 2005). These dimensional increases may reflect an increase in robustness of vertebrae with age to compensate for the loss of stability due to general decrease in bone mass or vertebral height compression (Rühli et al. 2005). The difference in the patterns of the age-related dimensional changes between macaques and humans may reflect the different mechanical stresses that act on the lumbar vertebrae that arise from the different positional behavior of macaques (quadrupedalism) and humans (bipedalism), and this is a subject for future study.

The dorsal and ventral heights of the vertebral body decreased with age in both sexes, except for a few lumbar vertebrae that slightly increased heights from young adulthood (7–10 years) to the oldest age category (>20 years). The increase appears contradictory with the fact that trunk length of macaques or stature in humans decreases with age in adult life (Roth et al. 2004; Hamada 2008). However, the reduction of stature (or trunk length) may stem from not

only a decrease in the bone (vertebral) height itself, but also from kyphosis, impairment of cartilage, narrowing of intervertebral discs and a reduction of muscles (Rogers 1982).

The VBHv/VBHd ratio consistently decreased with age to reach the lowest value in the older age groups in both sexes, indicating that the height decreased more rapidly on the ventral margin than on the dorsal. In addition, females showed a greater decrease than males (Hamada and Yamamoto 2010; Table 1.3, present study). The VBHv/VBHd ratio in humans was reported to either decrease (Ericksen 1976, 1978a, b; Evans et al. 1993; Hermann et al. 1993; Diacinti et al. 1995) or to show negligibly change with age (Sevinc et al. 2008) in either sex. The decrease found in these Japanese macaques was slight (from 0.82 to 0.79 in the L1 of females), and so partly contributes to kyphosis. The majority of the examined Japanese macaques did not show severe kyphosis, with about 8 % having VBHv/VBHd ratio smaller than 0.7 (threshold of abnormality or kyphosis).

Vertebral body mineral density

The parameters of the age-related changes in the BMD has been reported to differ between studies, including, for example, the peak BMD, the age at attaining the peak BMD, and whether the BMD remains constant or decreases with increasing age after the peak (Colman and Binkley 2002). Therefore, the general trend is discussed.

The vBMD, or bone mass, exhibited an age-related pattern, increasing to the peak and then decreasing with further age in rhesus macaques (Aguiló and Cabrera 1989; Pope et al. 1989; Champ et al. 1996; Colman et al. 1999b; Cerroni et al. 2000), in cynomolgus macaques (Jayo et al. 1994). In women, it increases with a peak around 30 years of age (Matkovic 1992). In men, the vBMD reached the peak later than in women, but with a greater magnitude of the increased vBMD to the peak level (Colman and Binkley 2002). Macaques and humans both attain a peak of bone mass or BMD considerably after sexual maturation. The present study on Japanese macaques showed that the vBMD decreased from that in young adulthood (7–10 years) through the oldest (>20 years). Females had a significantly higher vBMD in young adulthood than males, although it fell markedly to a lower value than that of males after 20 years of age. Reproductive aging, and in particular the depletion of sex hormones after menopause, is strongly associated with bone loss in female macaques showing marked decreases after 20 years of age. It is also true in women compared with men. Japanese macaques experience a natural menopause (Nigi 1975; Nozaki et al. 1995) and the average age at the last parturition was 21.7 years and menopause would be a few years later (Takahata et al. 1995) with considerable inter-individual variation in the post-menopausal life (Shimizu

2007). For the male Japanese macaques, no consistent age-related change patterns were found. For example, the vBMD of L1 increased from group 7–10 years to >20 years, but that of L5 decreased from group 10–15 years to >20 years. Age-related changes in the reproductive physiology of male Japanese macaques are not currently known.

Trabecular bone loss begins from young adulthood and the rate of loss then accelerated in older adulthood in both sexes (in Japanese macaques, present study, Table 1.4; in humans, Riggs et al. 2004, 2008; Christiansen et al. 2011; in rhesus macaques, Turnquist et al. 2011). Women showed a greater loss of TbBMD than men, particularly in postmenopausal life (Riggs et al. 2008). The same sex difference was also found here in Japanese macaques when the subjects were controlled for severe OST. The maximum decrease in the TbBMD was found between the age groups of 15–20 years to >20 years, except for the L5 and L7 vertebrae in females which showed the maximum decrease from group 10–15 years to 15–20 years. In the oldest female age group (>20 years of age), the TbBMD in all vertebrae except for L5 were lower than those in males (Table 1.4).

TbBMD is regarded as an indicator of bone deterioration and the fracture risk (Fujii et al. 1996). The vertebral TbBMD measured with QCT, which is equivalent to WHO Diagnostic Category, is tentatively given by the ACR–SPR–SSR (American College of Radiology, Society for Pediatric Radiology, and Society of Skeletal Radiology 2013) as a BMD >120 mg/cm^3 for normal, 80 to 120 mg/cm^3 for osteopenia and <80 mg/cm^3 for osteoporosis. In the present study on Japanese macaques, the threshold density for abnormally low BMD was tentatively determined, from the mean minus two standard deviations of the young adulthood age group, as 70 mg/cm^3. Seven females (age ranged from 7.0 to 22.5 years) and 13 males (age ranged from 7.1 to 23.7 years) had a lower BMD than this threshold.

Vertebral body osteophytosis

OST increased with age in adult Japanese macaques from an initial onset to a severe state. Male macaques tended to have OST earlier than females, as is also the case in humans (Beresford 1952). The onset age of OST in male macaques was 8.5 years (Japanese macaques, present study), before 10 years (rhesus macaques, Krueger et al. 1999) or 12 years (rhesus macaques, Duncan et al. 2012). The onset age of OST in female macaques was 11.1 years (Japanese macaques, present study), >12 years (rhesus macaques, Duncan et al. 2012), or 5.3 years (pig-tailed macaques, Kramer et al. 2002). These onset ages of OST in macaques

(equivalent ages) are relatively later than that in humans (20 years, Nathan 1962; Duncan et al. 2012).

The age at which most to all of the individuals had OST in these Japanese macaques was about 16.5 years in both sexes, which appears the same as that in humans (40–50 years, Nathan 1962; Snodgrass 2004). Whether female macaques (women) after 15 years (40 years) suffer more severe OST than males (men) has not been determined, and there are evidences both pro (Japanese macaques, Lipps et al. 2007; present study) and con (rhesus macaques and humans, Black et al. 2001; Duncan et al. 2012). Both pig-tailed and rhesus macaques had a more severe OST than humans at equivalent ages (Kramer et al. 2002; Duncan et al. 2012).

The first lumbar vertebrae tended to have the most severe OST of all the lumbar vertebrae in both sexes (in rhesus macaques, DeRousseau 1985; Duncan et al. 2012; in women and female pig-tailed macaques, Kramer et al. 2002; and in Japanese macaques, present study). The cranio-caudal cline of severity was found (DeRousseau 1985; present study), although a different ranked order of L1 >L7 >L5 >L3 was also reported in rhesus macaques (Duncan et al. 2012).

Mechanical stress on a bone at the joint is the major factor in OST development and severity (Nathan 1962). The quality and quantity of any such mechanical stresses would explain the sex differences and human-macaque difference in OST. Body mass (BM) may be one factor that explains the earlier onset of OST in males (men) than in females (women) and the relatively earlier onset of OST in humans than in macaques (Klaassen et al. 2011). In humans, the BM has been associated with OST (Pye et al. 2007a). Macaques have a greater sex difference in their BM, with the average mass of males and females being 12 kg and 8 kg, respectively (Hamada 2008). Although, both the single time and average life time BM does not seem to be clearly related to the severity of OST in macaques (Colman et al. 1999b; Kramer et al. 2002), Japanese macaques have greater sex difference, which would be reflected in the onset time of OST.

Positional behavior is another factor involved in promoting OST. The pronograde quadrupedalism of macaques versus the orthograde bipedalism in humans may result in different stresses to the vertebral body and so explain the more severe OST seen in macaques than in humans at equivalent ages and the cranio-caudal cline of severity of OST (Johnson and Shapiro 1998; Turnquist et al. 2011). Although prono- and orthograde difference or the difference in architecture of lumbar vertebrae between biped and quadruped (Duncan et al. 2012) can be discussed to explain the great difference of OST between humans and macaques, at present decisive mechanism has not been given. The female Japanese macaques suffered

more severe OST after 15 years of age than males could relate to the fact that adult females carry their infants (sometimes juveniles) below their abdomen or on their back.

Interrelationship between vertebral body morphometry, density, and osteophytosis

It was hypothesized that the loss of TbBMD leads to the susceptibility of bone fragility and causes diminution of the vertebral body height, especially in the ventral side, which leads to kyphosis; and that OST may develop using bone minerals absorbed from the bone, which results in a further decrease in the BMD. Therefore, significant relationships between these three aspects are possibly expected.

Using all the data from young adulthood through to the oldest age group of Japanese macaques, no significant relationship was found between these three aspects, even when the influence of age was removed. The fact that the age-related bone changes are complicated and do not always follow expectations likely arises from three principal reasons. Firstly, the inter-individual variation was wide, especially for the TbBMD and OST. Secondly, various patterns of age-related changes existed, such as monotonous increase/decrease and increase followed by decrease with advanced age. Thirdly, the TbBMD was greatly influenced by the OST.

When controlling for the influence of age, a negative relationship was found between TbBMD and OST in all the examined vertebrae in females. OST development could be an adaptation to a load-bearing function (Klaassen et al. 2011). Trabecular deterioration due to low TbBMD causes declines in weight-bearing property. OST is considered to increase the weight-bearing surface of the vertebra (Zhao et al. 2013), thus OST development may compensate for that deterioration. However, no such association was found in males. Significant relationships were also found between the depths of the vertebral body and OST (positive), and between the VBWcau and TbBMD (negative) in males, and between the VBWcau and vBMD (positive) in females. Although osteophyte was excluded from vertebral body morphometry by measuring at the outermost part of the epiphyseal area, OST may influence the body depths in males.

The relationship between VBHv/VBHd ratio and TbBMD was not tight. Seven female and 13 male subjects showed a lower TbBMD than the threshold of abnormality (70 mg/cm^3). The TbBMD of two female subjects out of three that had a lower than the threshold of the VBHv/VBHd ratio were not measured because they showed severe OST, whilst the third had a relatively high TbBMD of 105.8 mg/cm^3. Two of three male subjects that had a lower than the threshold of height ratio also had a TbBMD below the threshold, but the third male had a

higher TbBMD of 112.9 mg/cm^3. Thus, a lower TbBMD did not necessarily result in an abnormally low height ratio. Rather a significant height ratio decrease may result from other random phenomena, such as fracture caused by abrupt mechanical stresses.

The TbBMD and OST are usually assumed to be negatively correlated with each other (Stewart and Black 2000), and here in Japanese macaques the OST development showed a negative relationship with the TbBMD values in both sexes. However, OST tended to appear in vertebrae with a higher TbBMD in males although high TbBMD also observed in females. Almost all the vertebrae with a TbBMD of less than 50 mg/cm^3 had OST. The incidence of OST rapidly increased from TbBMD class of 50–100 mg/cm^3 to the class of <50 mg/cm^3. However, the inter-individual variation was wide, which may reflect that OST appears to be increased by mechanical stresses that are applied to the joint, and tend to be greater in males.

OST appearance and development was found to lack any relationship to the VBHv/VBHd ratio. Vertebral deterioration, such as compression or wedging, may arise from the microfracture in the bone caused by the bone resorption. Osteoarthritis, including OST, is associated with a decrease in rate of bone turnover (Peel et al. 1995). However, present study showed that OST appearance and development lack any relationship with compression (VBHv/VBHd ratio), and few subjects showed both a lower VBHv/VBHd ratio and an OST of >0. Physical stress biased to the ventral side of vertebral body would cause wedging, though we should wait study showing the ventrally biased physical stress, perhaps from posture or locomotion.

In conclusion, Japanese macaques particularly females showed similar age-related bone changes to those in women. However, the variations, especially abnormal in vertebral body heights and BMD should be examined. The most important difference between humans and macaques are the rapid OST aggravation in macaques (Duncan et al. 2012; present study), which influenced bone dimensions and density. Compared to morphometry and density in macaques, OST showed the strongest relationship with age, with greater severity than that in humans of an equivalent age. Longitudinal study is strongly recommended to follow the age change of bone and physiological change. Age change in reproductive physiology should be studied in male macaques.

Chapter 2

Effect of physical activity and body mass on osteoarthritis in Japanese macaques in different living conditions

Introduction

Lumbar vertebral osteoarthritis (OA) is one of the most prevalent disorders suffering older people worldwide. Lumbar vertebral OA includes narrowing of the intervertebral disc space, bony outgrowth or osteophyte, and vertebral endplate sclerosis (Pye et al. 2007b).

Lumbar vertebral OA has been studied in monkeys, particularly macaques considered as a useful animal model of human vertebral OA (e.g. Kramer et al. 2002). All previous studies in monkeys were from the captive condition where the monkeys were supplied with food though there are several kinds of restricted movement including individual cage, social cage and corral cage. From all previous studies, macaques in the captive condition had much more prevalent and severe vertebral OA than those of humans (in pig-tailed macaques, Kramer et al. 2002; in rhesus macaques, Duncan et al. 2012; in Japanese macaques using only osteophytosis (OST), Pomchote 2015). The differences are considered to arise from different biomechanical stresses, vertebral curvature or vertebral morphology between quadrupedal macaques and bipedal humans (Duncan et al. 2012).

The study on lumbar vertebral degenerative joint disorders, OST and OA from the apophyseal surfaces, in free-ranging African great apes was conducted by Jurmain (2000). The author found dramatically smaller frequency of vertebral degenerative disorders in chimpanzees, lowland gorillas and bonobos compared with those of humans. Great apes have four lumbar vertebrae compared with five lumbar vertebrae in humans, including the differences in the orientation of the facet joints on the lumbar vertebrae between great apes and humans (Aiello and Dean 2002) which may relate to prevalence of OA. The shortened vertebrae of great apes are rigid and resistant to forces which participated in lower prevalence of degenerative joint disorders (Jurmain 2000). The author also supported that different ape species which share similar number of vertebrae of larger body had more severity of degenerative joint disorders than those of smaller body size, whereas similar species with more number of vertebrae more developed disorders than those with smaller number of vertebrae.

Compared with other mammals, macaques share bone changes with humans that the antero-posterior diameter of vertebral body increases from cervical to lumbar region, and the

motion range absolutely high, all of which may contribute to the different intervertebral disc degeneration from that of other mammals (Alini et al. 2008). Macaques from captive colonies had significantly higher prevalence of degenerative joint disorders compared with humans or great apes. Several confounders were discussed for the higher prevalence of OA, including obesity in a captive group of macaques compared with free-ranging ones (Jurmain 2000).

From the above reasons, a comparative study on the same species which differ in body size or their living conditions is needed to understand an etiology of OA. Thus, the objective of this study was to compare OA prevalence and severity between Japanese macaques (*Macaca fuscata*) reared in individual cages (captive condition) and living in free-ranging (natural condition) using radiographic data, which are evaluated through disc space narrowing (DSN) and OST.

Materials and Methods

Captive group (abbreviated as CG)

Twenty one females and 22 males of Japanese macaques were selected. They were born in corral enclosures and then were moved to individual cages (76 x 90 x 85 cm) in an indoor room at the Primate Research Institute, Kyoto University, Japan. The temperature, humidity and lighting were controlled in the cage room. They were fed with standard monkey chows (AS, Oriental Yeast Co. Ltd., Tokyo, Japan) and supplemented with sweet potatoes. Water provides *ad libitum*. All animals could contact with others via vision, sound and scent. All of them had the exact birth dates and medical histories. Body mass (BM) (kg) was measured at the time of inspection. Then, body mass index like-index (BMI) was calculated by dividing BM by the square of length from the tip of crown to ischial callosity. I complied with guidelines of the Primate Research Institute of Kyoto University and carried out this study with permission from the ethical committee.

Free-ranging group (abbreviated as FRG)

Thirty one females and 27 males of Japanese macaques were studied. They lived on Koshima Islet where is located at the lower latitude of Japan. The area of Koshima Islet is about 0.3 km^2. The islet is mainly covered by evergreen forest with various species of trees described elsewhere (Iwamoto 1974; Watanabe 1989). Although the monkeys can freely roam around the islet to search for food, they have been artificially fed since 1952 but the amount of food was different during various periods and was limited to control their population size from 1975 (Mori 1979). Their exact ages were known because they have been observed for more than 60 years. BM was weighed at the time of inspection, and BMI was calculated by dividing BM by the square of crown-rump length (m).

Radiographic evaluation

Lateral lumbar radiographs were obtained from all animals using Portable X-ray 150 (Hitachi Co. Ltd., Japan). The distance between X-ray cube and film was 1 m. Conditions were set as follow: cube voltage was 60 kV, exposure was 20 mA with 0.2–0.8 sec depending on the size of the monkey. I used X-ray film (Fuji RX) with dimension 10 x 12 inches inserted in the cassette with a medium intensifier.

All films were selected and scored randomly for the presence and severity of OA by a single observer (Porrawee Pomchote). All films were read two different times by the same reader and the scores from the second reading were used. An atlas scoring method (Lane et al. 1993; Kramer et al. 2002) was used to determine presence and severity of OA. The scores were as follows: 0 for unaffected site, 1, 2, or 3 for slight, moderate, and severe involvement, respectively (Figure 2.1). I concentrated on development of DSN and OST at the lumbar region. Thus, each lumbar vertebral level was scored for DSN and OST (including OST-S and OST-V), separately described in detail elsewhere (Kramer et al. 2002). DSN was evaluated in each lumbar vertebral level (L1/L2 through L6/L7). OST was divided into two different scoring types: OST-S was from the highest score of OST between the two adjacent lumbar vertebrae (L1/L2 through L6/L7) and OST-V was the highest score of each lumbar vertebra (L1 through L7).

All monkeys had seven lumbar vertebrae as the majority of macaques (Schultz 1961). Unreadable radiographs due to bad quality obstructed scoring in few spaces or vertebrae in particular at L6/L7 in one male from CG; at L1/L2 or L2/L3 in five females and eight males from FRG. For OST, if only one side of the anterior vertebral margin was readable that side was scored.

All average scores of DSN, OST-S and OST-V were calculated from L1 to L7 for each individual. The pattern of OA along the lumbar vertebral region was calculated from average scores of each lumbar vertebra from all individuals. No combined scores between DSN, OST-S and OST-V were calculated.

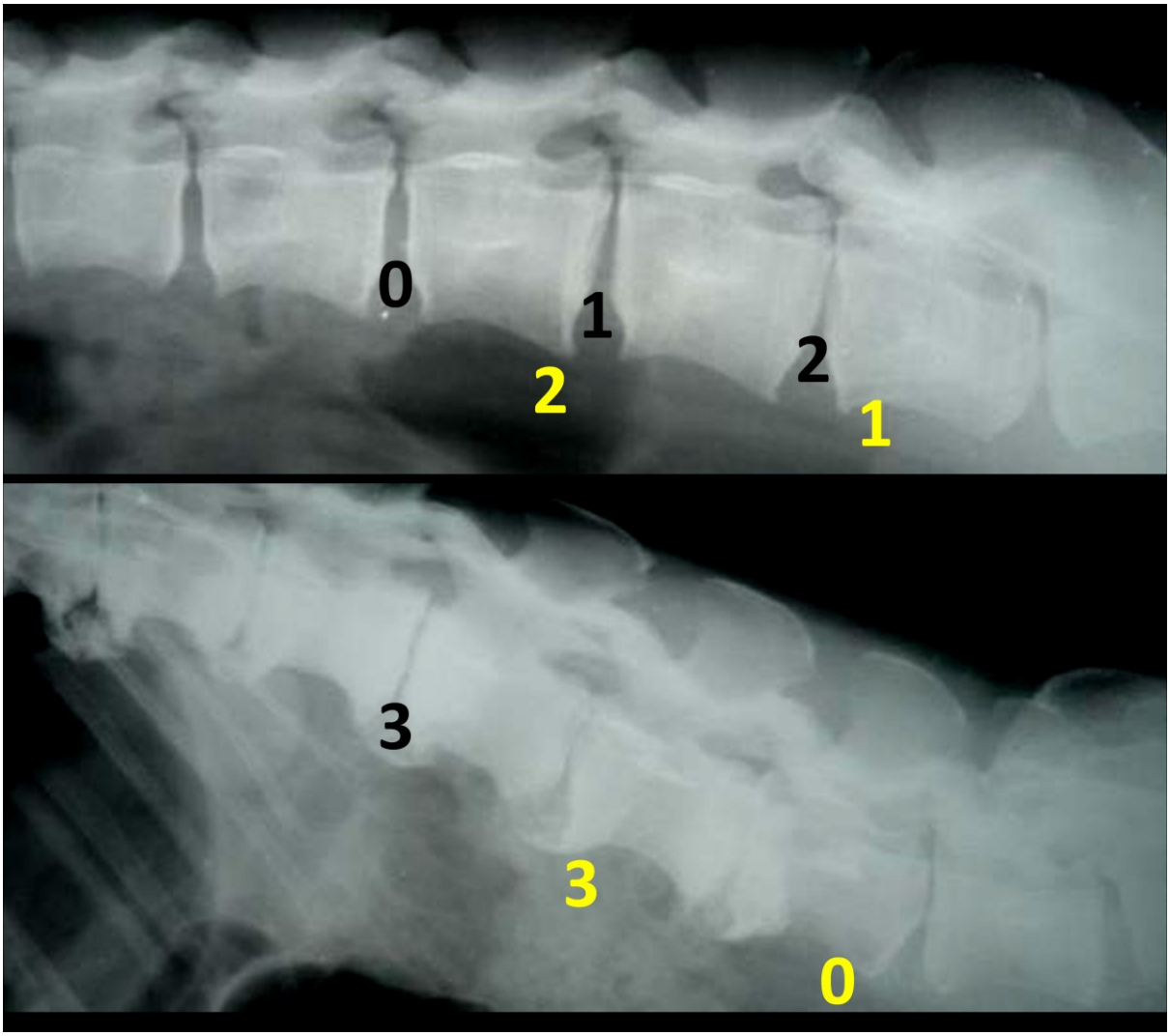

Figure 2.1 The atlas scoring method (Lane et al. 1993; Kramer et al. 2002) for disc space narrowing (DSN) and osteophytosis (OST) of the lumbar vertebrae of Japanese macaques. The scores are 0 for unaffected site, 1, 2, or 3 for slight, moderate, and severe involvement, respectively. *Numbers in black* = DSN, *numbers in yellow* = OST

Statistical analysis

All statistical analyses were performed using SPSS (windows XP, version 16.0) or Excel (Microsoft Co. Ltd., version 2007). I used as many as samples that I had, thus age was not matched between females and males of CG and FRG. Independent *t* test and analysis of covariance (ANCOVA) were used to detect significant differences between both sexes of different groups. Macaques clearly develop OA in particular DSN including kyphosis with advancing age, so BMI is possibly affected by DSN development and was not included in ANCOVA. Statistical significance was accepted at the $P < 0.05$ level.

I compared age-related changes in DSN and OST-V scores between Japanese macaques from CG and FRG with those of rhesus macaques and humans, which used similar scoring system, by adjusting Japanese macaque ages to represent human equivalent ages based on human-to-monkey age ratio of 1:3.5 years (Duncan et al. 2012).

Results

From Table 2.1, both sexes of Japanese macaques from CG had significantly higher average age, BM, BMI, DSN, OST-S and OST-V scores than those of FRG, except for age between males of the two groups (Independent *t* test). After the effect of BM was controlled using ANCOVA, only significant differences in DSN, OST-S and OST-V scores between female CG and FRG still remained, while DSN, OST-S and OST-V were not significantly different between males of the two groups.

The average scores of DSN, OST-S and OST-V in both sexes of two different groups increased with advancing age. FRG monkeys showed clearly less prevalence of DSN, OST-S and OST-V compared with CG counterparts. Rather with variation was found in DSN, OST-S and OST-V (Figures 2.2–2.4). Using Pearson's correlation coefficient, significantly positive correlations were observed between age and DSN, OST-S and OST-V (in female CG; r = 0.550 to 0.582, P <0.006 to 0.01; in female FRG; r = 0.561 to 0.581, P <0.001; in male CG; r = 0.537 to 0.722, P <0.001 to 0.01; in male FRG; r = 0.431 to 0.598, P <0.001 to 0.025) except for age vs. DSN of female FRG monkeys that showed no significant correlation (r = 0.176, P <0.344).

For CG monkeys, DSN was firstly found at 9.1 and 10.9 years of age, and OST was firstly observed at 13.4 and 10.0 years of age in females and males, respectively. The youngest female and male with DSN were 8.8 and 6.6 years of age and those with OST were 8.6 and 13.6 years of age, respectively in FRG monkeys. The earliest score of DSN and OST was 1 in monkeys from both groups. The maximum score of DSN and OST were 3 and 2 in monkeys from CG and FRG, respectively (data not shown).

Among CG monkeys, 17 females showed DSN (DSN = 0.17–2.83) and one of them had no OST; 20 females had OST (OST-S = 0.17–2.33, OST-V = 0.14–2.57) and four of them had OST without DSN. In males, 13 monkeys showed DSN (DSN = 0.17–1.83) and two of them had no OST; 15 monkeys had OST (OST-S = 0.17–2.33, OST-V = 0.29–2.43) and four of them presented OST without DSN; five monkeys had no DSN and OST (Table 2.2).

From FRG monkeys, seven females had DSN (DSN = 0.17–0.5) and one of them had no OST; 10 females showed OST (OST-S = 0.33–1, OST-V = 0.29–0.83) and four of them had no DSN; 20 monkeys had no DSN and OST. In males, nine monkeys had DSN (DSN =

0.17–0.83) and six of them had no OST; six monkeys had OST (OST-S = 0.25–1.17, OST-V = 0.25–1.29) and three of them had no DSN; 15 monkeys had no DSN and OST (Table 2.2).

The prevalent patterns of DSN, OST-S and OST-V along the lumbar vertebral region were compared between both sexes of CG and FRG monkeys (Figures 2.5–2.7). In general, I observed clearly less prevalence in DSN, OST-S and OST-V in FRG monkeys than CG monkeys. In females, the maximum DSN was at upper region in CG, but in FRG the maximum appeared at the middle region; OST in both CG and FRG showed similar trend that upper region had maximum. Female CG had significantly higher DSN, OST-S and OST-V scores than female FRG at every lumbar vertebral level (P <0.001 to 0.035; P <0.001 to 0.002; and P <0.001, respectively). In males, DSN showed rather similar pattern between the two groups that DSN dominantly appeared at upper and middle regions, while OST had slightly different pattern that CG had the maximum at upper region but FRG dominantly appeared at upper and middle regions. Male CG had significantly higher DSN scores than those of FRG at only L2/L3 (P <0.031); OST-S scores at every lumbar vertebral level (P <0.001 to 0.04) except for L3/L4 that showed no significant difference; and OST-V scores at every lumbar vertebral level (P <0.001 to 0.042).

I compared the present result and previous study which showed DSN and OST-V severity of rhesus macaques and humans with age (Figures 2.8–2.9) (Duncan et al. 2012). In general, macaques and humans increased severity of DSN and OST-V with advancing age with different degrees of changes. In captive group of Japanese macaques and rhesus macaques prevalence of DSN and OST-V significantly increased with age, while FRG Japanese macaques and humans had rather similar degrees of age-related changes in DSN and OST-V with less development.

Table 2.1 Means ± SD (range) of parameters from Japanese macaques in captive group (CG) and free-ranging group (FRG)

Parameters	CG		FRG		*P* values	
	Females	Males	Females	Males	Females CG vs. FRG	Males CG vs. FRG
Age (years)	19.9 ± 5.57 (9.1–29.3)	14.1 ± 4.61 (7.9–28.7)	11.9 ± 5.58 (5.7–22.6)	12.1 ± 5.89 (5.5–22.6)	< 0.000*	< 0.193
BM (kg)	9.50 ± 2.49 (6.02–16.74)	11.91 ± 1.78 (9.22–15.92)	6.12 ± 0.86 (4.75–7.90)	7.84 ± 2.02 (4.25–10.70)	< 0.000*	< 0.000*
BMI (kg/m^2)	30.51 ± 8.85 (20.34–59.15)	29.65 ± 3.91 (24.68–39.98)	23.47 ± 1.77 (19.57–26.36)	26.47 ± 2.93 (21.59–30.81)	< 0.002*	< 0.002*
DSN scores	0.75 ± 0.82 (0–2.83)	0.37 ± 0.52 (0–1.83)	0.06 ± 0.13 (0–0.5)	0.11 ± 0.21 (0–0.83)	< 0.001*,< 0.026+	< 0.039*,< 0.963
OST-S scores	1.10 ± 0.81 (0–2.33)	0.66 ± 0.77 (0–2.33)	0.18 ± 0.29 (0–1)	0.13 ± 0.29 (0–1.17)	< 0.000*,< 0.001+	< 0.005*,< 0.335
OST-V scores	1.18 ± 0.79 (0–2.57)	0.75 ± 0.80 (0–2.43)	0.16 ± 0.25 (0–0.83)	0.12 ± 0.28 (0–1.29)	< 0.000*,< 0.000+	< 0.002*,< 0.136

BM = body mass; BMI = body mass index like-index; DSN = disc space narrowing; OST = osteophytosis; *statistical significant differences between groups (Independent *t* test); +statistical significant differences between groups from analysis of covariance (ANCOVA) after controlling for influence of BM

Table 2.2 Disc space narrowing (DSN) and osteophytosis (OST) scores of captive (CG) and free-ranging (FRG) Japanese macaques; number of subject (% from all subjects)

		CG (female, N = 21; male, N = 22)		FRG (female, N = 31; male, N = 27)	
Female		OST >0	OST = 0	OST >0	OST = 0
	DSN >0	16 (76.19)	1 (4.76)	6 (19.36)	1 (3.23)
	DSN = 0	4 (19.05)	N/A	4 (12.90)	20 (64.52)
Male		OST >0	OST = 0	OST >0	OST = 0
	DSN >0	11 (50.00)	2 (9.09)	3 (11.11)	6 (22.22)
	DSN = 0	4 (18.18)	5 (22.73)	3 (11.11)	15 (55.56)

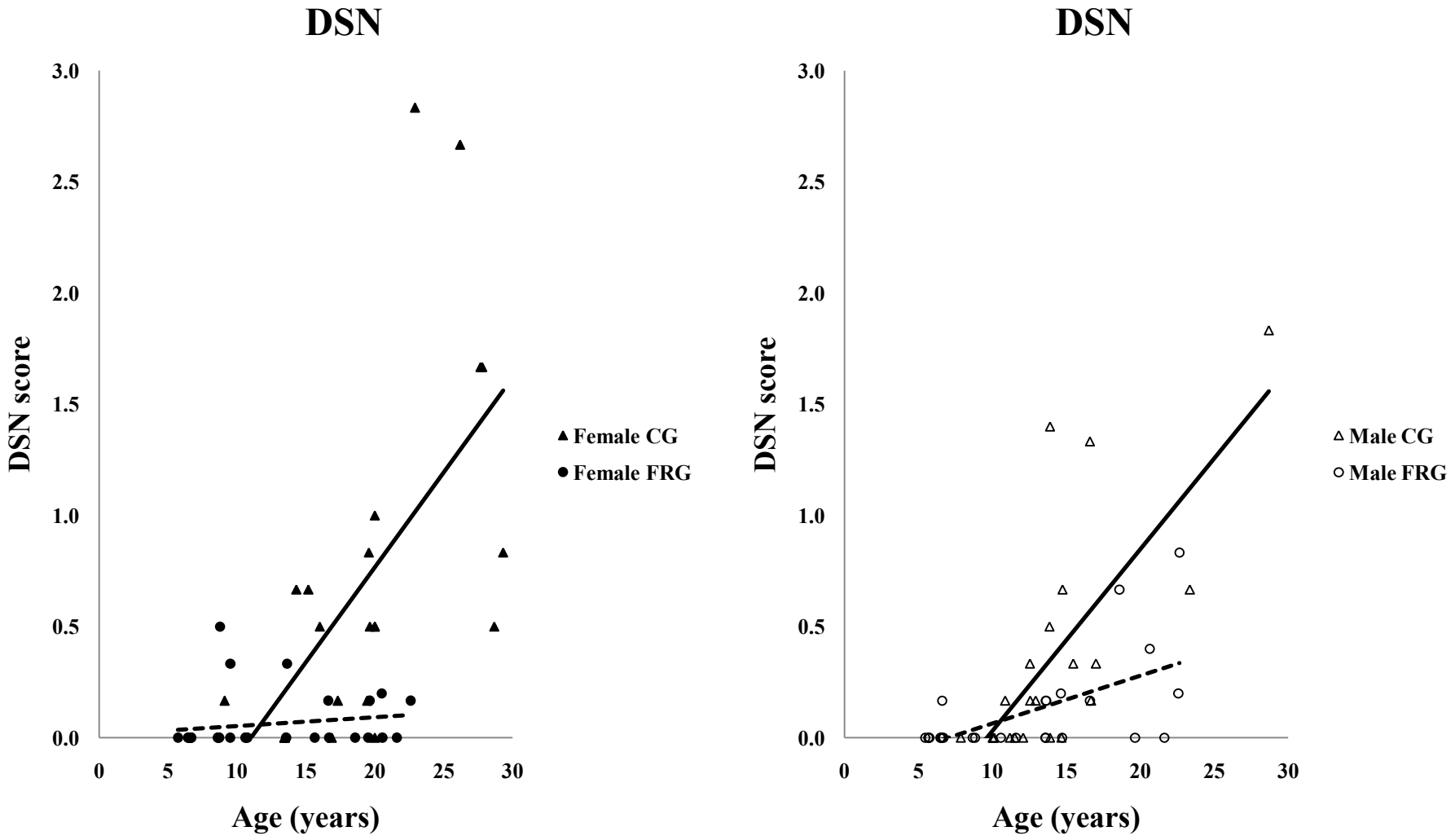

Figure 2.2 Comparisons of average disc space narrowing (DSN) scores between females from captive group (CG) and free-ranging group (FRG), and between males from CG and FRG. *Solid trend lines* = monkeys from CG, *dotted trend lines* = monkeys from FRG

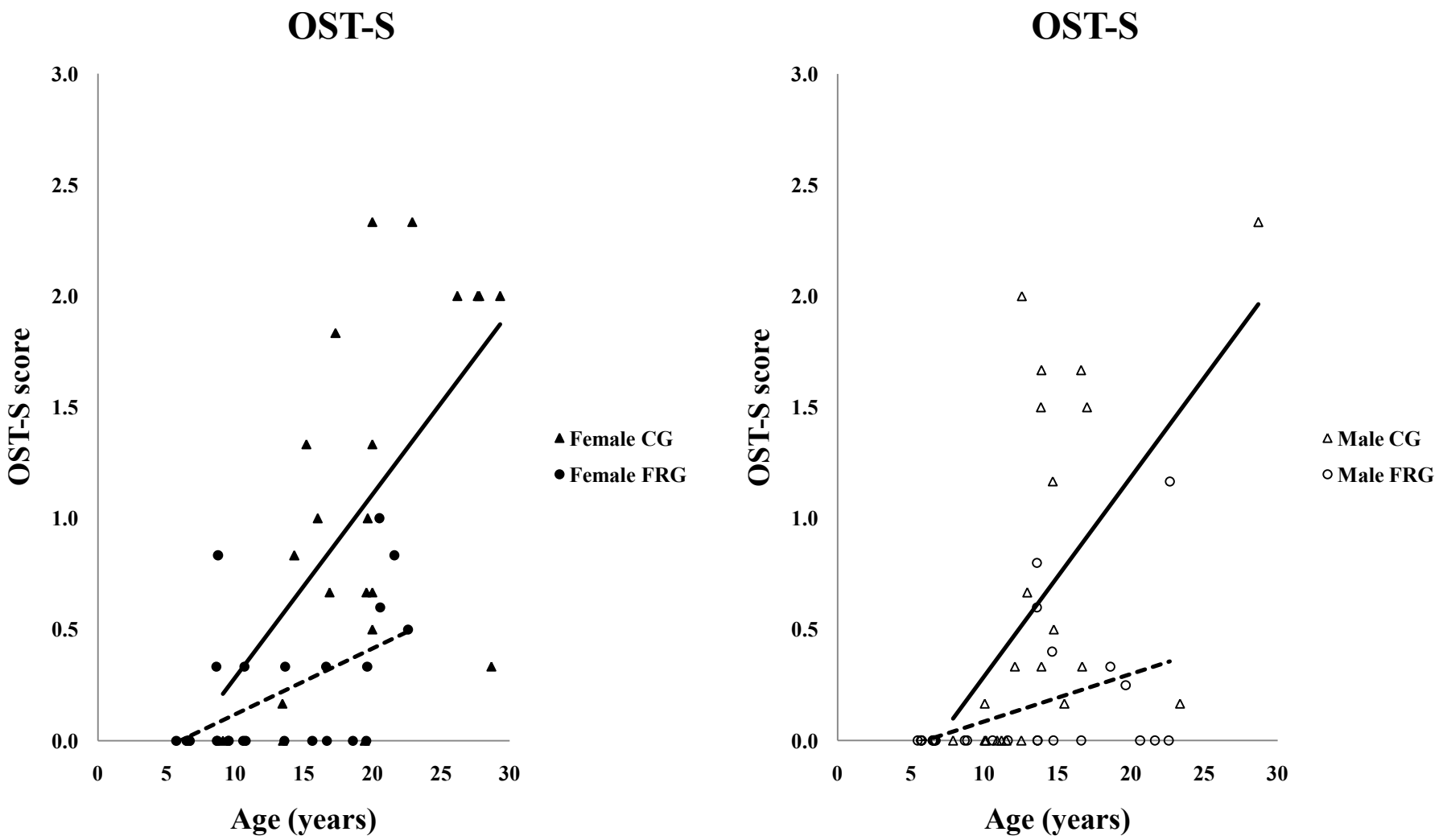

Figure 2.3 Comparisons of osteophytosis (OST-S) scores between females from captive group (CG) and free-ranging group (FRG), and between males from CG and FRG. *Solid trend lines* = monkeys from CG, *dotted trend lines* = monkeys from FRG

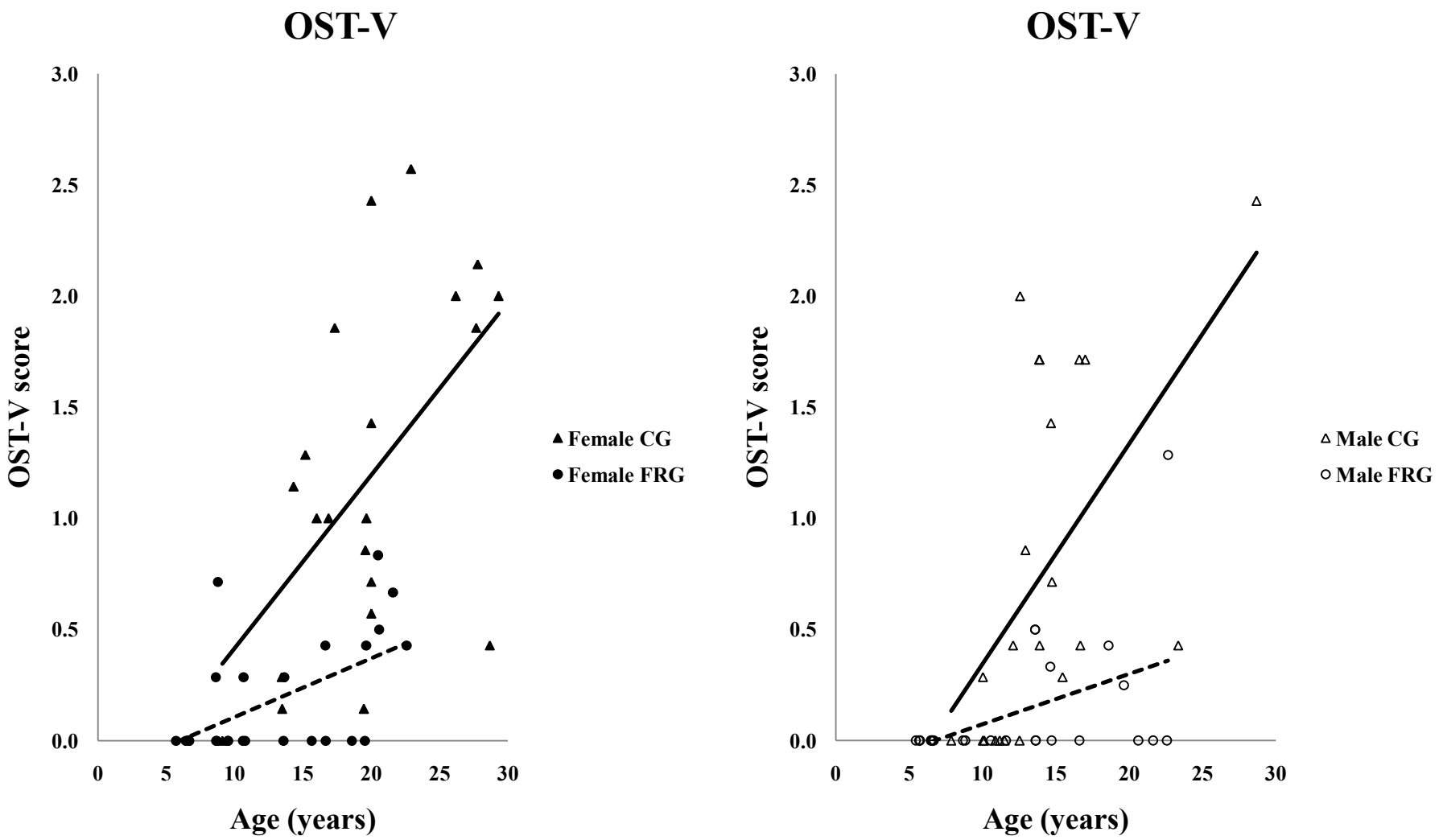

Figure 2.4 Comparisons of osteophytosis (OST-V) scores between females from captive group (CG) and free-ranging group (FRG), and between males from CG and FRG. *Solid trend lines* = monkeys from CG, *dotted trend lines* = monkeys from FRG

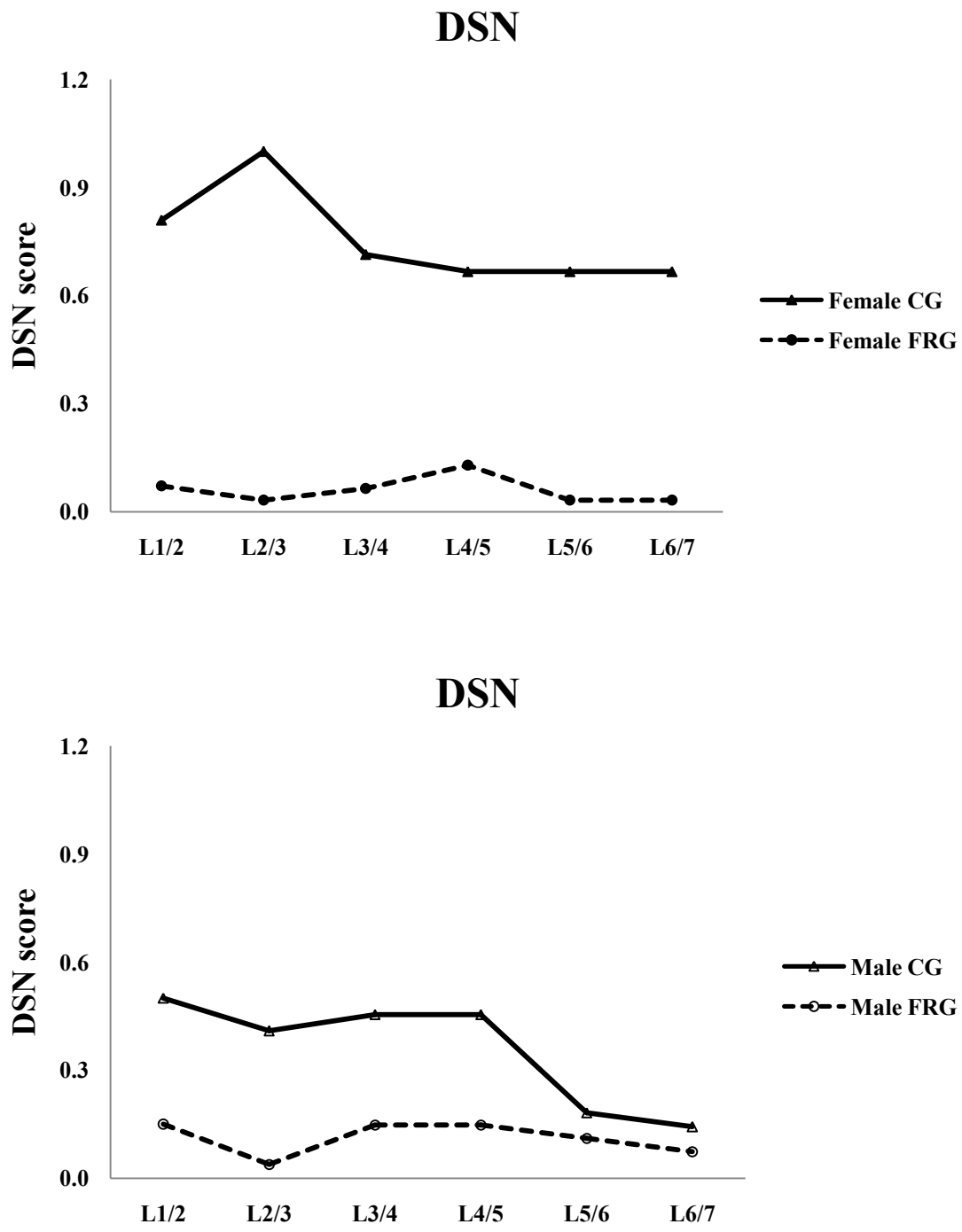

Figure 2.5 Patterns of disc space narrowing (DSN) along the lumbar vertebral region of monkeys from captive group (CG) and free-ranging group (FRG)

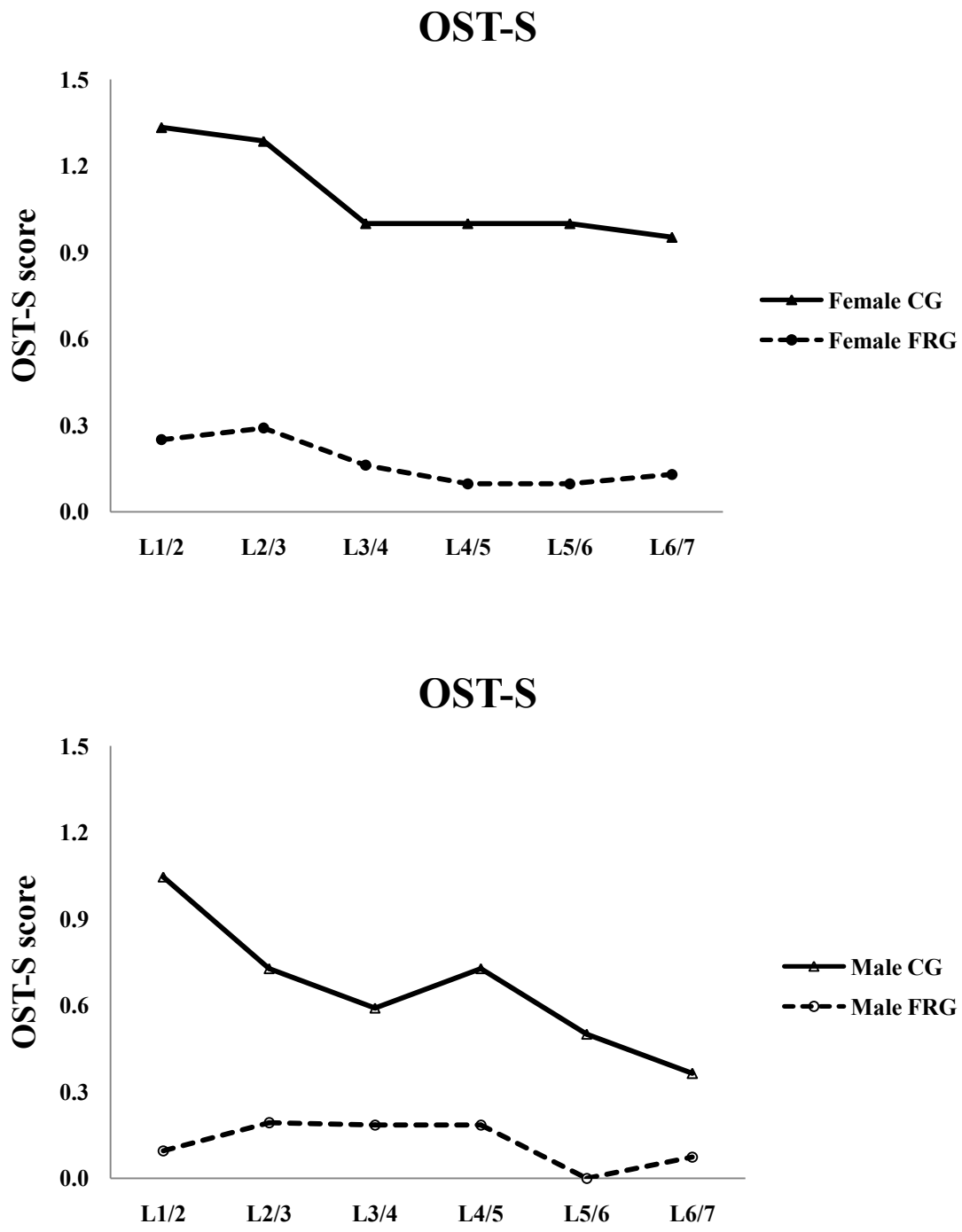

Figure 2.6 Patterns of osteophytosis (OST-S) along the lumbar vertebral region of monkeys from captive group (CG) and free-ranging group (FRG)

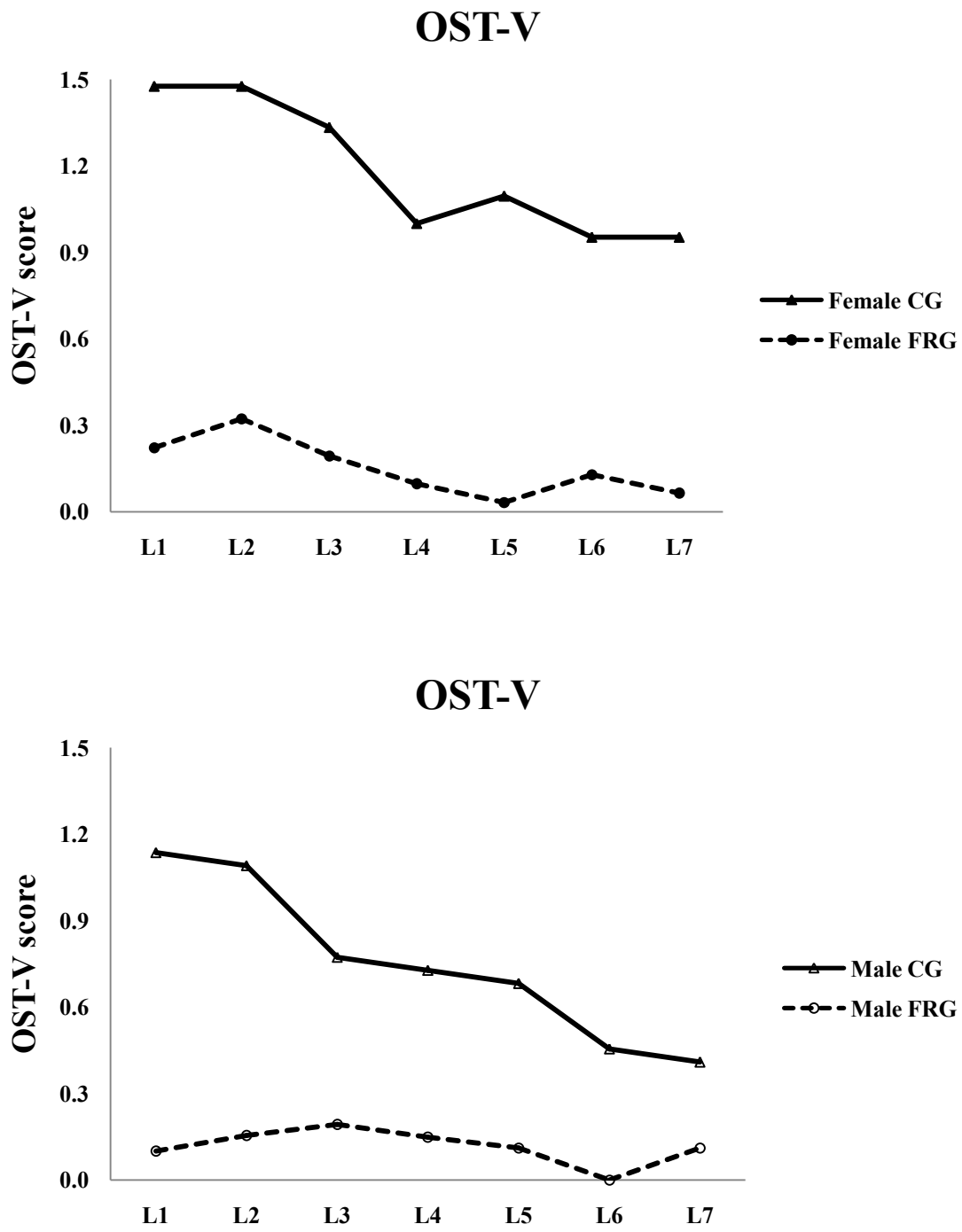

Figure 2.7 Patterns of osteophytosis (OST-V) along the lumbar vertebral region of monkeys from captive group (CG) and free-ranging group (FRG)

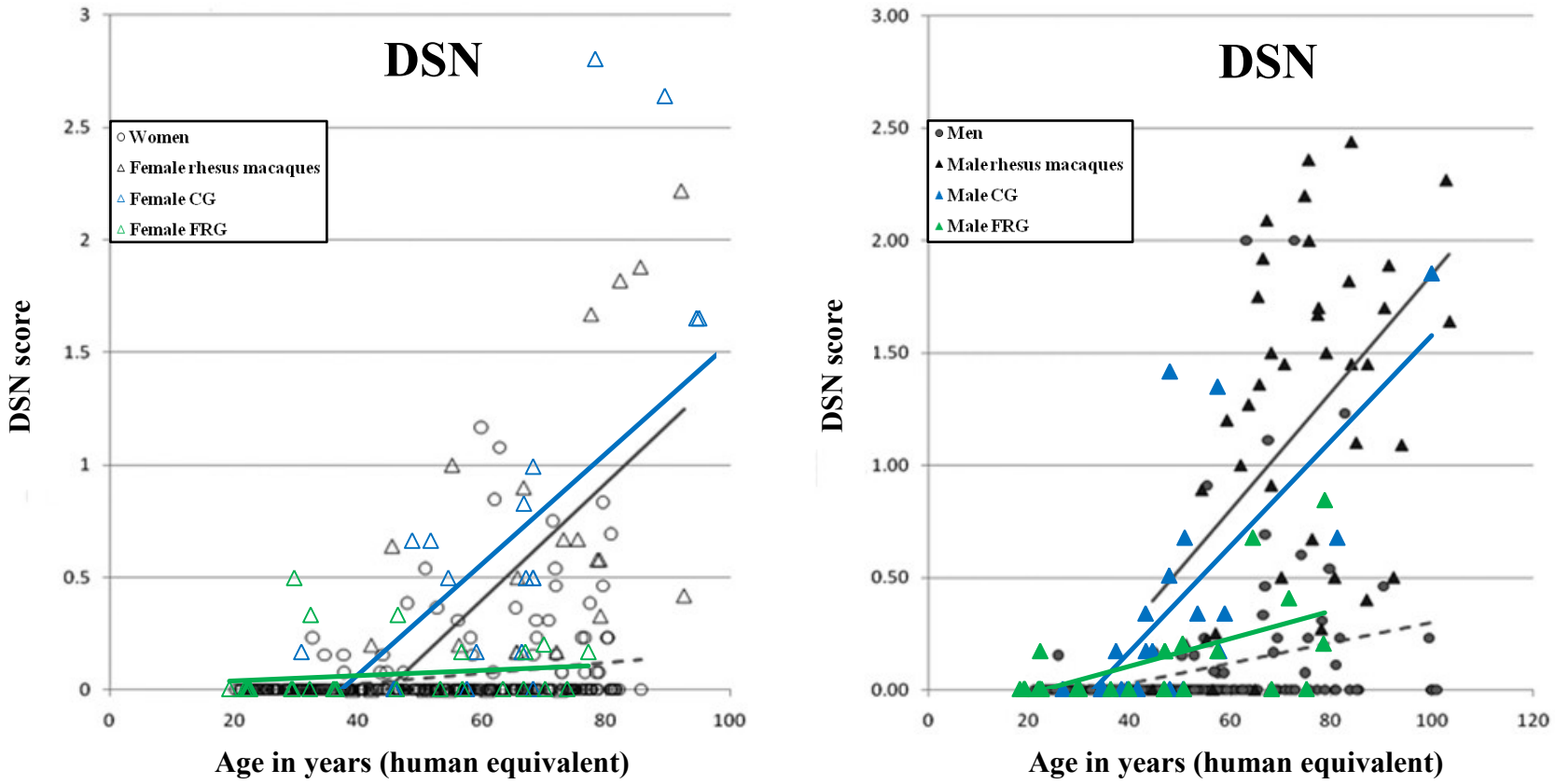

Figure 2.8 The comparisons between age (human equivalent) and disc space narrowing (DSN) scores in humans, rhesus macaques, and Japanese macaques from captive group (CG) and free-ranging group (FRG). *Left panel* = women vs. female macaques, *right panel* = men vs. male macaques, *black dash trend lines* = humans, *black solid trend lines* = rhesus macaques, *blue solid trend lines* = Japanese macaques from CG, *green solid trend lines* = Japanese macaques from FRG

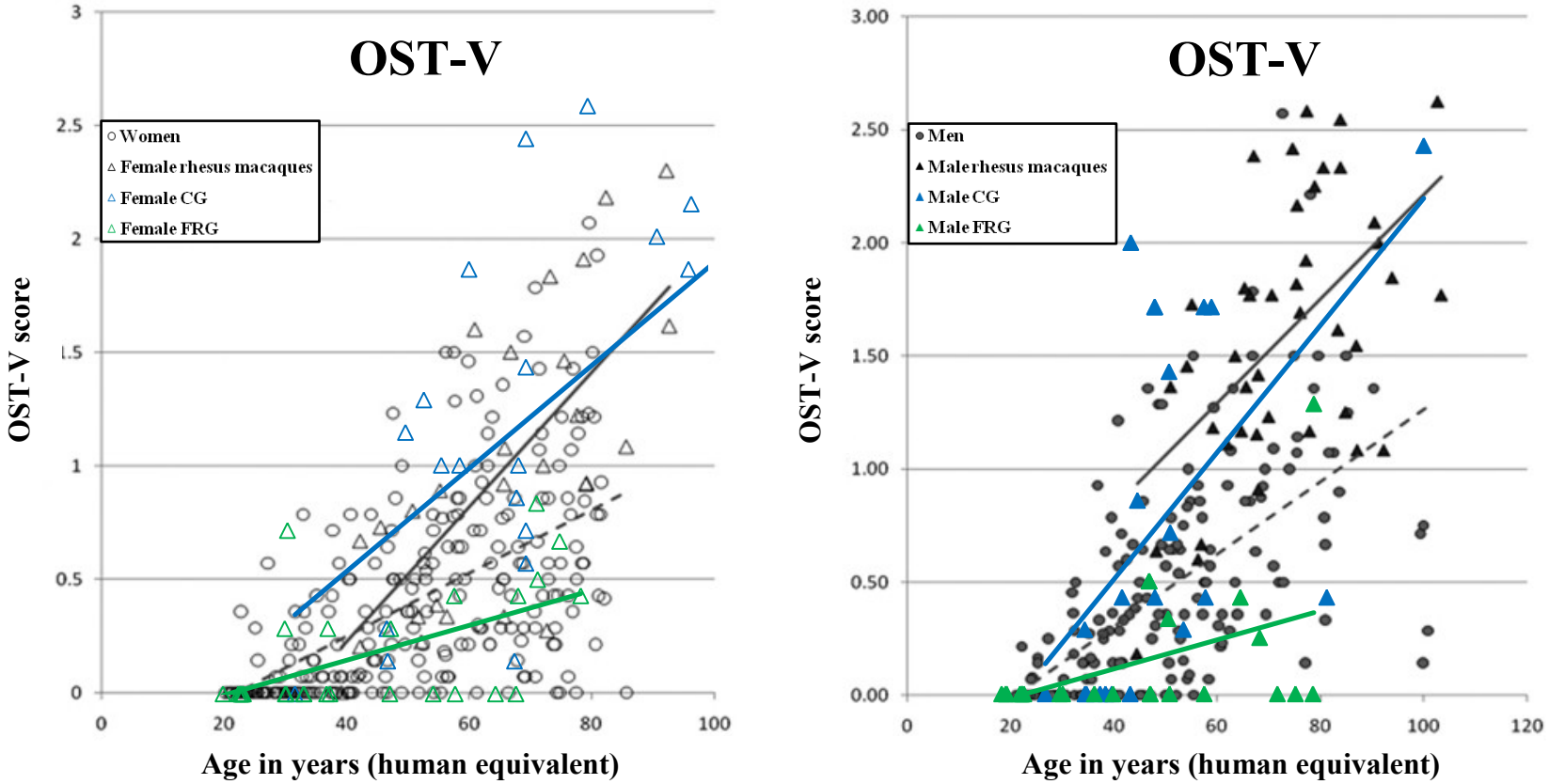

Figure 2.9 The relationship between age (human equivalent) and osteophytosis (OST-V) scores in humans, rhesus macaques, and Japanese macaques from captive group (CG) and free-ranging group (FRG). *Left panel* = women vs. female macaques, *right panel* = men vs. male macaques, *black dash trend lines* = humans, *black solid trend lines* = rhesus macaques, *blue solid trend lines* = Japanese macaques from CG, *green solid trend lines* = Japanese macaques from FRG

Discussion

The prevalence and severity of OA were greatly different between macaques and humans (Kramer et al. 2002; Duncan et al. 2012; present study). It is also noticeable that captive macaques have dramatically higher prevalence and severity of OA than humans, even though adopting parameter as high as 3.5 for age equivalence. Anatomy of vertebrae, loading, size, vertebral orientation and vertebral curvature (Alini et al. 2008; Duncan et al. 2012) possibly cause the differences of OA. However, at present decisive cause has not been given. The finding is FRG Japanese macaques had significantly less prevalence and severity of DSN and OST than CG counterparts, which is rather similar to those in humans.

There are potentially several factors related to OA such as age, sex, genetic predisposition, obesity, nutrition, lifestyle and physics (Williams and Spector 2006; Kalichman and Hunter 2007). In this study, however, I mainly focus on BM and physical activity that may influence on OA in Japanese macaques from CG and FRG.

In the present study, I tried to control the influence of age and BM which were considered to relate with OA development in humans and to highlight on physical activity. After controlling for the influence of BM, the significant differences of OA among females remained. Thus, other factors; for instance, uncontrolled factor like age in females and physical factor should play an important role among the two groups.

Vertebral OA clearly increases severity with advancing age in macaques (DeRousseau 1985; Kramer et al. 2002; present study) and in humans (Duncan et al. 2012). Although the variations of DSN and OST with age are wide (Kramer et al. 2002; Duncan et al. 2012), after Japanese macaques reach puberty around 4 years of age (Black and Lane 2002) DSN and OST can be detected. The youngest monkey presented DSN was 6.6 years of age that is later than the youngest pig-tailed macaque of Kramer et al. (2002) having DSN at 4.7 years of age. Caged-living monkeys had more DSN and OST severity than FRG monkeys and achieved the severest stage of DSN and OST (maximum score is 3) compared with those in FRG (maximum score is 2).

From the patterns of DSN and OST along the lumbar vertebral region, Japanese macaques studied tend to have maximum OA at the upper lumbar region (thoracolumbar) in both sexes like that in female pig-tailed macaques (Kramer et al. 2002). It is suggested that macaques have the pattern of cranio-caudal cline of severity in DSN and OST along the lumbar vertebrae (DeRousseau 1985; Kramer et al. 2002; present study). DSN and OST may

be also severe in lumbosacral joints (Duncan et al. 2012) which is not found in the present study.

In humans, high BM or high BMI is associated with high prevalence and severity of OA at weight-bearing joints (e.g. Felton et al. 2000). OA is caused by compressive stresses on the weight-bearing joints (Rogers 1982). There are scarcity of reports examining relationship between diet restriction influenced on BM, and then on the development of OA in monkeys. However, the prevalence and severity of OA in several joints were less observed in restricted-feed dogs with significantly lower BM compared with dogs without food restriction (Kealy at al. 2000; Smith et al. 2006). It seems likely that lighter BM of FRG monkeys relates with less severe OA compared with CG monkeys having higher BM and more severe OA.

Over BM or obesity has been observed in individual-caged macaques (e.g. Hansen and Bodkin 1986). CG Japanese macaques housed in individual cages were significantly heavier than those from FRG. There are some reasons possibly associated with the heavier of CG and smaller BM in FRG monkeys. Firstly, since monkeys reared in individual cages lack of exercise and their physical activity was limited which may lead to higher fat mass. Secondly, FRG monkeys living in Koshima Islet have the smallest body size, in linear dimension, among Japanese macaque populations distributed in mainland Japan, including CG monkeys (from Arashiyama, Takahama and Wakasa) (Hamada et al. 1986; Kimura and Hamada 1995). In addition, because the habitat of FRG monkeys, Koshima Islet, has small area, about 0.3 km^2, the monkeys have been under the poor nutritional condition which may affect their BM (Kimura and Hamada 1995).

In females, even though BM was controlled, the differences of OA between groups still remained, which cannot be fully explained by different average age between the two groups. Thus physical activity which has been considered to be highly related to prevalence and severity of OA was discussed.

In human studies, both excessive stress and lack of normal stress correlated with development of OA (Whiting and Zernicke 2008). Excessive stress is observed in persons engaged with strenuous sports (Arokoski et al. 2000) or who engaged in heavy load working occupation (Felson et al. 1991). Lack of stress from sedentary lifestyle or sedentary occupation tended to increase risk of OA (Cooper et al. 1994). Therefore, normal or optimal stress is crucial for the preservation of normal joint condition that might be associated with FRG Japanese macaques.

Sitting posture should be considered as another possible factor on development of OA. Free-ranging monkeys commonly perform a variety of positional behavior compared with captive monkeys in particular those reared in individual cages. To my knowledge, there are few reports on positional behavior of free-ranging Japanese macaques (Kawai et al. 1970; Chatani 2003). A juvenile male monkey spent most of daily activity in sitting rather than other postures or locomotion (Kawai et al. 1970). Other ages and sexes of monkeys may have different patterns of positional activity, though the frequency of sitting is not less than that in the juvenile male. Chatani (2003) studied positional behavior of adult free-ranging monkeys and the author found that they used sitting posture most frequently and in the longest duration.

Vertebral balance and curvature determine the stress pattern, which influence on development of OA (Lauerman et al. 1992), and thus the posture of lumbar vertebrae is important. Vertebral OA in humans arises from stresses due to vertebral curvature and weight-bearing in upright posture of bipedalism (Bridges 1994). The sitting posture has been considered to relate with the development of vertebral OA in humans (Hadjipavlou et al. 1999) and in monkeys (Lauerman et al. 1992; Kramer et al. 2002). Although the monkeys are quadruped but they mainly spend time in the upright sitting posture, thus the upright alignment of vertebrae undergoes the accumulatively and repetitively stress of gravity force involved in development of OA (Lauerman et al. 1992; Kramer et al. 2002). Furthermore, the frequency and incidence of OST were clearly observed at the points of maximum curvature of the human vertebrae, while the areas lying along with the center of gravity showed the lowest frequency of OST (Nathan 1962). In the findings, I hold the view that in sitting posture, the vertebral column of Japanese macaques is apparently kyphotic at the thoracolumbar part and flat at the lumbosacral part (Kramer et al. 2002). The lumbar vertebrae especially at the thoracolumbar part would experience repetitively the greatest stress, which finally results in development of OST (Nathan 1962) and degeneration of intervertebral discs if the discs continue in flexed posture or hyperflexion for a long time (Adams and Hutton 1985). Even though free-ranging Japanese macaques have a tendency to use sitting posture more frequently than other positions in their activity life, the physical activity of Japanese macaques reared in individual cages was clearly limited that they spent most of their time in sitting position (personal observation). It is possible that the sitting posture in Japanese macaques combining with vertebral curvature especially at the thoracolumbar region causes onset and severity of OA. Thus, individual-caged monkeys tend to be more suffered from the development of OA by restricted movement as described above.

In conclusion, CG macaques, in particular those reared in individual cages had dramatically high prevalence and severity of OA compared with humans and FRG Japanese macaques. FRG Japanese macaques showed significantly less development of OA, which is rather comparable with humans. Although there are several risk factors such as genetics influencing on development of OA, I suggest that BM and physical activity should be considered as the factors influencing on the prevalence and severity of OA between CG and FRG Japanese macaques.

Chapter 3

Relation between bone mass and osteoarthritis in female Japanese and cynomolgus macaques

Introduction

Osteoporosis (OP) and osteoarthritis (OA) are two common age-related disorders affecting older people worldwide (Stewart and Black 2000). OP is the state of low bone mass, measured as bone mineral density (BMD) and bone mineral content (BMC), represented by deterioration of microarchitecture of bone tissue, and finally results in fragility and higher fracture risk, especially at the distal radius, proximal femur, and vertebra (Cerroni et al. 2000). OA is a joint disorder which is observed using radiographs, represented by joint space narrowing, bone spur or osteophyte, and endplate sclerosis (Lane et al. 1993). The most commonly joints suffered from OA is the weight-bearing joints such as knee, hand, hip and vertebra (Rogers et al. 2004).

OP and OA increase severity with advancing age but the relation between these disorders is still in controversy (Sambrook and Naganathan 1997). Bone mass at the sites affected by OA is abnormally high because of the development of osteophytosis (OST) and sclerosis (Peel et al. 1995; Agarwal 2001; Pomchote 2015). Furthermore, the etiology of OP and OA is multifactorial such as hormonal status, body mass (BM), food or physical activity (Dequeker et al. 2003; Chapter 2).

Previous studies conducted on humans found correlation between BMD at several sites of skeleton and disc space narrowing (DSN) at the vertebral region (e.g. Ichchou et al. 2010), while others only reported the relationship between BMD and OST (e.g. Pye et al. 2006). On the contrary, the other studies found no association between BMD and OA (e.g. Muraki et al. 2004; in rhesus macaques, Grynpas et al. 1993).

Macaques (genus *Macaca*) show acceleration in bone loss due to estrogen depletion (Champ et al. 1996; Colman et al. 1999a; Chen et al. 2000) and an increase in prevalence and severity of OA (Kramer et al. 2002; Duncan et al. 2011). Therefore, they have been considered as useful subjects for studies on aging and maintenance of skeletal structure (Black and Lane 2002).

Japanese macaques (*Macaca fuscata*) and cynomolgus macaques (*M. fascicularis*) are closely related with each other (Fooden 1976), though they showed various species specific characteristics mainly related to BM, which may influence their bone mass and OA. Japanese

macaques have more than two times of the adult BM compared with cynomolgus macaques (Black and Lane 2002). The two macaque species also differ in lifestyle, that is, semi-terrestrial (Japanese macaques) vs. arboreal (cynomolgus macaques) (Kikuchi 2004).

Reproductive aging in particular estrogen depletion resulting from loss of ovarian activity plays a crucial role in bone loss (Clarke and Khosla 2010a, b) and possibly relates to the development of OA (Tanamas et al. 2011). Macaques have a short postmenopausal life, therefore older females provided for menopausal research are very limited (Bellino and Wise 2003). Colman et al. (1999a) found low bone mass correlated with postmenopausal life in female rhesus macaques (*M. mulatta*) (averaged age 25.5 years); in contrast Kikuchi (2003) did not observe decrease in bone mass in female Japanese macaques (aged range from 0–27 years). Using postmenopausal female rhesus macaques (N = 10, >28 years), Champ et al. (1996) did not show any relation between period of estrogen depletion and bone mass.

In the present study, I investigated age-related and reproductive aging-related bone changes in terms of bone mass (BMDs and BMCs), OA (DSN and OST) and body size (BM and body mass index like-index (BMI)), and relation between bone mass and OA at the different skeleton sites in female Japanese and cynomolgus macaques which have different BM and lifestyle using cross-sectional data.

Materials and Methods

Japanese macaques (*M. fuscata*)

Sixty one female Japanese macaques were used in the present study. They were maintained at the Primate Research Institute of Kyoto University in Japan. They were born and reared at the outdoor corral enclosures (length about 20 to 100 m, width about 20 to 30 m). They originate from Arashiyama, Takahama and Wakasa groups having similar body size and growth patterns (Hamada et al. 1986). Among 61 monkeys, 21 were moved and kept in individual cages in an indoor room in which the temperature and light were controlled, and 19 monkeys were kept in one-male harem groups. They were fed daily with commercial monkey chows (AS, Oriental Yeast Co. Ltd., Tokyo, Japan) and supplemented with sweet potatoes. Water was available *ad libitum*. They had the exact birth records and complete medical histories. Monkeys were weighed (BM, kg) and were measured crown-rump length (CRL, m) at the time of inspection after anesthesia using ketamine hydrochloride (Ketalar, Sankyo Co. Ltd.; 5 mg/BM) and medetomidine hydrochloride (Domitor, Meiji Co. Ltd.; 0.025 mg/BM), and supplemented with 0.5–2.0 % sevoflurane (Pfizer and Mylan, Co. Ltd.) (for old monkeys). Next, BMI was calculated by dividing BM (kg) by the square of CRL (m). I followed the guidelines of the Primate Research Institute of Kyoto University and carried out this study with permission from the ethical committee.

Since the menstrual records were incomplete, I could not classify Japanese macaques into different menstrual groups. The average age of Japanese macaques at the last parturition was 21.7 years and menopause would be a few years later (Takahata et al. 1995) with considerable inter-individual variation in the post-menopausal life (Shimizu 2007). From 61 monkeys, 55 monkeys (age ranged from 6.3–24.1 years) were classified as <25 years of age group (U_{25}) and the remaining 6 monkeys (age ranged from 25.8–29.3 years) were determined as ≥25 years of age group (O_{25}).

Cynomolgus macaques (*M. fascicularis*)

Eighty four female cynomolgus macaques born at the Tsukuba Primate Research Center, National Institute of Biomedical Innovation in Japan were used. They were housed in individual cages in an indoor room. The temperature, humidity and artificial light were controlled and described elsewhere (Chen et al. 2000; Yoshida 2005). They were fed with commercial monkey chows (CSK-2, Clea Japan Co. Ltd.) and apples. Water was available *ad*

libitum. They were anesthetized using 5–10 mg/kg BM ketamine hydrochloride (Ketalar, Daiichi-Sankyo Co. Ltd.) and 2 mg/kg BM xylazine (Selactar, Bayer Health Care Co. Ltd.), and combined with 0.3–0.5 % isoflurane (Forane, Abbott Co. Ltd.) (for old monkeys). BM (kg) and CRL (m) were recorded after anesthesia and BMI was calculated as BM/CRL^2. Birth dates and medical histories have been recorded accurately. Treatment and care of monkeys were followed the guidelines and permission from the ethical committee of the Tsukuba Primate Research Center.

All monkeys were observed daily for menstrual bleeding and then the data were recorded. Seventy three monkeys (age ranged from 5 to 27 years) were classified as premenopausal (Pre) because they were continuing menstrual cycles defined by menstrual bleeding. Nine monkeys (age ranged from 31 to 37 years) were considered as postmenopausal (Post) as determined by the absence of menstrual bleeding and no menstrual cycle for ≥2 years since the last menstrual bleeding has occurred.

Peripheral quantitative computed tomography (pQCT)

A peripheral quantitative computed tomography (pQCT) machine (XCT Research SA+; Norland Stratec Inc.) was used to examine distal parts of right radius. Before the scout scan, the radius length was measured from the radial head to the end of the styloid process using a spreading caliper. Then, the distal forearm of a monkey was inserted to the gantry of pQCT machine. Next, the distal forearm was fixed by a holding stand perpendicularly to the gantry plane, and the fingers were placed on an adjustable base. After the scout scan, the reference line was set manually at the intersection of the joint space with the radius-ulnar junction (Riggs et al. 2008). From this reference line, the 5 % (trabecular site) and 20 % (cortical site) positions of the radius length from the reference line were scanned. Scan setting was as follows: tube voltage/current = 50.4 kV, 0.281 mA; collimation B, 0.25 x 0.9 mm for small animals and excised bones, 0.5 mm slice thickness, 1 block (160 projections), 0.1 mm voxel size and a 6 mm/s scan speed. The 5 % position was measured for trabecular BMD (TbBMD) and trabecular BMC (TbBMC) with the following parameters: a threshold of 350 mg/cm^3 at contour mode 3 was used to separate the bone areas from soft tissue. An inner threshold of 500 mg/cm^3 was set to separate the cortical areas from the trabecular areas, followed by an additional 10.0 % contraction of the endosteal borders to eliminate any possible cortical parts (peel mode 4 with concentric peel 10 %). The cortical BMD (CorBMD) and cortical BMC (CorBMC) were obtained using a threshold of 700 mg/cm^3 with

threshold algorithm and filtration. Coefficient of variation (CV) of repositioning was smaller than 3 %.

Radiographic assessment

A lateral radiograph of lumbar vertebral region was obtained from all animals in the similar way described in Chapter 2. Every radiograph was read for DSN at each intervertebral space and OST at each anterior vertebral margin, blinded to age and species by a single observer (Porrawee Pomchote). The atlas scoring method (Lane et al. 1993; Kramer et al. 2002) was used to assess prevalence and severity of DSN and OST separately. The scores were 0 for unaffected site, 1, 2 or 3 for slight, moderate and severe involvement, respectively (see figure 2.1 in Chapter 2). Each lumbar vertebral level was scored for DSN and OST. DSN was defined by narrowing of the intervertebral disc height relative to the adjacent intervertebral spaces. OST was defined by the higher score of OST obtained from cranial and caudal sites in each vertebral body as previously described (Kramer et al. 2002).

Since the present study was concentrated on prevalence and severity of DSN and OST at the lumbar region, the evaluated sites were confined to intervertebral spaces L1/L2 through L6/L7 for DSN and vertebral bodies L1 to L7 for OST. Normally macaques have seven lumbar vertebrae (Schultz 1961). Only one cynomolgus monkey has six lumbar vertebrae, so L4 is assigned as unreadable vertebra as previous study (Kramer et al. 2002). Among 84 cynomolgus monkeys, four could not be scored for both DSN and OST because of bad quality of radiographs. All radiographs were read two different times by the same reader and the scores from the second reading were used.

Average scores of DSN and OST were calculated from L1 to L7 for each individual. The pattern of OA along the lumbar vertebral region was obtained from average scores of each lumbar vertebra from all individuals.

Statistical analysis

Statistical analyses were performed using SPSS (windows XP, version 16.0) or Excel (Microsoft Co. Ltd., version 2007). Both parametric and nonparametric tests were used, depending upon the normal distributions of the data. All variables are expressed as means ± SD (range). Independent *t* test was applied to determine significant differences of means between the two species. Whereas significant differences of means between U_{25} and O_{25} in Japanese macaques and between Pre and Post in cynomolgus macaques were determined using Mann-Whitney *U* test. After each macaque species was divided into two groups, I

analyzed relation between bone mass at the radius and OA at the lumbar region with age using linear regression in U_{25} and Pre in Japanese and cynomolgus macaques, respectively, while means ± SD were analyzed in the O_{25} of Japanese macaques and Post of cynomolgus macaques. Significant differences between the two species comparing between U_{25} and Pre, and between O_{25} and Post were determined using analysis of covariance (ANCOVA) and Mann-Whitney *U* test, respectively. Since BM is well-known to influence on bone mass and OA prevalence, BM-adjusted (Δ) bone mass and OA changes with age were assessed using the linear regression for U_{25} and Pre, and means ± SD for O_{25} and Post, respectively and Pearson's correlation (r) coefficient, then, was applied for U_{25} and Pre.

The relationships between all variables were examined using bivariate correlation analysis, Pearson's correlation coefficient. Partial correlation (r_p) analysis was applied to control for the influence of age and BM on the association between a pair of variables. DSN clearly developed in macaques with advancing age, resulting in decrease of trunk length or kyphosis, thus BMI was not included in ANCOVA and partial correlation analysis. The level of statistical significance was set at $P < 0.05$.

I compared age-related changes in DSN and OST scores between female Japanese (present study), cynomolgus (present study) and rhesus macaques (Duncan et al. 2012), and humans (Duncan et al. 2012) using the same scoring method. Macaque ages were adjusted to represent human equivalent ages with a ratio of 1:3.5 years (Duncan et al. 2012).

Results

All variables of body size and bone mass in Japanese macaques (*M. fuscata*) were significantly higher than those in cynomolgus macaques (*M. fascicularis*) except for CorBMD (Independent *t* test, Table 3.1). Since there is no significant difference in age between the two species, BM is considered to affect densitometric variables. After the influence of BM was controlled using ANCOVA, the differences in TbBMD, TbBMC and CorBMC between the two species still remained. Only CorBMC still related with BM (P <0.001) and BM explained about 84.8 % of variability in CorBMC (see table 3.4 in the appendix).

As listed in Tables 3.2 and 3.3, macaques from U_{25} in *M. fuscata* and Pre in *M. fascicularis* had higher bone mass but lower DSN and OST scores than those from O_{25} and Post, respectively; however, only *M. fascicularis* showed significant differences in all bone mass and OA between groups except for CorBMD, while *M. fuscata* showed significant differences in OA between U_{25} and O_{25}.

M. fuscata had higher BM and BMI than *M. fascicularis* between U_{25} and Pre (all P < 0.001), and between O_{25} and Post (BM, P <0.001; BMI, P <0.181), respectively. BM and BMI in the two species slightly increased in U_{25} and Pre of *M. fuscata* and *M. fascicularis*, respectively (Figure 3.1). Only the averages of BM in O_{25} and Post of *M. fuscata* and *M. fascicularis*, respectively were lower than the regression lines of U_{25} and Pre, respectively (Figure 3.1 (left panel)).

TbBMD and TbBMC of *M. fuscata* had significantly higher than *M. fascicularis* (U_{25} vs. Pre, all P <0.001; O_{25} vs. Post, P <0.003 and P <0.001, respectively) (Figure 3.2). The average TbBMD of O_{25} was higher than the extrapolation line of U_{25}, while the average TbBMD of Post was lower than the regression line of Pre (Figure 3.2 (left panel)). TbBMC also showed similar change between U_{25} and O_{25} in *M. fuscata*, and between Pre and Post in *M. fascicularis* (Figure 3.2 (right panel)). *M. fascicularis* had higher CorBMD than *M. fuscata* only between U_{25} and Pre (P <0.001), while no significant difference between O_{25} and Post was found. Decrease of CorBMD was found from U_{25} to O_{25} in *M. fuscata*, and from Pre to Post in *M. fascicularis* (Figure 3.3 (left panel)). For CorBMC, no change between the two age groups in *M. fuscata* was observed, while slight decrease from Pre to Post was observed in *M. fascicularis* (Figure 3.3 (right panel)). *M. fuscata* had significantly higher

CorBMC than *M. fascicularis* between U_{25} and Pre, and between O_{25} and Post (all P <0.001) (Figure 3.3 (right panel)).

Age changes (human equivalent) in DSN and OST scores were compared between women, female rhesus macaques (*M. mulatta*), *M. fuscata* and *M. fascicularis* (Figure 3.4, modified from Duncan et al. 2012). Women and female macaques exhibited wide inter-individual variations of DSN and OST. However, both DSN and OST prevalence significantly increased with advancing age in women and female macaques (P <0.001). Female macaques had similar trend of DSN and OST with age and they showed much higher prevalence and severity of DSN and OST than women (Figure 3.4).

The patterns of DSN and OST along the lumbar vertebral region were compared between *M. fuscata* and *M. fascicularis* (Figure 3.5). DSN and OST showed rather similar trend that the maximum DSN and OST were observed at the thoracolumbar region. Both of them showed then decrease to the midlumbar region and slight increase and final decrease. No significant difference was observed in all vertebral levels in both DSN and OST of both species.

Both DSN and OST in *M. fuscata* and *M. fascicularis* increased with age and had comparable scores both in U_{25} and O_{25}, and in Pre and Post (Figure 3.6). No significant difference in both DSN and OST between the two groups of the two species was observed (U_{25} vs. Pre, P <0.149 and P <0.875; O_{25} vs. Post, P <0.662 and P <0.199, respectively). DSN of *M. fuscata* showed significant regression line against age in U_{25}, while did not show significant regression in O_{25} (Figure 3.6 (left panel)). Extrapolation of regression line of U_{25} came close to the average DSN of O_{25}. The similar trend was observed in *M. fascicularis* (Figure 3.6 (left panel)). OST in U_{25} of *M. fuscata* showed significantly linear increase with age (Figure 3.6 (right panel)). On the contrary, in O_{25} of *M. fuscata* did not show linear change with age, and the average of the group came lower than the regression line of U_{25}. *M. fascicularis* also showed similar trend with *M. fuscata* (Figure 3.6 (right panel)).

Bone mass at the radial site and OA in lumbar vertebrae age changes which were adjusted by BM were determined within the two age groups of *M. fuscata* and the two menstrual status groups of *M. fascicularis* (Figures 3.7–3.12).

Δ bone mass of the U_{25} and Pre of *M. fuscata* and *M. fascicularis*, respectively showed significant regression lines against age (P <0.001 to 0.017), except for Δ CorBMC of Pre (Figures 3.7–3.10). Δ TbBMD, TbBMC and CorBMC manifested different age change patterns from non-adjusted TbBMD, TbBMC and CorBMC, that is, average of O_{25} of *M. fuscata* came higher than the regression line of U_{25}, and the averages Δ TbBMD, TbBMC and

CorBMC of O_{25} correspond to about 15 years of age of U_{25} (Figures 3.7, 3.8 and 3.10 (left panels)). Similar age change patterns were found in *M. fascicularis* between Pre and Post (Figures 3.7, 3.8 and 3.10 (right panels)). The averages Δ CorBMD of O_{25} in *M. fuscata* and Post in *M. fascicularis* came lower than the regression lines of U_{25} and Pre in *M. fuscata* and *M. fascicularis*, respectively and the averages Δ CorBMD of O_{25} and Post were corresponding to 15 years of age of U_{25} and Pre in *M. fuscata* and *M. fascicularis*, respectively (Figure 3.9).

Δ DSN and OST of the U_{25} and Pre of *M. fuscata* and *M. fascicularis*, respectively showed significant linear relationship with age (all P <0.001) (Figures 3.11–3.12). Δ DSN exhibited different age change pattern from non-adjusted DSN, that is, average of O_{25} of *M. fuscata* came lower than the regression line of U_{25}, and the average Δ DSN of O_{25} correspond to about 15 years of age of U_{25} (Figure 3.11 (left panel)). Similar age change patterns were found in *M. fascicularis* between Pre and Post (Figure 3.11 (right panel)). Δ OST of the average of O_{25} corresponded to about 15 years of age of U_{25} (Figure 3.12 (left panel)). Similar age change patterns were presented in *M. fascicularis* (Figure 3.12 (right panel)).

Table 3.1 Means ± SD (range) of age, body size, bone mass and osteoarthritis in female *M. fuscata* and *M. fascicularis*

	Age (years)	**BM[#] (kg)**	**BMI[#] (kg/m^2)**	**TbBMD[#] (mg/cm^3)**	**TbBMC[#] (mg/mm)**	**CorBMD[#] (mg/cm^3)**	**CorBMC[#] (mg/mm)**	**DSN[#]**	**OST[#]**
M. fuscata	16.09 ± 6.10 (6.3–29.3)	9.24 ± 1.86 (6.02–16.74)	28.93 ± 6.29 (20.34–59.15)	137.07 ± 36.95 (51.80–205.4)	4.25 ± 1.88 (1.34–8.82)	1198.02 ± 25.41 (1134.90–1255.40)	39.94 ± 4.93 (28.76–48.51)	0.48 ± 0.68 (0.00–2.83)	0.95 ± 0.84 (0.00–2.57)
M. fascicularis	16.81 ± 8.13 (5.0–37.0)	3.49 ± 0.79 (2.36–6.11)	20.39 ± 4.06 (14.57–32.73)	101.99 ± 40.62 (27.80–210.50)	1.09 ± 0.70 (0.10–3.10)	1223.13 ± 32.11 (1116.50–1283.60)	21.94 ± 3.86 (14.41–31.89)	0.56 ± 0.67 (0.00–2.50)	0.89 ± 0.80 (0.00–2.57)
P value between species									
Independent *t* test	*NS*	**	**	**	**	**	**	*NS*	*NS*
ANCOVA				++	++	*NS*	++		

[#] = abbreviations; BM = body mass; BMI = body mass index like-index; TbBMD = trabecular bone mineral density; TbBMC = trabecular bone mineral content; CorBMD = cortical bone mineral density; CorBMC = cortical bone mineral content; DSN = disc space narrowing; OST = osteophytosis; [**]statistically significant differences between the two species (Independent *t* test) at the $P < 0.01$ level; [++]statistically significant differences between the two species from analysis of covariance (ANCOVA) after controlling for BM at the $P < 0.01$ level

Table 3.2 Means ± SD (range) of age, body size, bone mass and osteoarthritis in <25 years of age group and ≥25 years of age group of female *M. fuscata*

M. fuscata	Age (years)	BM (kg)	BMI (kg/m^2)	TbBMD (mg/cm^3)	TbBMC (mg/mm)	CorBMD (mg/cm^3)	CorBMC (mg/mm)	DSN	OST
<25 years of age	14.8 ± 4.99	9.39 ± 1.86	28.91 ± 6.34	139.67 ± 37.52	4.29 ± 1.91	1196.79 ± 25.26	40.07 ± 4.91	0.38 ± 0.58	0.86 ± 0.82
	(6.3–24.1)	(6.27–16.74)	(21.32–59.15)	(51.80–205.4)	(1.34–8.82)	(1134.90–1244.30)	(28.76–48.51)	(0.00–2.83)	(0.00–2.50)
≥25 years of age	27.6 ± 1.38	8.82 ± 1.85	28.99 ± 6.36	113.70 ± 21.38	3.87 ± 1.78	1209.32 ± 226.27	38.71 ± 5.46	1.42 ± 0.85	1.71 ± 0.64
	(25.8–29.3)	(6.02–13.10)	(20.34–42.51)	(82.70–149.10)	(2.02–7.24)	(1174.30–1255.40)	(33.18–47.12)	(0.17–2.67)	(0.33–2.00)
Difference *P*	***	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	***	*

Table 3.3 Means ± SD (range) of age, body size, bone mass and osteoarthritis in premenopausal and postmenopausal of female *M. fascicularis*

M. fascicularis	Age (years)	BM (kg)	BMI (kg/m^2)	TbBMD (mg/cm^3)	TbBMC (mg/mm)	CorBMD (mg/cm^3)	CorBMC (mg/mm)	DSN	OST
Premenopausal	14.5 ± 5.64	3.52 ± 0.83	20.07 ± 4.09	106.78 ± 39.54	1.15 ± 0.72	1224.59 ± 25.97	22.52 ± 3.64	0.47 ± 0.60	0.80 ± 0.77
	(5.0–27.0)	(2.36–6.11)	(14.57–32.73)	(40.30–210.50)	(0.10–3.10)	(1160.50–1280.70)	(16.25–31.89)	(0.00–2.50)	(0.00–2.43)
Postmenopausal	34.1 ± 1.90	3.23 ± 0.44	23.14 ± 2.77	60.14 ± 28.22	0.61 ± 0.45	1207.51 ± 61.56	17.20 ± 2.54	1.67 ± 0.68	2.00 ± 0.53
	(31.0–37.0)	(2.48–4.00)	(20.16–28.14)	(72.30–177.20)	(0.19–1.47)	(1116.50–1275.30)	(14.41–22.72)	(0.50–2.50)	(1.00–2.57)
Difference *P*	***	*NS*	**	***	**	*NS*	***	***	***

Abbreviations of variables are as in Table 3.1; $^{*\text{-}***}$statistical significance in differences between groups (Mann-Whitney *U* test) at the * P <0.05, ** P <0.01 and *** P <0.001

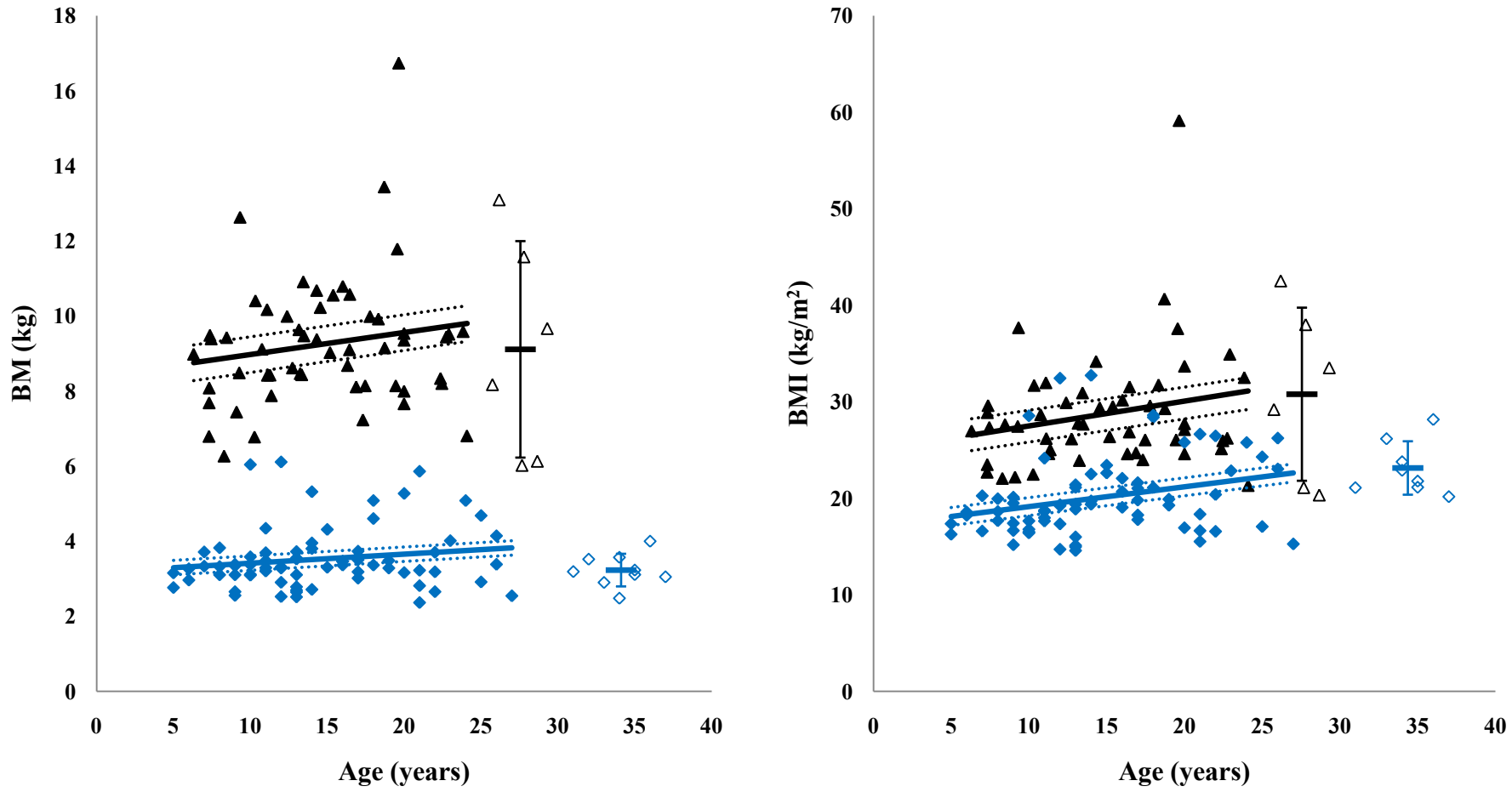

Figure 3.1 Relationships between age and body mass (BM) (left panel), and between age and body mass index-like index (BMI) (right panel) of <25 years of age group (filled triangle, black solid line) and ≥25 years of age group (open triangle) in *M. fuscata* and premenopausal (filled diamond, blue solid line) and postmenopausal (open diamond) in *M. fascicularis*. The round dotted lines indicate 95 % confidence interval of the intercept. Vertical bars indicate means ± SD

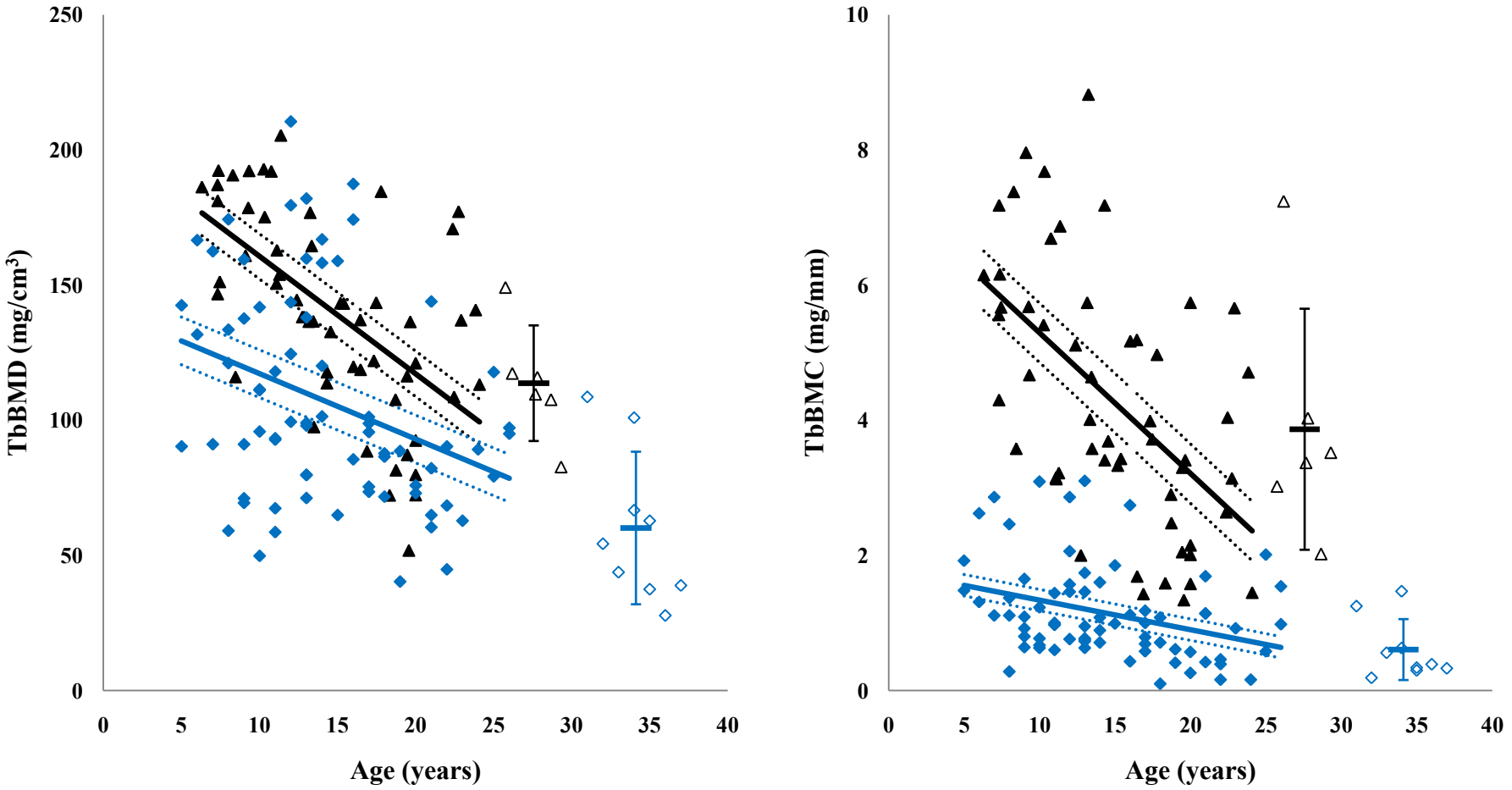

Figure 3.2 Relationships between age and trabecular bone mineral density (TbBMD) (left panel), and between age and bone mineral content (TbBMC) (right panel) of <25 years of age group (filled triangle, black solid line) and ≥25 years of age group (open triangle) in *M. fuscata* and premenopausal (filled diamond, blue solid line) and postmenopausal (open diamond) in *M. fascicularis*. The round dotted lines indicate 95 % confidence interval of the intercept. Vertical bars indicate means ± SD

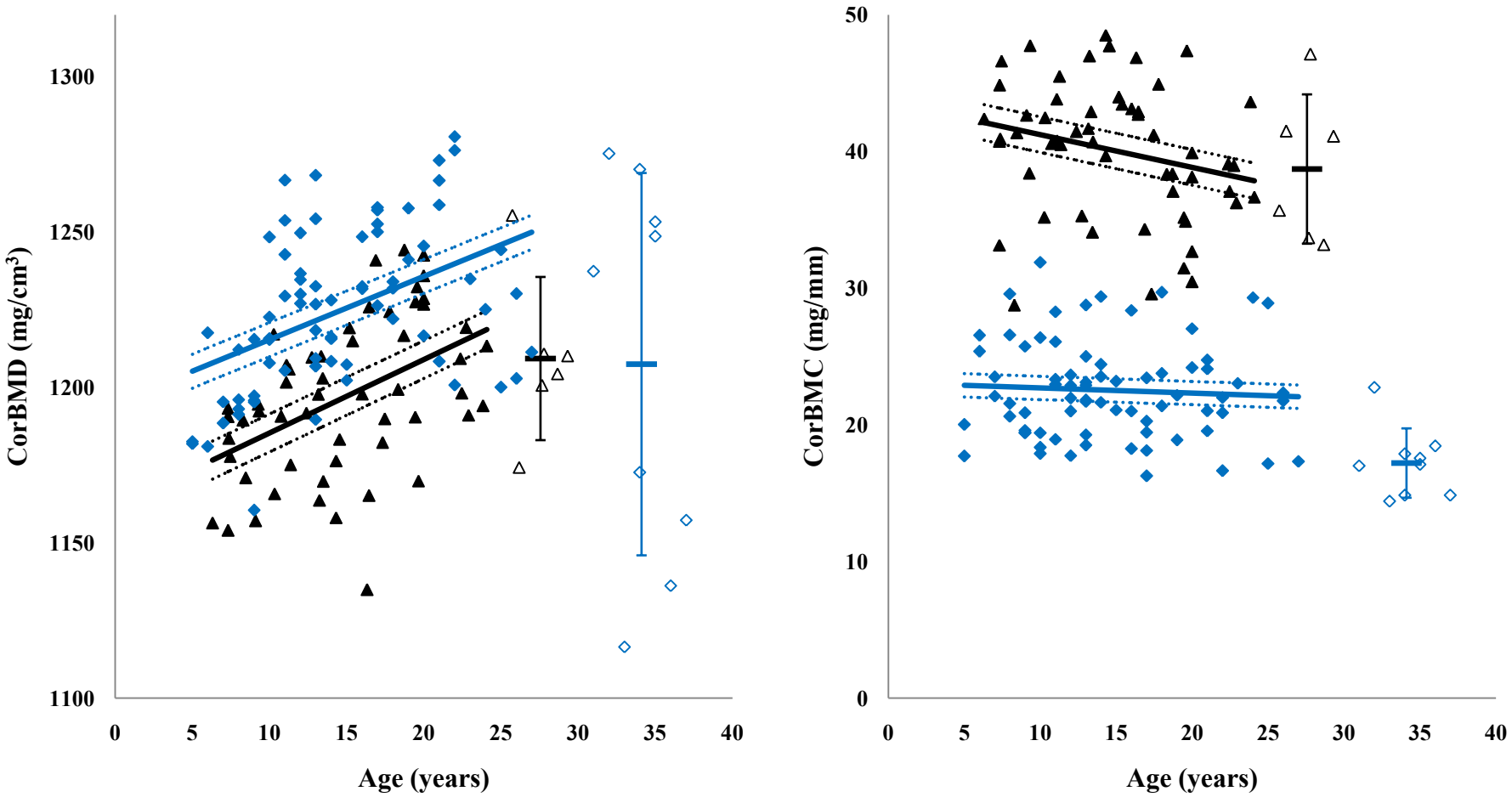

Figure 3.3 Relationships between age and cortical bone mineral density (CorBMD) (left panel), and between age and bone mineral content (CorBMC) (right panel) of <25 years of age group (filled triangle, black solid line) and ≥25 years of age group (open triangle) in *M. fuscata* and premenopausal (filled diamond, blue solid line) and postmenopausal (open diamond) in *M. fascicularis*. The round dotted lines indicate 95 % confidence interval of the intercept. Vertical bars indicate means ± SD

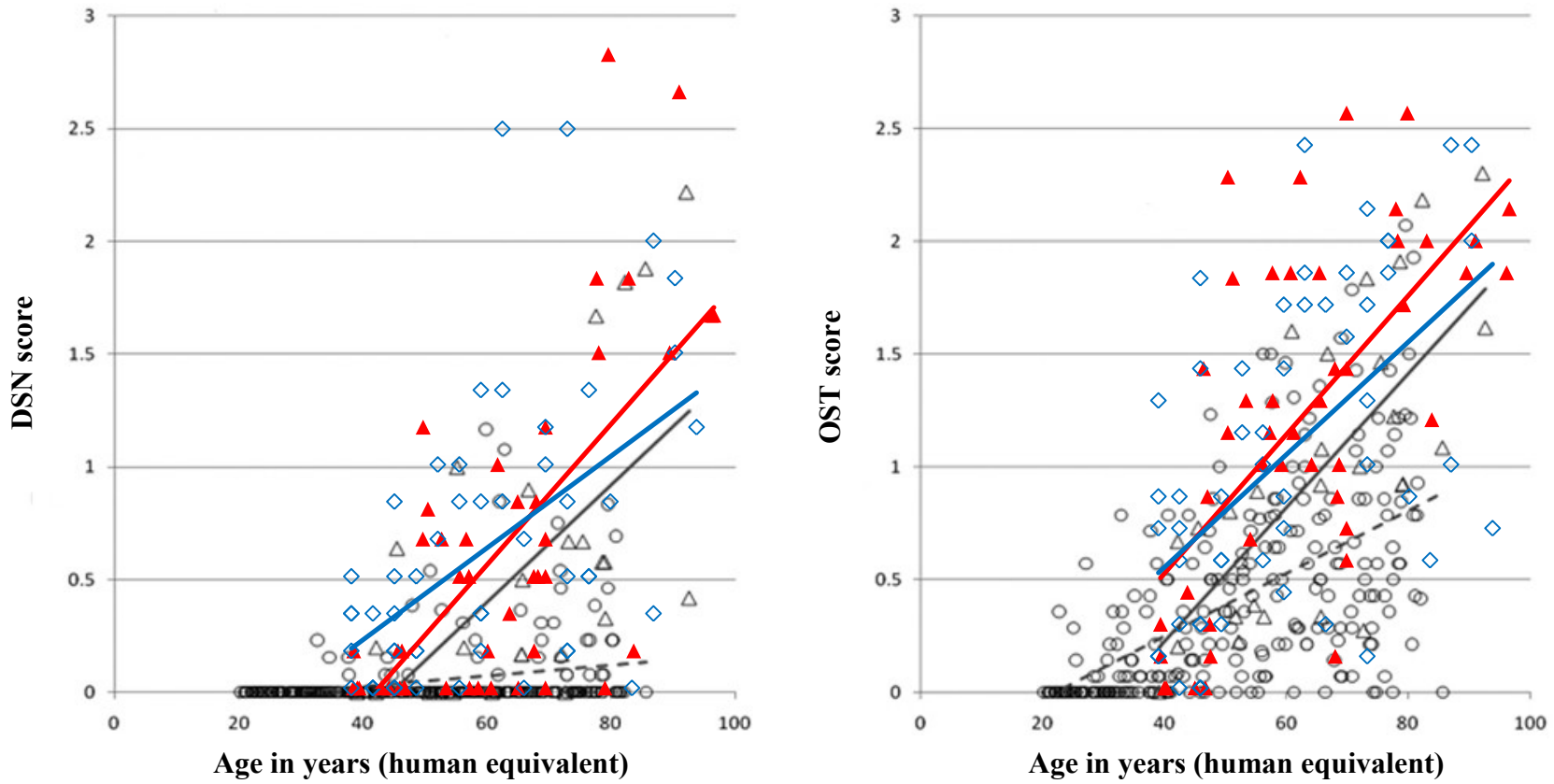

Figure 3.4 The comparisons between age (human equivalent) and disc space narrowing (DSN) (left panel) and osteophytosis (OST) (right panel) scores in women, female rhesus, Japanese and cynomolgus macaques. *Open circle* (○) *and black dash trend lines* = women, *open triangle* (△) *and black solid trend lines* = female rhesus macaques, *filled triangle* (▲) *and red solid trend lines* = female Japanese macaques, *open diamond* (◇) *and blue solid trend lines* = female cynomolgus macaques. *Note* to directly compare with women and female rhesus macaques, ages of Japanese and cynomolgus macaques start from 11.1–27.8 and 11–27 years, respectively

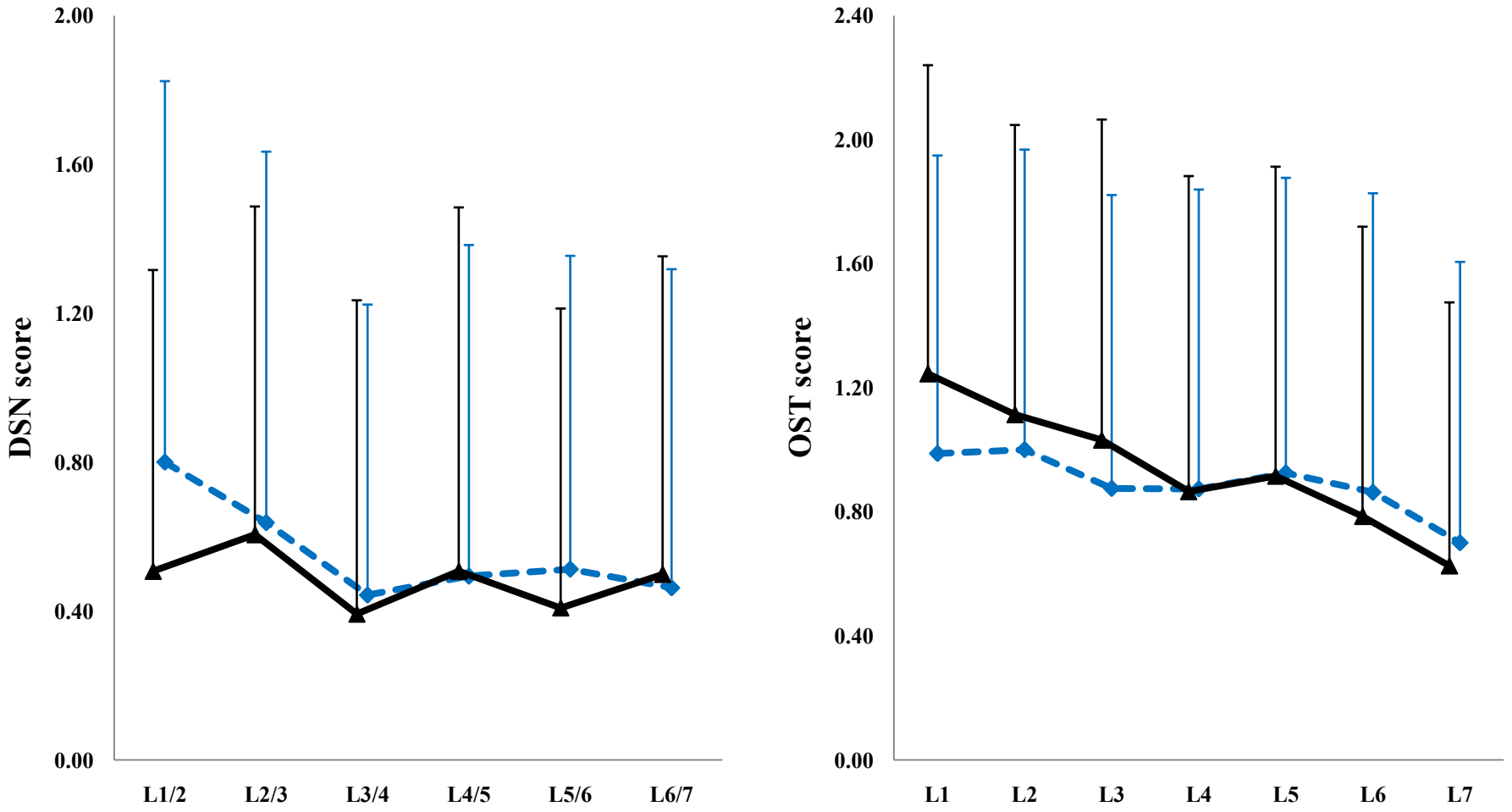

Figure 3.5 Patterns of disc space narrowing (DSN) (left panel) and osteophytosis (OST) (right panel) along the lumbar vertebral region of *M. fuscata* (black solid line) and *M. fascicularis* (blue square dotted line). Vertical bars indicate means + SD

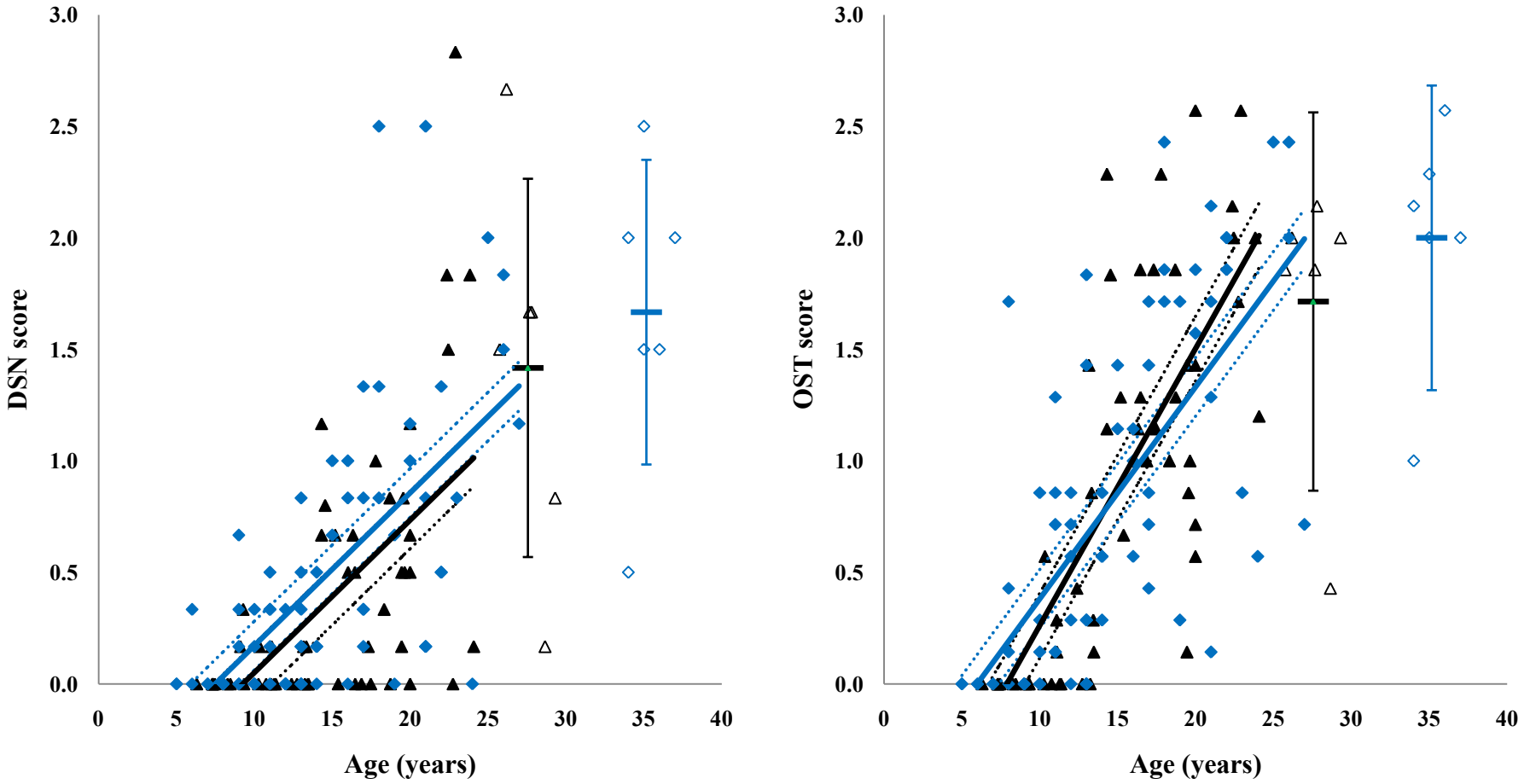

Figure 3.6 Relationships between age and disc space narrowing (DSN) scores (left panel), and between age and osteophytosis (OST) scores (right panel) of <25 years of age group (filled triangle, black solid line) and ≥25 years of age group (open triangle) in *M. fuscata* and premenopausal (filled diamond, blue solid line) and postmenopausal (open diamond) in *M. fascicularis*. The round dotted lines indicate 95 % confidence interval of the intercept. Vertical bars indicate means ± SD

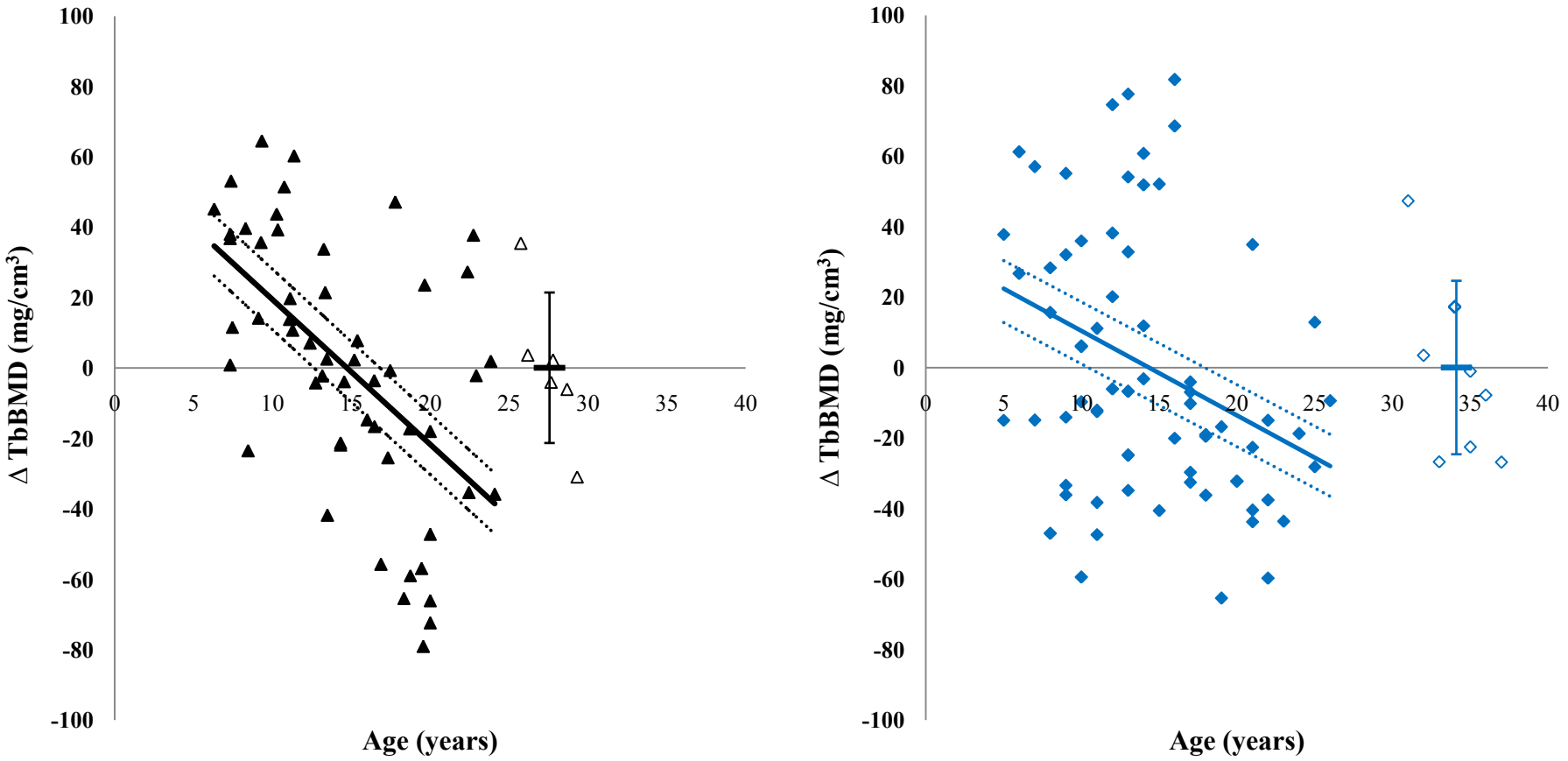

Figure 3.7 Relationships between age and body mass-adjusted trabecular bone mineral density (Δ TbBMD) of <25 years of age group (filled triangle, black solid line) and ≥25 years of age group (open triangle) in *M. fuscata* (left panel), and premenopausal (filled diamond, blue solid line) and postmenopausal (open diamond) in *M. fascicularis* (right panel). The round dotted lines indicate 95 % confidence interval of the intercept. Vertical bars indicate means ± SD

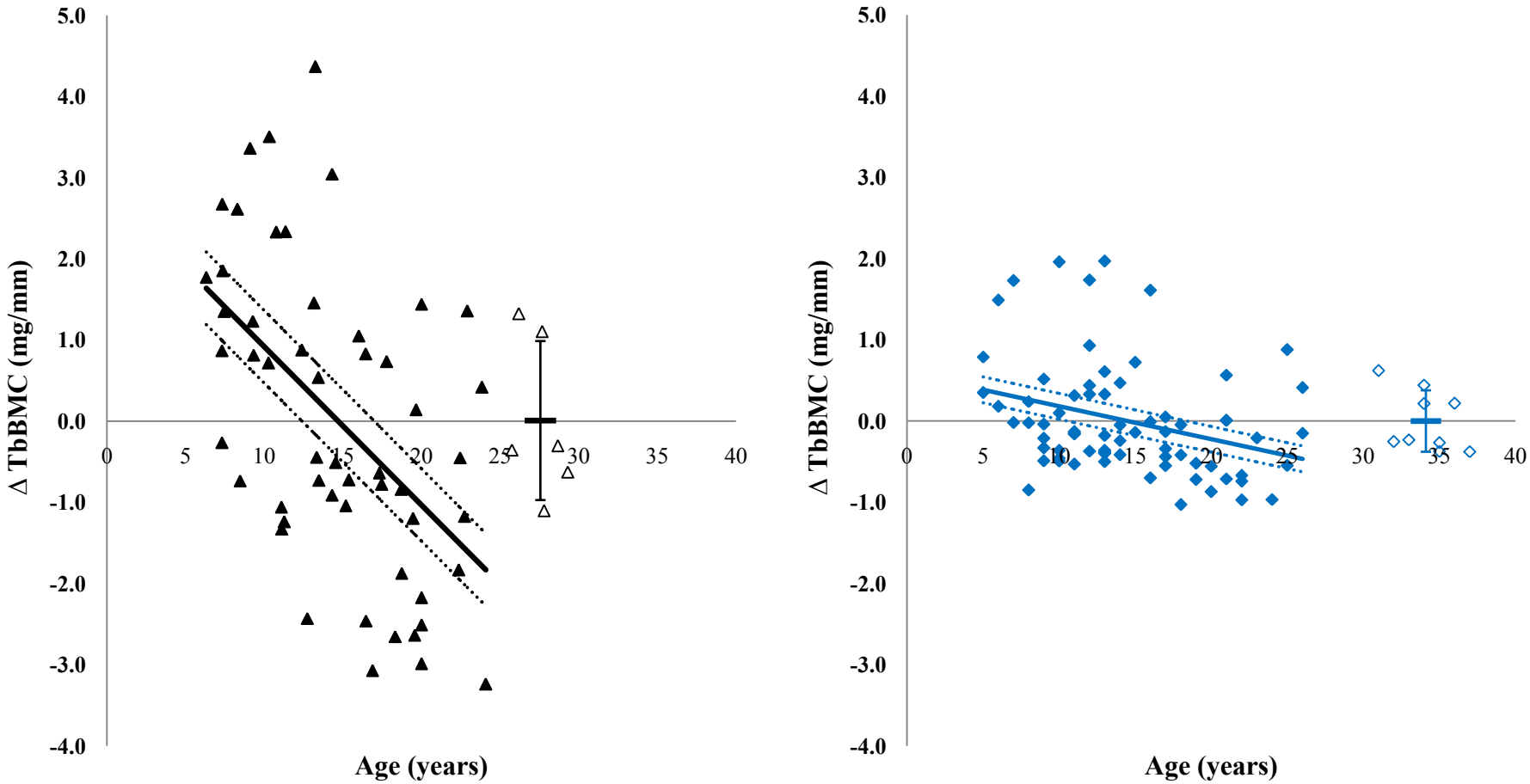

Figure 3.8 Relationships between age and body mass-adjusted trabecular bone mineral content (Δ TbBMC) of <25 years of age group (filled triangle, black solid line) and ≥25 years of age group (open triangle) in *M. fuscata* (left panel), and premenopausal (filled diamond, blue solid line) and postmenopausal (open diamond) in *M. fascicularis* (right panel). The round dotted lines indicate 95 % confidence interval of the intercept. Vertical bars indicate means ± SD

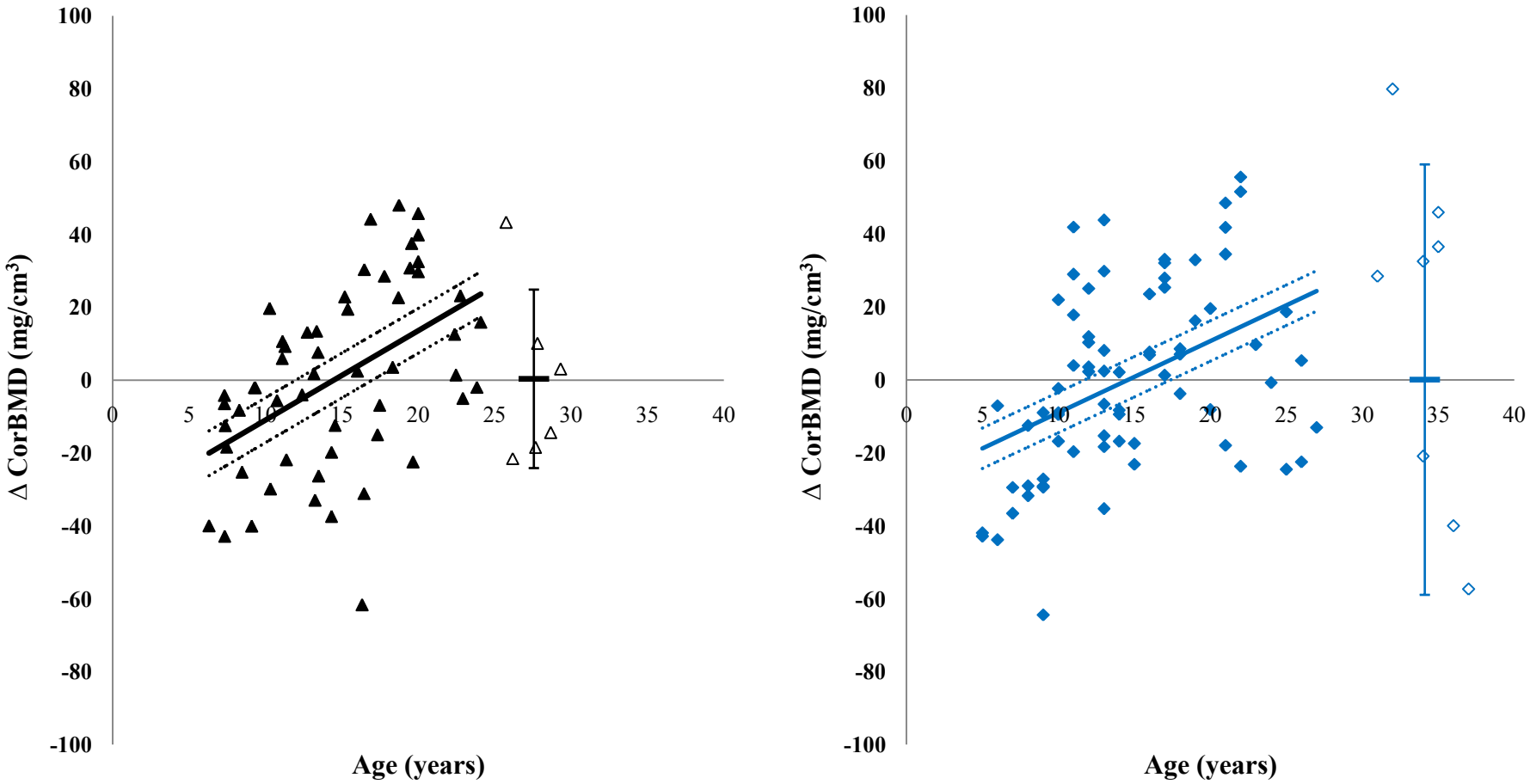

Figure 3.9 Relationships between age and body mass-adjusted cortical bone mineral density (Δ CorBMD) of <25 years of age group (filled triangle, black solid line) and ≥25 years of age group (open triangle) in *M. fuscata* (left panel), and premenopausal (filled diamond, blue solid line) and postmenopausal (open diamond) in *M. fascicularis* (right panel). The round dotted lines indicate 95 % confidence interval of the intercept. Vertical bars indicate means ± SD

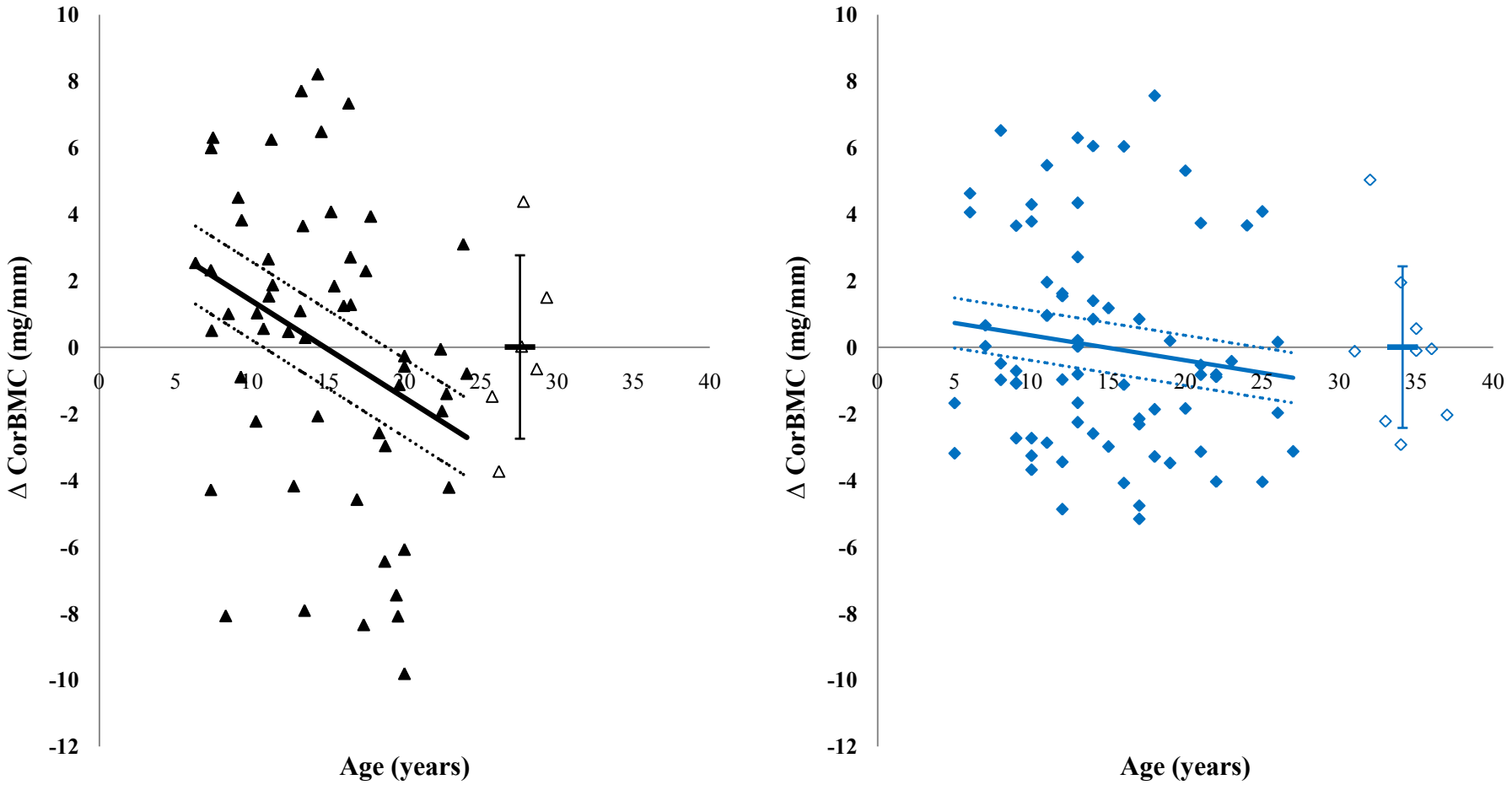

Figure 3.10 Relationships between age and body mass-adjusted cortical bone mineral content (Δ CorBMC) of <25 years of age group (filled triangle, black solid line) and ≥25 years of age group (open triangle) in *M. fuscata* (left panel), and premenopausal (filled diamond, blue solid line) and postmenopausal (open diamond) in *M. fascicularis* (right panel). The round dotted lines indicate 95 % confidence interval of the intercept. Vertical bars indicate means ± SD

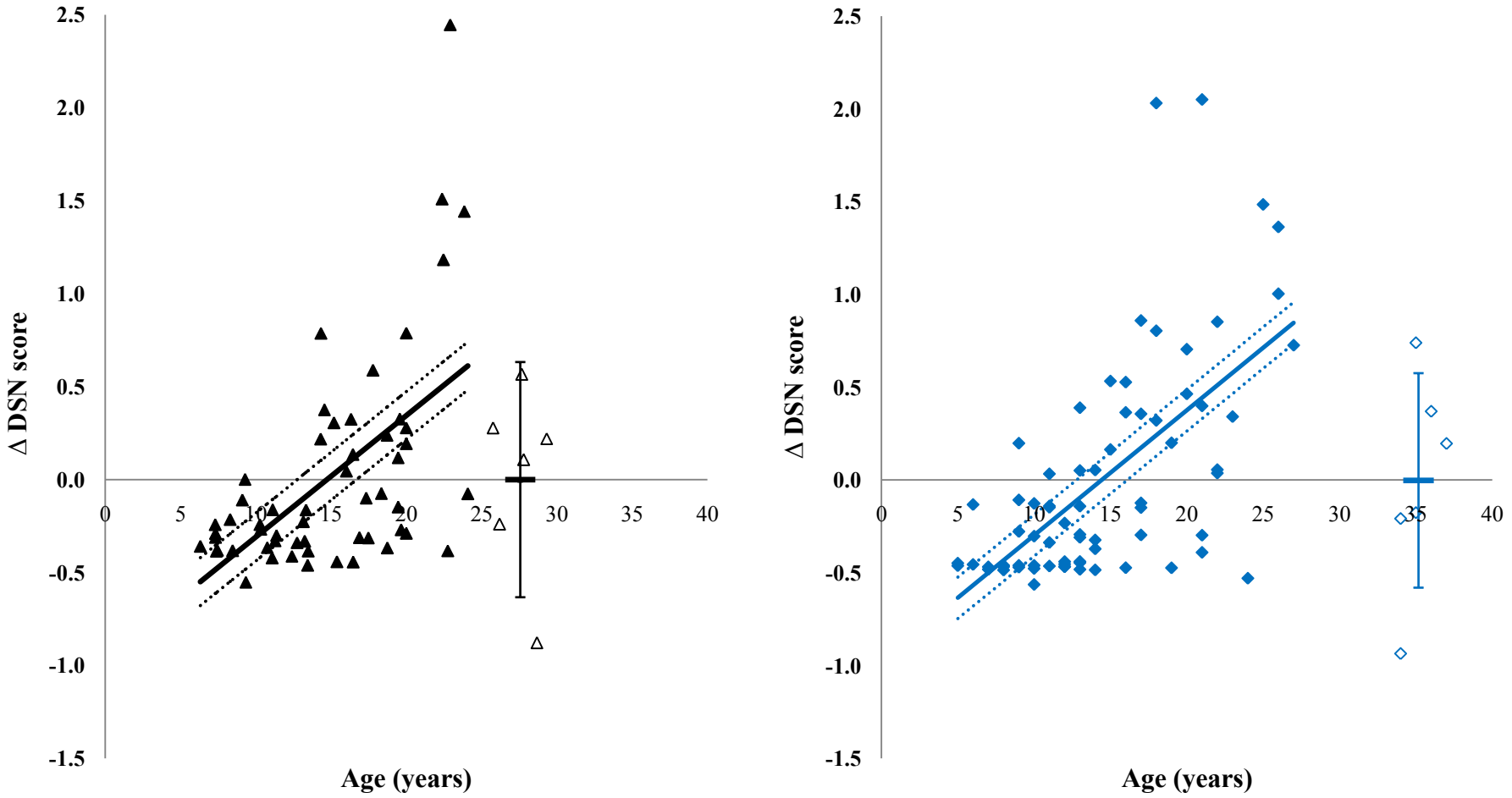

Figure 3.11 Relationships between age and body mass-adjusted disc space narrowing (Δ DSN) of <25 years of age group (filled triangle, black solid line) and ≥25 years of age group (open triangle) in *M. fuscata* (left panel), and premenopausal (filled diamond, blue solid line) and postmenopausal (open diamond) in *M. fascicularis* (right panel). The round dotted lines indicate 95 % confidence interval of the intercept. Vertical bars indicate means ± SD

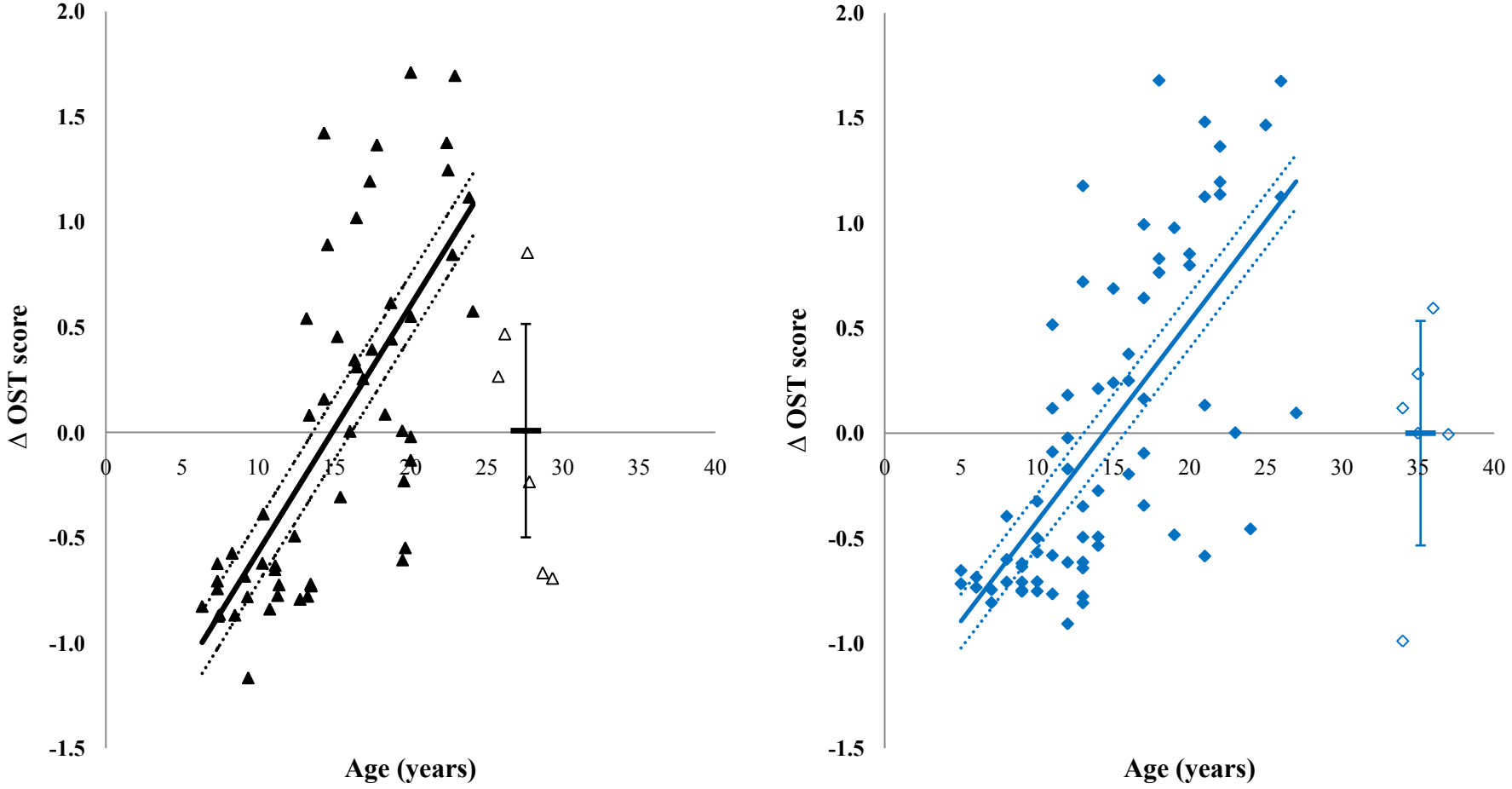

Figure 3.12 Relationships between age and body mass-adjusted osteophytosis (Δ OST) of <25 years of age group (filled triangle, black solid line) and ≥25 years of age group (open triangle) in *M. fuscata* (left panel), and premenopausal (filled diamond, blue solid line) and postmenopausal (open diamond) in *M. fascicularis* (right panel). The round dotted lines indicate 95 % confidence interval of the intercept. Vertical bars indicate means ± SD

Interrelationships between variables

A correlation was found between age, bone mass (BMDs and BMCs), OA (DSN and OST) and BM. The associations between age and bone mass: TbBMD, TbBMC and CorBMD in *M. fuscata* were r = -0.579, -0.460 and 0.430 (all P <0.001), respectively; those in *M. fascicularis* were r = -0.357 to -0.467 (all P <0.001). As for the associations between age and DSN and OST in *M. fuscata* were r = 0.641 to 0.733 (all P <0.001) and in *M. fascicularis* were r = 0.700 to 0.712 (all P <0.001). The correlations between bone mass and OA were, between TbBMD and OST, r = -0.386 (P <0.001) in *M. fuscata*; and between TbBMD and DSN and OST, r = -0.378 to -0.390 (all P <0.001) in *M. fascicularis*; between TbBMC and OST, r = -0.280 (P <0.05) in *M. fuscata*; and between TbBMC and DSN and OST, r = -0.316 to -0.351 (all P <0.001) in *M. fascicularis*; and between CorBMD and OST, r = 0.225 and 0.255 (all P <0.05) in *M. fascicularis* and *M. fuscata*, respectively. The correlation between BM and bone mass was between BM and CorBMC, r = 0.449 and 0.461 (all P <0.001) in *M. fascicularis* and *M. fuscata*, respectively (Tables 3.4–3.5).

After the influences of age and BM were controlled using partial correlation analysis, some positively significant correlations were observed between bone mass and OA in both species: between TbBMD and DSN (r_p = 0.283, P <0.05) and between TbBMC and DSN (r_p = 0.439, P <0.01) in *M. fuscata*, and between CorBMC and DSN in *M. fascicularis* (r_p = 0.360, P <0.01) (see tables 3.5–3.6 in the appendix).

Table 3.4 Correlations between age, bone mass, osteoarthritis and body size in female *M. fuscata*

	TbBMD	TbBMC	CorBMD	CorBMC	DSN	OST	BM
Age	-.579**	-.460**	.430**	-.230	.641**	.733**	.093
TbBMD		.637**	-.401**	.302*	-.230	-.386**	-.140
TbBMC			-.665**	.279*	-.014	-.280*	-.006
CorBMD				-.443**	.079	.255*	-.082
CorBMC					-.005	-.019	.461**
DSN						.761**	.214
OST							.218
BM							

Table 3.5 Correlations between age, bone mass, osteoarthritis and body size in female *M. fascicularis*

	TbBMD	TbBMC	CorBMD	CorBMC	DSN	OST	BM
Age	-.467**	-.397**	.093	-.357**	.700**	.712**	.013
TbBMD		.719**	-.053	.168	-.390**	-.378**	.052
TbBMC			-.204	.102	-.316**	-.351**	.012
CorBMD				.095	.168	.225*	-.018
CorBMC					-.028	-.036	.449**
DSN						.766**	-.025
OST							.115
BM							

Abbreviations of variables as in Table 3.1; Pearson's correlation coefficient at the * P <0.05, ** P <0.01 and *** P <0.001 level

Discussion

OP and OA are common age-related disorders in humans. It has been suggested that the two disorders uncommonly appear together in the same subject (Dai 1998). An inverse relationship was suggested, that is, OA is related with higher bone mass, that is, less OP (Sambrook and Naganathan 1997). Lifestyle and health-related factors such as exercise or diet, which are not possible to be controlled in human study, have been considered to relate with the occurrence of OP (Turner 2001) and OA (Bennell et al. 2012). Postmenopausal estrogen depletion would influence both OP and OA. Several aspects of bone changes in macaques such as relationship between OP and OA, and bone loss with natural estrogen depletion are scarce or are still unclear.

To understand etiology and relation of OP and OA, I examined age-related and reproductive aging-related bone changes and interrelationships in bone mass, OA and body size in female Japanese (*M. fuscata*) and cynomolgus macaques (*M. fascicularis*). Several factors, that is, age, BM, physical activity and menstrual status were able to be controlled. *M. fuscata* and *M. fascicularis* clearly differ in BM and lifestyle, that is, *M. fuscata* is heavier and semi-terrestrial, and *M. fascicularis* is lighter and arboreal, which may contribute to bone mass and OA prevalence.

M. fuscata had significantly higher average bone mass than *M. fascicularis*, except for CorBMD, which may relate with the greater BM. Bones are crucial mechanical components of the musculoskeletal system, acting as the rigid levers for muscle operating to produce movement (White et al. 2012). Increases in bone length, muscle force and BM with growth cause adaptational changes in bone mass and bone architecture to improve stability (Pollock 2008). A larger BM causes a greater mechanical loading on the subject bone (radius), and that bone mass increases to accommodate that greater load as observed in *M. fuscata* as commonly reported (Edelstein and Barrett-Connor 1993; Zhao et al. 2007). The present results found significant differences of bone mass between species, however, after BM was controlled, not only CorBMC still showed significant correlation with BM but also significant difference in CorBMD disappeared, while bone mass at the trabecular site (distal radius) did not correlate with BM. The present findings support the general knowledge that cortical part is mainly responsible for load bearing (Wang 2011).

The BM and BMI of the two species in the present study showed no noticeable changes with advancing age. In adult women, commonly BM increases with age (e.g.

Samson et al. 2000). There are several factors involved in BM of macaques such as dietary intake, reproduction or life conditions. The pattern of age-related changes of BM in the present study may reflect that captive macaques are healthy under excellent caring and provisioning. The subjects also gave births and caring infants, that is, paying greatly to reproduction.

M. fuscata and *M. fascicularis* shared the similar pattern of age-related changes in TbBMD and TbBMC, both of which linearly decreased with advancing age. Trabecular bone loss started from young adulthood and continued to older adulthood in the present study, both concords with those in women (Fujii et al. 1996; Riggs et al. 2004, 2008) and in female Japanese macaques (Pomchote 2015). However, acceleration of trabecular bone loss in postmenopausal women (Block et al. 1989) was not observed in the two macaque species in the present study. Alterations of TbBMD are resulted from changes in size and number of trabeculi (Rüegsegger et al. 1991). The continuous decline of TbBMD is consistent with loss of TbBMC. Trabecular bone loss started in young adulthood in the absence of sex hormone deficiency was reported at the distal tibia (Fujii et al. 1996) and distal radius (Riggs et al. 2004) in women. Although genetic factors or decrease of sex steroid before menopause were suggested (Riggs et al. 2004) for the decrease of trabecular bone mass, at present definite mechanism has not been given.

CorBMD of macaques increased from young adulthood to reach the peak and then decreased. The age at peak would be perimenopausal period about 25–30 years in *M. fuscata* and *M. fascicularis*. In women, CorBMD decreases after middle age and rapidly decreases after menopause (Riggs et al. 2004). The decrease after reaching the peak in CorBMD is considered resulting from the increase in porosity (Rüegsegger et al. 1991; Riggs et al. 2002) and endosteal resorption (Riggs et al. 2004) which has higher rate than that of periosteal apposition, causing decrease of cortical area and thickness with age (Clarke and Khosla 2010b). CorBMC of the two macaque species decreased from young adulthood with advancing age, which is comparable to that of women (Thompson 1980; Aspray et al. 1996; Grampp et al. 1996).

OA prevalence and severity clearly increase with advancing age in the two species of macaques. This pattern of OA changes with age is similar to that in women (Kramer 2006; Duncan et al. 2012) and in female macaques (in pig-tailed macaques, Kramer et al. 2002; in rhesus macaques, Duncan et al. 2011, 2012). The variation among subjects was wide in female macaques (Kramer et al. 2002; Duncan et al. 2011, 2012; present study) and in women (Kramer 2006; Duncan et al. 2012).

In *M. fuscata* and *M. fascicularis*, the thoracolumbar region is the most susceptible area for both DSN and OST (also in rhesus macaques, Duncan et al. 2012; in pig-tailed macaques, Kramer et al. 2002). The patterns of DSN and OST along the lumbar vertebral region between female rhesus macaques and women were directly compared (Duncan et al. 2012) using the similar scoring method as the present study. There found intergeneric differences in that maximum OA occurred around midlumbar region in women, and at the thoracolumbar region in female macaques (Duncan et al. 2012; present study). Changes in vertebral balance and curvature which are determined by loading pattern are associated with development of OA (Lauerman et al. 1992). Although macaques are quadruped, they use upright sitting posture when resting (Kramer et al. 2002) and the lumbar vertebral region is kyphotic at the thoracolumbar area (personal observation). On the other hand, in human vertebrae the frequency and incidence of OST were clearly higher at midlumbar region (L3–L4) where the maximum curvature (lordosis) is found (Nathan 1962; Bridges 1994), which confirms the finding of Miller et al. (1988) from autopsy that the most disc degeneration appeared at the L3–L4. Accordingly it is probable that the severe DSN and OST occur at the point of the maximum curvature of lumbar vertebrae.

I found that *M. fuscata* in captivity (individual cages) has significantly higher OA severity and BM than free-ranging conspecific individuals (Chapter 2). It is suggested that BM and physical activity are the primary causes of OA. BM alone is able to put a small load on bones, but the effect of weight at many skeletal sites is amplified by muscle operation (Pollock 2008). In the present study, I expected to observe certain differences in DSN and/or OST between the two species that differ in BM and lifestyle, heavier and semi-terrestrial Japanese macaques, and lighter and arboreal cynomolgus macaques. However, no significant differences in DSN and OST from both each lumbar vertebral level and average of all vertebrae were observed between the two species. Thus, OA prevalence is not influenced by BM in macaque species (interspecific comparison). The fact that subject macaques of the two species were born and reared in captivity where their physical activity was restricted in common may partly contribute to rather similar OA severity, in spite of different lifestyle in the wild.

BM is considered as a risk factor of OA in humans (Lementowski and Zelicof 2008), but in macaques BM does not seem to clearly relate with the severity of OA (Carlson et al. 1996; Colman et al. 1999b; Kramer et al. 2002; present study). In humans (bipedal), the vertebral region has dual curvature, that is, kyphosis and lordosis differing from that in macaque (quadrupedal). Thus, excessive lordosis resulted from excessive BM (Murrie et al.

2003) possibly plays a crucial role in development of vertebral OA in humans, however, on which there are no evidence or studies in nonhuman primates or macaques. Another thing is that those OA studies in macaques are cross-sectional but not longitudinal.

Since body size is a fundamental specific character of every species, it plays a role in evolution (Damuth and Macfadden 1990) including bone characteristics. Interspecific trend in relationship between BM and OA would be different from intraspecific trend. Alterations of loading conditions, especially BM, either adaptively affect structures of bone and cartilage or causes joint degeneration (Weiss and Jurmain 2007). Comparative studies on diverse mammals including nonhuman primates showed the relation between body size and OA, that is, most of larger species had more OA prevalence than smaller ones (Fox 1939; Schultz 1956). However, it is proposed that locomotive forces or stresses in different sizes of animals do not scale in proportion to their BM (McNeill Alexander 1985 in Scherf 2008). Weiss (2006) found negative correlation between BM and OA in prehistoric adult human skeletons, that is, the smaller and lighter individuals have higher OA severity. From the present study, the fact that the greater size did not cause severe or less severe OA in macaque species may in part be explained that the smaller vertebral joints of *M. fascicularis* having small surface area to distribute stresses or loads, thus leading to high prevalence and severity of OA as *M. fuscata*, which agrees with previous report (Weiss 2006).

Female macaques exhibited greater decrease in bone mass and increase in bone turnover, which are due to estrogen depletion from both naturally occurring menopause (in rhesus macaques, Colman et al. 1999a) or ovariectomy (in cynomolgus macaques, Jerome et al. 1995; Jerome et al. 1997), resembling to those in postmenopausal women. It was expected to observe a dramatic decrease of bone mass at the trabecular site in O_{25} of *M. fuscata* and Post of *M. fascicularis*, representing the effect of estrogen depletion. However, such was not found in those groups. Although marked trabecular bone loss was found in the O_{25} (in *M. fuscata*) and in the Post (in *M. fascicularis*), both groups had higher TbBMD than expected from averages of the U_{25} (in *M. fuscata*) and the Pre (in *M. fascicularis*), respectively. On the other hand, CorBMD showed great decrease after menopause in the two macaque species.

Postmenopausal estrogen depletion is also considered to influence OA. Both DSN and OST showed significant prevalence in the U_{25} (in *M. fuscata*) and in the Pre (in *M. fascicularis*), which can be mainly resulted from the influence of age. Furthermore, the important finding was contrary to the expectation that the Δ DSN and OST scores of the O_{25} of *M. fuscata* and the Post of *M. fascicularis* were rather comparable to those of middle age adults.

The lack of significant decline in bone mass and comparable development of OA from U_{25} to O_{25} in *M. fuscata* and Pre to Post in *M. fascicularis* may be explained by the fact that the smaller proportion of macaques experience menopause and have short duration of postmenopausal life (Champ et al. 1996). Macaques which experience menopause are healthier in bone conditions than those that decreased bone mass with age and die before reaching menopause. Furthermore, the survival rate of macaques with good bone conditions (high bone mass and low prevalence of OA) may higher than that of macaques suffered from degenerative bone disorders, thus the former can survive and reach the postmenopausal life as observed in the present study. In addition, nutritional efficiency especially high calcium and vitamin D contained in monkey chows may prevent bone loss (Lips 2001) as reported in older humans. It is highly possible that captive macaques were healthy under excellent provision of caretaker and veterinarian, except physical activity (Chapter 2), and neither decline of bone mass nor aggravation of OA with advancing age was observed, which contributes to good condition of bone (Felton et al. 2000).

Interrelationships between variables

I used adult macaques from young adulthood through the oldest to examine association between bone mass and OA. Significant (positive) relationships were found between bone mass and OA scores at the different sites (radius vs. lumbar vertebrae) in *M. fuscata* and *M. fascicularis* (present study), congruent with studies in women (Nevitt et al. 1995; Jiang et al. 2008). Growth factors may play a role in the relationship between OA and BMD at remote sites, such as insulin-like growth factor (IGF) type I and II and transforming growth factor *ß* (TGF *ß*) (Dequeker et al. 1993). However, the mechanism has not been fully elucidated (Dequeker et al. 1993). The present findings support the idea that vertebral OA is correlated with not only higher BMD at various sites of skeleton (in humans, see Stewart and Black 2000 for review) but also with higher BMC at distal radius (in macaques, present study). Bone loss and OA were found to be unrelated (in rhesus macaques, Grynpas et al. 1993), though relationship was examined only between total BMD and OST at lumbar vertebrae. On the other hand, I used volumetric BMD at radial trabecular and cortical sites and lumbar vertebral DSN and OST evaluated separately.

In conclusion, female Japanese and cynomolgus macaques, though having different BM, had similar age-related and reproductive aging-related bone changes. The two macaque species shared certain similarities with humans, that is, increased bone loss and OA development with advancing age. However, macaques had much severer OA development

than women, probably by the smaller amount of physical activity in subject macaques. Macaques lack significant bone loss with estrogen depletion at the trabecular site but may be lost at the cortical site. Positive relations between bone mass and OA at the different sites were found in the two macaque species which is congruent with that in humans.

General Discussion and Conclusion

Macaques are considered as a useful animal model for osteoporosis (OP) and osteoarthritis (OA) because they show vertebral bone loss (e.g. Cerroni et al. 2000) and an increasing severity of OA (e.g. Duncan et al. 2012) with advancing age. Moreover, several confounding factors influencing bone mass and development of OA in humans such as lifestyle, exercise or diet (Turner 2001; Bennell et al. 2012) can be controlled using a macaque model.

In the present study, the vertebral body of lumbar vertebra is focused because the main function of this part is to support the body weight and to absorb shock (Ankel-Simons 2007). Changes in the morphometry (Ericksen 1976, 1978a, b), bone mineral density (BMD) (Twomey et al. 1983) and OA (Rogers and Waldron 1995) are three major aspects of age-related changes in human vertebrae. However, there are no studies on the relationships in these aspects.

In Chapter 1, I examined age-related bone changes at the odd-numbered lumbar vertebrae of Japanese macaque (*Macaca fuscata*) skeletons. I hypothesized that the loss of trabecular BMD (TbBMD) contributes to bone fragility and causes decrease in vertebral body height, particularly in the ventral side, leading to kyphosis; and that osteophytosis (OST) may develop using bone minerals absorbed from the bone, which causes a further decrease in the TbBMD. From the findings, the major pattern of the age-related dimensional changes of Japanese macaques was an increase from young adulthood to the peak and subsequent decrease with age. Males reached this peak earlier (age group 10–15 years) than females (age group 15–20 years) in the depths and widths of the vertebral body, however, respective dimensions increase with age in humans (Ericksen 1976, 1978a, b; Rühli et al. 2005). The difference may be attributed to the different mechanical stresses on vertebral body that stem from the different positional behavior of quadrupedalism (macaques) and bipedalism (humans). The ventro-dorsal height (VBHv/VBHd) ratio of Japanese macaques decreased with age, which is similar to that of humans (Ericksen 1976, 1978a, b; Evans et al. 1993; Hermann et al. 1993; Diacinti et al. 1995). Trabecular bone loss started from young adulthood and then the loss was accelerated in older adulthood in both sexes in Japanese macaques, which was reported in humans (Riggs et al. 2004, 2008; Christiansen et al. 2011). OST of Japanese macaques significantly aggravated with advancing age as that of humans (Duncan et al. 2012). No significant relationship was found between VBHv/VBHd ratio,

TbBMD and OST when the influence of age was removed. It is true that age-related bone changes are complicated and do not always follow the expectations, which likely arises from the inter-individual variations, existence of various patterns of age-related changes, and other factors affecting bone properties that I could not control such as menstrual status, body mass (BM) or physical activity. Furthermore, although I excluded some monkeys with severe OST, the TbBMD was greatly increased by the OST (Agarwal 2001). Other limitations of the Chapter 1 are only TbBMD and OST were chosen to assess bone mass and OA, respectively.

Thus studies in Chapters 2 and 3 were conducted to fill the gaps from Chapter 1, that is, to identify major risk factors in live macaques, that is, influence of BM and physical activity (in Chapter 2) or menstrual status (in Chapter 3) on bone mass and OA, including to study relationship between bone mass from trabecular and cortical parts and OA (disc space narrowing (DSN) and OST) (in Chapter 3).

In Chapter 2, I compared lumbar vertebral OA prevalence and severity between Japanese macaques reared in individual cages (captive condition) and living in free-ranging (natural condition) using radiographs on which I evaluated DSN and OST. Although macaques are quadruped, correlation between lumbar vertebral properties and BM supports the use of macaques as models of the bipedal humans (Elliott and Sarver 2004). This is consistent with biomechanical analyses showing that vertebrae of quadruped are highly loaded in axial compression through the action of the muscles and ligaments during quadrupedally standing and walking (Smit 2002). In general, it supports the concept that quadrupedal animals are appropriate model of the bipedal human vertebrae (Elliott and Sarver 2004). In present study, captive (CG) Japanese macaques were not only significantly heavier than free-ranging (FRG) macaques, but also CG had dramatically higher prevalence and severity of OA than FRG and reached the severest stage of DSN and OST which FRG never reached. When macaque age was adjusted to human equivalence using the parameter of 3.5 (Duncan et al. 2012), CG Japanese macaques had dramatically higher prevalence and severity of OA than humans, which is supposed to be caused by the differences of vertebral anatomy, loading, size, vertebral orientation and vertebral curvature (Alini et al. 2008; Duncan et al. 2012) and comparable to those in other species of macaques in captivity (Kramer et al. 2002; Duncan et al. 2012). While FRG Japanese macaques had significantly less prevalence and severity of DSN and OST than CG, which is rather similar to those in humans. This finding suggests that BM and physical activity should be considered as factors influencing on the prevalence and severity of OA between CG and FRG Japanese macaques.

Therefore, I focused on female macaques of two species, that is, Japanese macaques and cynomolgus macaques (*M. fascicularis*), which are closely related with each other (Fooden 1976), however, they showed species specific characteristics mainly related to BM (Black and Lane 2002) and lifestyle (Kikuchi 2004). These characteristics may influence on their bone mass and OA prevalence. I also investigated age-related and reproductive aging-related bone changes in terms of bone mass at the trabecular and cortical sites of distal radius, OA at lumbar vertebrae and body size, and relation between radial bone mass and lumbar vertebral OA.

Japanese macaques had significantly higher bone mass than cynomolgus macaques, except for Cortical BMD (CorBMD), relating with the difference of BM (Table 3.1 in Chapter 3, 9.2 and 3.5 kg in Japanese and cynomolgus macaques, respectively). Higher BM causes higher mechanical loading on bone resulting in increases in bone mass to accommodate (Edelstein and Barrett-Connor 1993; Zhao et al. 2007). Both BM and body mass index like-index (BMI) showed clearly significant differences between the two species, however, no significant differences were observed in DSN and OST values between the two species. This finding supports earlier reports that BM does not seem to clearly relate with the severity of OA in macaques (Carlson et al. 1996; Colman et al. 1999b; Kramer et al. 2002), but the relation is commonly found in humans (Lementowski and Zelicof 2008). In humans (bipedal), the vertebral region has dual curvature, that is, kyphosis and lordosis differing from that in macaque (quadrupedal). Excessive lordosis caused by some factors such as excessive BM (Murrie et al. 2003) possibly plays an important role in vertebral OA development in humans, however, on which there are no evidence or studies in macaques. Another matter is that those OA studies in macaques are cross-sectional but not longitudinal.

High BM poses heavy loads and stresses to joints and then causes degeneration of the joints (Weiss and Jurmain 2007). However, locomotive stresses in different sizes of animals may not necessarily scale with their BM (McNeill Alexander 1985 in Scherf 2008). I hypothesized that the smaller vertebral joints of cynomolgus macaques having smaller surface area to distribute stresses or loads, so causing prevalence and severity of OA as high as those of heavier Japanese macaques, which is discussed on human skeletons (Weiss 2006). Other OA-related factors such as genetics or physical activity (Duncan et al. 2012) may influence the two macaque species in the present study.

Japanese and cynomolgus macaques shared the similar pattern of age-related and reproductive aging-related bone changes in bone mass and OA. Trabecular bone mass (density and content) of the two species started decreasing in young adulthood in the absence

of sex hormone deficiency. This result accords with previous studies in women (Fujii et al. 1996; Riggs et al. 2004). CorBMD of the two species increased from young adulthood to reach the peak and then decreased, while cortical bone mineral content (CorBMC) continuously decreased with advancing age, which is comparable to those in women (Grampp et al. 1996; Riggs et al. 2004). Bone loss due to estrogen depletion has been reported for both humans (see Clarke and Khosla 2010b for review) and macaques (natural menopause in rhesus macaques, Colman et al. 1999a; ovariectomy in cynomolgus macaques, Jerome et al. 1994; Jerome et al. 1995; Jerome et al. 1997). Estrogen deficiency resulting in bone loss via cellular and molecular mechanisms is well understood (Clarke and Khosla 2010b). However, significant trabecular bone loss in postmenopausal life (for women, Block et al. 1989; Aspray et al. 1996; Riggs et al. 2004) was not found in female Japanese macaques of ≥25 year age group (O_{25}) and postmenopausal cynomolgus macaque group (Post). On the contrary, CorBMD showed great decrease after menopause in the two macaque species.

Prevalence and severity of OA clearly increased with advancing age in the two macaque species (present study), which is similar to that found in women (Kramer 2006; Duncan et al. 2012) and in other macaque species (in pig-tailed macaques, Kramer et al. 2002; in rhesus macaques, Duncan et al. 2011, 2012). The most important finding is that the trends of DSN and OST development are almost similar among macaque species reared in captive conditions, meaning that BM does not primarily influence the development of OA in captive macaques as it was suspected in Chapter 2. I propose that physical activity seems to greatly affect the development of OA more than BM in macaques.

In humans, evidences of estrogen deficiency associated OA are increasing (Roman-Blas et al. 2009). Sowers et al. (2006) found significant relations of lower levels of estradiol and its metabolite 2-hydroxyestrone with postmenopausal women who developed knee OA. The authors claimed that higher 2-hydroxyestrone levels inhibited leukotriene synthesis in the arachidonic acid pathway which possibly delayed the development of knee OA. However, I did not found accelerated OA development with estrogen depletion in O_{25} and Post of Japanese and cynomolgus macaques, respectively. I suggest that the lack of significant decrease in bone mass and comparable development of OA may be responsible for the limited number of postmenopausal macaques and short period of postmenopausal life (Champ et al. 1996). Moreover, high calcium and vitamin D from monkey chows may prevent bone loss (Lips 2001). It is highly possible that captive macaques were healthy under excellent provision of caretaker and veterinarian, and neither decline of bone mass nor aggravation of OA with advancing age was observed, which contributes to good condition of

bone (Felton et al. 2000). In addition, the survival rate of macaques having higher bone mass and lower prevalence of OA may higher than those suffered from bone loss and severe OA or *vice versa*, and thus the former can survive and have postmenopausal life as observed in the present study.

The present study supports the inverse relationship between OP and OA at different skeletal sites (Sambrook and Naganathan 1997), that is, OA is related with higher bone mass (Nevitt et al. 1995; Jiang et al. 2008), though this relation was not found in rhesus macaques (Grynpas et al. 1993). Growth factors may play a role in relationship between OA and bone mass at remote sites. Increased levels of growth factors, that is, insulin-like growth factor (IGF) type I and II and transforming growth factor *ß* (TGF *ß*) that are known to stimulate bone formation, may relate with higher CorBMD in patients with hand OA (Dequeker et al. 1993) and may influence both radial bone mass and vertebral OA in the present study. However, the mechanism has not been fully elucidated (Dequeker et al. 1993).

From the present study, macaques shared most age-related bone changes with humans, in particular female macaques. Macaques exhibited vertebral morphometric changes with age. Decreases of vertebral body heights, widths and depths, especially decrease in the VBHv/VBHd ratio may contribute to kyphosis as found in humans. Although widths and depths increase with age in humans, the difference may stem from the different mechanical stresses on vertebral body because of quadrupedalism (macaques) and bipedalism (humans). Bone mass changes with age in macaques are similar to that observed in humans. Trabecular bone mass continuously decreases with advancing age in the absence of sex hormone deficiency. This result accords with previous studies in women, partly supporting similarity of reproductive physiology and mechanisms between female macaques and women. Cortical bone mass also showed the similar trend with age in macaques and humans. Japanese macaques have higher bone mass than cynomolgus macaques, relating with differences in BM. OA in macaques and humans significantly increase severity with advancing age. However, macaques from captive conditions had dramatically higher prevalence and severity of OA than that of humans, which possibly is attributed to the differences in vertebral anatomy, loading, size, vertebral orientation and vertebral curvature between macaques and humans. In contrast, FRG Japanese macaques showed significantly less development of OA rather comparable with that in humans. Although there are several factors influencing bone mass and OA such as genetics or environment, the present findings suggest that not only age commonly influences bone mass and OA but also different BM causes different bone mass between Japanese and cynomolgus macaques and that physical activity should affect

development of OA. Free-ranging macaques may be in the optimal condition for the less OA development, whereas captive macaques may be in the low physical activity resulting in high prevalence and severity of OA which is similar to that observed in humans with sedentary lifestyle.

In conclusion although the number of old or postmenopausal macaques to study age-related bone changes is very limited, there is no ideal or perfect animal model which can answer all questions and elucidate all aspects of age-related bone changes in humans. For that purpose, longitudinal study is necessary. Macaques, however, provide similarities in age-related bone changes with humans more than other animals, such as decreasing in vertebral height and bone mass, and spontaneous OA with age. Much useful information can be obtained from macaques and various confounding factors which are uncontrolled in humans can be controlled in macaques.

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Appendices

Table 1.1 Pearson's correlation of all variables from L1, 3, 5 and 7 of female Japanese macaques

	L1	Age	VBHd	VBHv	VBDcra	VBDcau	VBWcra	VBWcau	OST	vBMD	TbBMD
Age	Correlation		.172	-.034	-.293	.169	-.130	-.005	.885**	-.265	-.452**
	Sig. (2-tailed)		.296	.841	.074	.303	.437	.978	.000	.130	.007
	N		39	38	38	39	38	38	40	34	34
VBHd	Correlation			.791**	.237	.384*	.581**	.636**	.209	-.140	-.246
	Sig. (2-tailed)			.000	.153	.016	.000	.000	.202	.431	.160
	N			38	38	39	38	38	39	34	34
VBHv	Correlation				.205	.278	.572**	.500**	-.006	-.189	-.198
	Sig. (2-tailed)				.216	.092	.000	.002	.971	.285	.262
	N				38	38	38	37	38	34	34
VBDcra	Correlation					.727**	.397*	.441**	-.319	.406*	.371*
	Sig. (2-tailed)					.000	.014	.006	.051	.017	.031
	N					38	38	37	38	34	34
VBDcau	Correlation						.443**	.523**	.141	.211	.093
	Sig. (2-tailed)						.005	.001	.392	.231	.599
	N						38	38	39	34	34
VBWcra	Correlation							.804**	-.165	.074	-.034
	Sig. (2-tailed)							.000	.323	.679	.847
	N							37	38	34	34
VBWcau	Correlation								-.023	.112	.012
	Sig. (2-tailed)								.889	.527	.945
	N								38	34	34
OST	Correlation									-.200	-.456**
	Sig. (2-tailed)									.256	.007
	N									34	34
vBMD	Correlation										.924**
	Sig. (2-tailed)										.000
	N										34
TbBMD	Correlation										
	Sig. (2-tailed)										
	N										

Table 1.1 (continued)

	L3	Age	VBHd	VBHv	VBDcra	VBDcau	VBWcra	VBWcau	OST	vBMD	TbBMD
Age	Correlation		.253	.000	-.118	.160	-.227	-.200	.901**	-.363*	-.493**
	Sig. (2-tailed)		.120	1.000	.480	.325	.164	.216	.000	.032	.003
	N		39	38	38	40	39	40	40	35	35
VBHd	Correlation			.795**	.203	.277	.320	.592**	.289	-.136	-.306
	Sig. (2-tailed)			.000	.227	.088	.050	.000	.074	.437	.074
	N			37	37	39	38	39	39	35	35
VBHv	Correlation				.048	.171	.414**	.570**	.021	-.067	-.133
	Sig. (2-tailed)				.774	.305	.010	.000	.900	.703	.445
	N				38	38	38	38	38	35	35
VBDcra	Correlation					.607**	.515**	.485**	-.189	.257	.196
	Sig. (2-tailed)					.000	.001	.002	.256	.136	.259
	N					38	38	38	38	35	35
VBDcau	Correlation						.408**	.583**	.063	.102	.111
	Sig. (2-tailed)						.010	.000	.698	.561	.527
	N						39	40	40	35	35
VBWcra	Correlation							.759**	-.292	.143	.170
	Sig. (2-tailed)							.000	.071	.412	.329
	N							39	39	35	35
VBWcau	Correlation								-.257	.129	.073
	Sig. (2-tailed)								.109	.459	.675
	N								40	35	35
OST	Correlation									-.373*	-.538**
	Sig. (2-tailed)									.027	.001
	N									35	35
vBMD	Correlation										.927**
	Sig. (2-tailed)										.000
	N										35
TbBMD	Correlation										
	Sig. (2-tailed)										
	N										

Table 1.1 (continued)

	L5	Age	VBHd	VBHv	VBDcra	VBDcau	VBWcra	VBWcau	OST	vBMD	TbBMD
Age	Correlation		.054	-.139	.304	.412*	-.293	.253	.901**	-.335*	-.470**
	Sig. (2-tailed)		.740	.404	.063	.010	.074	.121	.000	.049	.004
	N		40	38	38	38	38	39	40	35	35
VBHd	Correlation			.587**	.322*	.365*	.274	.480**	-.037	.136	-.114
	Sig. (2-tailed)			.000	.048	.024	.097	.002	.822	.435	.513
	N			38	38	38	38	39	40	35	35
VBHv	Correlation				-.053	-.063	.206	.284	-.259	.268	.156
	Sig. (2-tailed)				.752	.705	.214	.084	.116	.119	.370
	N				38	38	38	38	38	35	35
VBDcra	Correlation					.832**	.224	.375*	.280	.114	.038
	Sig. (2-tailed)					.000	.177	.020	.088	.514	.829
	N					38	38	38	38	35	35
VBDcau	Correlation						.228	.520**	.453**	.071	-.004
	Sig. (2-tailed)						.168	.001	.004	.686	.982
	N						38	38	38	35	35
VBWcra	Correlation							.693**	-.273	.215	.108
	Sig. (2-tailed)							.000	.097	.214	.538
	N							38	38	35	35
VBWcau	Correlation								.200	.058	-.094
	Sig. (2-tailed)								.222	.742	.592
	N								39	35	35
OST	Correlation									-.341*	-.509**
	Sig. (2-tailed)									.045	.002
	N									35	35
vBMD	Correlation										.914**
	Sig. (2-tailed)										.000
	N										35
TbBMD	Correlation										
	Sig. (2-tailed)										
	N										

Table 1.1 (continued)

	L7	Age	VBHd	VBHv	VBDcra	VBDcau	VBWcra	VBWcau	OST	vBMD	TbBMD
Age	Correlation		.069	-.159	.615**	.467**	-.158	.076	.860**	-.384*	-.510**
	Sig. (2-tailed)		.670	.346	.000	.004	.344	.659	.000	.023	.002
	N		40	37	38	37	38	36	40	35	35
VBHd	Correlation			.555**	.143	.487**	.202	.312	.014	.039	.017
	Sig. (2-tailed)			.000	.392	.002	.224	.064	.932	.822	.922
	N			37	38	37	38	36	40	35	35
VBHv	Correlation				-.126	.090	.360*	.289	-.294	.003	.047
	Sig. (2-tailed)				.459	.595	.029	.088	.077	.986	.787
	N				37	37	37	36	37	35	35
VBDcra	Correlation					.670**	.030	.128	.688**	-.228	-.375*
	Sig. (2-tailed)					.000	.858	.459	.000	.188	.026
	N					37	38	36	38	35	35
VBDcau	Correlation						.136	.624**	.554**	.000	-.054
	Sig. (2-tailed)						.421	.000	.000	.996	.757
	N						37	36	37	35	35
VBWcra	Correlation							.311	-.208	-.025	-.067
	Sig. (2-tailed)							.065	.210	.886	.704
	N							36	38	35	35
VBWcau	Correlation								.028	.294	.326
	Sig. (2-tailed)								.870	.087	.056
	N								36	35	35
OST	Correlation									-.452**	-.565**
	Sig. (2-tailed)									.006	.000
	N									35	35
vBMD	Correlation										.950**
	Sig. (2-tailed)										.000
	N										35
TbBMD	Correlation										
	Sig. (2-tailed)										
	N										

** Correlation is significant at the <0.01 level (2-tailed)
* Correlation is significant at the <0.05 level (2-tailed)
VBHd = dorsal vertebral body height; VBHv = ventral vertebral body height; VBDcra = cranial vertebral body depth; VBDcau = caudal vertebral body depth; VBWcra = cranial vertebral body width; VBWcau = caudal vertebral body width; OST = osteophytosis; vBMD = total bone mineral density; TbBMD = trabecular bone mineral density

Table 1.2 Pearson's correlation of all variables from L1, 3, 5 and 7 of male Japanese macaques

	L1	Age	VBHd	VBHv	VBDcra	VBDcau	VBWcra	VBWcau	OST	vBMD	TbBMD
Age	Correlation		.250	.028	-.165	.035	-.436**	-.245	.798**	.074	-.326
	Sig. (2-tailed)		.136	.870	.330	.839	.007	.144	.000	.670	.052
	N		37	36	37	36	37	37	37	36	36
VBHd	Correlation			.801**	.501**	.640**	-.009	.289	.362*	-.008	-.153
	Sig. (2-tailed)			.000	.002	.000	.956	.082	.028	.961	.373
	N			36	37	36	37	37	37	36	36
VBHv	Correlation				.386*	.445**	.119	.207	.168	.154	.114
	Sig. (2-tailed)				.020	.007	.490	.225	.328	.369	.507
	N				36	36	36	36	36	36	36
VBDcra	Correlation					.814**	.306	.331*	.013	.096	.151
	Sig. (2-tailed)					.000	.066	.045	.940	.576	.378
	N					36	37	37	37	36	36
VBDcau	Correlation						.079	.496**	.227	.047	.028
	Sig. (2-tailed)						.647	.002	.184	.785	.872
	N						36	36	36	36	36
VBWcra	Correlation							.454**	-.407*	-.213	-.035
	Sig. (2-tailed)							.005	.012	.212	.837
	N							37	37	36	36
VBWcau	Correlation								-.124	-.348*	-.358*
	Sig. (2-tailed)								.466	.038	.032
	N								37	36	36
OST	Correlation									.289	-.050
	Sig. (2-tailed)									.087	.771
	N									36	36
vBMD	Correlation										.872**
	Sig. (2-tailed)										.000
	N										36
TbBMD	Correlation										
	Sig. (2-tailed)										
	N										

Table 1.2 (continued)

	L3	Age	VBHd	VBHv	VBDcra	VBDcau	VBWcra	VBWcau	OST	vBMD	TbBMD
Age	Correlation		.293	.028	-.360*	-.114	-.425**	-.099	.770**	-.007	-.378*
	Sig. (2-tailed)		.079	.867	.029	.500	.009	.562	.000	.969	.023
	N		37	37	37	37	37	37	37	36	36
VBHd	Correlation			.781**	.394*	.588**	.160	.478**	.346*	-.061	-.244
	Sig. (2-tailed)			.000	.016	.000	.343	.003	.036	.723	.151
	N			37	37	37	37	37	37	36	36
VBHv	Correlation				.155	.252	.327*	.313	.066	-.031	-.088
	Sig. (2-tailed)				.361	.132	.048	.059	.697	.859	.609
	N				37	37	37	37	37	36	36
VBDcra	Correlation					.843**	.181	.313	-.027	-.035	.123
	Sig. (2-tailed)					.000	.284	.059	.872	.839	.475
	N					37	37	37	37	36	36
VBDcau	Correlation						.109	.324	.156	-.132	-.119
	Sig. (2-tailed)						.520	.050	.356	.444	.490
	N						37	37	37	36	36
VBWcra	Correlation							.657**	-.392*	-.349*	-.164
	Sig. (2-tailed)							.000	.016	.037	.340
	N							37	37	36	36
VBWcau	Correlation								-.071	-.158	-.201
	Sig. (2-tailed)								.676	.358	.240
	N								37	36	36
OST	Correlation									.163	-.148
	Sig. (2-tailed)									.343	.388
	N									36	36
vBMD	Correlation										.865**
	Sig. (2-tailed)										.000
	N										36
TbBMD	Correlation										
	Sig. (2-tailed)										
	N										

Table 1.2 (continued)

	L5	Age	VBHd	VBHv	VBDcra	VBDcau	VBWcra	VBWcau	OST	vBMD	TbBMD
Age	Correlation		.407*	.085	-.081	-.137	-.328*	-.148	.671**	-.133	-.359*
	Sig. (2-tailed)		.013	.617	.638	.420	.047	.382	.000	.441	.032
	N		37	37	36	37	37	37	37	36	36
VBHd	Correlation			.710**	.483**	.447**	.242	.293	.445**	.103	-.102
	Sig. (2-tailed)			.000	.003	.005	.149	.079	.006	.551	.553
	N			37	36	37	37	37	37	36	36
VBHv	Correlation				.289	.285	.157	.100	.149	.187	.084
	Sig. (2-tailed)				.087	.087	.353	.557	.378	.275	.627
	N				36	37	37	37	37	36	36
VBDcra	Correlation					.927**	.179	.505**	.066	-.091	-.176
	Sig. (2-tailed)					.000	.295	.002	.702	.601	.312
	N					36	36	36	36	35	35
VBDcau	Correlation						.240	.582**	.113	.021	-.047
	Sig. (2-tailed)						.152	.000	.504	.902	.785
	N						37	37	37	36	36
VBWcra	Correlation							.598**	-.330*	.070	.131
	Sig. (2-tailed)							.000	.046	.685	.446
	N							37	37	36	36
VBWcau	Correlation								-.058	.075	.031
	Sig. (2-tailed)								.734	.662	.858
	N								37	36	36
OST	Correlation									.052	-.171
	Sig. (2-tailed)									.765	.318
	N									36	36
vBMD	Correlation										.924**
	Sig. (2-tailed)										.000
	N										36
TbBMD	Correlation										
	Sig. (2-tailed)										
	N										

Table 1.2 (continued)

	L7	Age	VBHd	VBHv	VBDcra	VBDcau	VBWcra	VBWcau	OST	vBMD	TbBMD
Age	Correlation		.431**	.176	-.119	-.036	-.409*	-.321	.733**	.254	.029
	Sig. (2-tailed)		.008	.296	.482	.833	.012	.056	.000	.135	.868
	N		37	37	37	37	37	36	37	36	36
VBHd	Correlation			.722**	.357*	.430**	.179	.502**	.178	.141	.039
	Sig. (2-tailed)			.000	.030	.008	.288	.002	.293	.412	.820
	N			37	37	37	37	36	37	36	36
VBHv	Correlation				.202	.024	.262	.347*	-.104	.038	-.037
	Sig. (2-tailed)				.231	.890	.118	.038	.539	.826	.831
	N				37	37	37	36	37	36	36
VBDcra	Correlation					.816**	.189	.611**	.033	-.289	-.318
	Sig. (2-tailed)					.000	.263	.000	.848	.088	.058
	N					37	37	36	37	36	36
VBDcau	Correlation						.178	.742**	.192	-.114	-.118
	Sig. (2-tailed)						.293	.000	.255	.509	.495
	N						37	36	37	36	36
VBWcra	Correlation							.584**	-.403*	-.120	-.044
	Sig. (2-tailed)							.000	.013	.487	.798
	N							36	37	36	36
VBWcau	Correlation								-.335*	-.253	-.171
	Sig. (2-tailed)								.046	.136	.318
	N								36	36	36
OST	Correlation									.239	-.014
	Sig. (2-tailed)									.160	.937
	N									36	36
vBMD	Correlation										.937**
	Sig. (2-tailed)										.000
	N										36
TbBMD	Correlation										
	Sig. (2-tailed)										
	N										

** Correlation is significant at the <0.01 level (2-tailed)
* Correlation is significant at the <0.05 level (2-tailed)
VBHd = dorsal vertebral body height; VBHv = ventral vertebral body height; VBDcra = cranial vertebral body depth; VBDcau = caudal vertebral body depth; VBWcra = cranial vertebral body width; VBWcau = caudal vertebral body width; OST = osteophytosis; vBMD = total bone mineral density; TbBMD = trabecular bone mineral density

Table 1.3 Partial correlation of all variables from L1, 3, 5 and 7 of female Japanese macaques after controlling for age

Control variable		L1	VBHd	VBHv	VBDcra	VBDcau	VBWcra	VBWcau	OST	vBMD	TbBMD
Age	**VBHd**	Correlation		.823**	.374*	.331	.595**	.624**	.132	-.033	-.070
		Sig. (2-tailed)		.000	.032	.060	.000	.000	.466	.857	.699
		df		31	31	31	31	31	31	31	31
	VBHv	Correlation			.208	.225	.524**	.477**	.063	-.148	-.132
		Sig. (2-tailed)			.245	.208	.002	.005	.728	.411	.464
		df			31	31	31	31	31	31	31
	VBDcra	Correlation				.844**	.415*	.484**	-.087	.371*	.317
		Sig. (2-tailed)				.000	.016	.004	.632	.033	.073
		df				31	31	31	31	31	31
	VBDcau	Correlation					.411*	.505**	-.018	.308	.257
		Sig. (2-tailed)					.017	.003	.919	.082	.149
		df					31	31	31	31	31
	VBWcra	Correlation						.800**	-.129	.126	.050
		Sig. (2-tailed)						.000	.474	.484	.781
		df						31	31	31	31
	VBWcau	Correlation							-.046	.163	.098
		Sig. (2-tailed)							.799	.363	.587
		df							31	31	31
	OST	Correlation								.029	-.167
		Sig. (2-tailed)								.873	.353
		df								31	31
	vBMD	Correlation									.936**
		Sig. (2-tailed)									.000
		df									31
	TbBMD	Correlation									
		Sig. (2-tailed)									
		df									

Table 1.3 (continued)

Control variable		L3	VBHd	VBHv	VBDcra	VBDcau	VBWcra	VBWcau	OST	vBMD	TbBMD
Age	**VBHd**	Correlation		.829**	.237	.112	.544**	.652**	.237	.082	-.049
		Sig. (2-tailed)		.000	.177	.529	.001	.000	.178	.646	.785
		df		32	32	32	32	32	32	32	32
	VBHv	Correlation			.107	.004	.453**	.532**	.116	.039	.006
		Sig. (2-tailed)			.548	.983	.007	.001	.512	.826	.973
		df			32	32	32	32	32	32	32
	VBDcra	Correlation				.721**	.567**	.524**	-.176	.260	.202
		Sig. (2-tailed)				.000	.000	.001	.320	.138	.252
		df				32	32	32	32	32	32
	VBDcau	Correlation					.510**	.538**	-.172	.272	.361*
		Sig. (2-tailed)					.002	.001	.330	.120	.036
		df					32	32	32	32	32
	VBWcra	Correlation						.803**	-.174	.065	.065
		Sig. (2-tailed)						.000	.326	.716	.713
		df						32	32	32	32
	VBWcau	Correlation							-.157	.166	.123
		Sig. (2-tailed)							.374	.349	.487
		df							32	32	32
	OST	Correlation								-.147	-.275
		Sig. (2-tailed)								.408	.116
		df								32	32
	vBMD	Correlation									.923**
		Sig. (2-tailed)									.000
		df									32
	TbBMD	Correlation									
		Sig. (2-tailed)									
		df									

Table 1.3 (continued)

Control variable		L5	VBHd	VBHv	VBDcra	VBDcau	VBWcra	VBWcau	OST	vBMD	TbBMD
Age	**VBHd**	Correlation		.680**	.136	.255	.376*	.475**	-.053	.366*	.151
		Sig. (2-tailed)		.000	.444	.146	.028	.005	.765	.033	.393
		df		32	32	32	32	32	32	32	32
	VBHv	Correlation			-.029	.175	.197	.346*	-.125	.365*	.292
		Sig. (2-tailed)			.872	.321	.264	.045	.481	.034	.094
		df			32	32	32	32	32	32	32
	VBDcra	Correlation				.856**	.365*	.346*	.068	.279	.267
		Sig. (2-tailed)				.000	.034	.045	.702	.111	.126
		df				32	32	32	32	32	32
	VBDcau	Correlation					.418*	.488**	.210	.256	.254
		Sig. (2-tailed)					.014	.003	.234	.144	.148
		df					32	32	32	32	32
	VBWcra	Correlation						.792**	-.025	.167	.027
		Sig. (2-tailed)						.000	.887	.344	.882
		df						32	32	32	32
	VBWcau	Correlation							-.023	.170	.048
		Sig. (2-tailed)							.897	.337	.788
		df							32	32	32
	OST	Correlation								-.137	-.261
		Sig. (2-tailed)								.439	.136
		df								32	32
	vBMD	Correlation									.909**
		Sig. (2-tailed)									.000
		df									32
	TbBMD	Correlation									
		Sig. (2-tailed)									
		df									

Table 1.3 (continued)

Control variable		L7	VBHd	VBHv	VBDcra	VBDcau	VBWcra	VBWcau	OST	vBMD	TbBMD
Age	**VBHd**	Correlation		.591**	.236	.692**	.199	.586**	-.022	.128	.141
		Sig. (2-tailed)		.000	.178	.000	.258	.000	.901	.469	.425
		df		32	32	32	32	32	32	32	32
	VBHv	Correlation			.021	.413*	.337	.459**	-.193	.009	.064
		Sig. (2-tailed)			.908	.015	.051	.006	.274	.958	.720
		df			32	32	32	32	32	32	32
	VBDcra	Correlation				.501**	.182	.101	.344*	-.057	-.178
		Sig. (2-tailed)				.003	.303	.569	.047	.747	.315
		df				32	32	32	32	32	32
	VBDcau	Correlation					.329	.616**	.116	.091	.066
		Sig. (2-tailed)					.057	.000	.512	.611	.711
		df					32	32	32	32	32
	VBWcra	Correlation						.421*	-.128	-.058	-.122
		Sig. (2-tailed)						.013	.471	.743	.492
		df						32	32	32	32
	VBWcau	Correlation							-.194	.233	.255
		Sig. (2-tailed)							.271	.184	.145
		df							32	32	32
	OST	Correlation								-.281	-.344
		Sig. (2-tailed)								.108	.047
		df								32	32
	vBMD	Correlation									.950**
		Sig. (2-tailed)									.000
		df									32
	TbBMD	Correlation									
		Sig. (2-tailed)									
		df									

** Correlation is significant at the <0.01 level (2-tailed)

* Correlation is significant at the <0.05 level (2-tailed)

VBHd = dorsal vertebral body height; VBHv = ventral vertebral body height; VBDcra = cranial vertebral body depth; VBDcau = caudal vertebral body depth; VBWcra = cranial vertebral body width; VBWcau = caudal vertebral body width; OST = osteophytosis; vBMD = total bone mineral density; TbBMD = trabecular bone mineral density

Table 1.4 Partial correlation of all variables from L1, 3, 5 and 7 of male Japanese macaques after controlling for age

Control variable		L1	VBHd	VBHv	VBDcra	VBDcau	VBWcra	VBWcau	OST	vBMD	TbBMD
Age	**VBHd**	Correlation		.829**	.575**	.659**	.094	.363*	.327	-.031	-.064
		Sig. (2-tailed)		.000	.000	.000	.590	.032	.055	.857	.717
		df		33	33	33	33	33	33	33	33
	VBHv	Correlation			.395*	.444**	.137	.215	.217	.153	.131
		Sig. (2-tailed)			.019	.008	.433	.214	.210	.381	.454
		df			33	33	33	33	33	33	33
	VBDcra	Correlation				.829**	.266	.306	.249	.109	.111
		Sig. (2-tailed)				.000	.122	.074	.150	.535	.526
		df				33	33	33	33	33	33
	VBDcau	Correlation					.097	.510**	.297	.045	.042
		Sig. (2-tailed)					.580	.002	.083	.799	.812
		df					33	33	33	33	33
	VBWcra	Correlation						.389*	-.081	-.201	-.166
		Sig. (2-tailed)						.021	.642	.248	.341
		df						33	33	33	33
	VBWcau	Correlation							.153	-.341*	-.443**
		Sig. (2-tailed)							.380	.045	.008
		df							33	33	33
	OST	Correlation								.348*	.297
		Sig. (2-tailed)								.040	.083
		df								33	33
	vBMD	Correlation									.950**
		Sig. (2-tailed)									.000
		df									33
	TbBMD	Correlation									
		Sig. (2-tailed)									
		df									

Table 1.4 (continued)

Control variable		L3	VBHd	VBHv	VBDcra	VBDcau	VBWcra	VBWcau	OST	vBMD	TbBMD
Age	**VBHd**	Correlation		.812**	.559**	.638**	.329	.525**	.217	-.063	-.128
		Sig. (2-tailed)		.000	.000	.000	.054	.001	.211	.720	.462
		df		33	33	33	33	33	33	33	33
	VBHv	Correlation			.172	.247	.373*	.312	.075	-.030	-.069
		Sig. (2-tailed)			.322	.153	.027	.068	.669	.862	.692
		df			33	33	33	33	33	33	33
	VBDcra	Correlation				.873**	.031	.294	.425*	-.039	.013
		Sig. (2-tailed)				.000	.860	.086	.011	.825	.942
		df				33	33	33	33	33	33
	VBDcau	Correlation					.061	.302	.409*	-.132	-.129
		Sig. (2-tailed)					.727	.077	.015	.451	.461
		df					33	33	33	33	33
	VBWcra	Correlation						.684**	-.111	-.378*	-.352*
		Sig. (2-tailed)						.000	.526	.025	.038
		df						33	33	33	33
	VBWcau	Correlation							.015	-.158	-.232
		Sig. (2-tailed)							.933	.364	.180
		df							33	33	33
	OST	Correlation								.241	.191
		Sig. (2-tailed)								.163	.271
		df								33	33
	vBMD	Correlation									.931**
		Sig. (2-tailed)									.000
		df									33
	TbBMD	Correlation									
		Sig. (2-tailed)									
		df									

Table 1.4 (continued)

Control variable		L5	VBHd	VBHv	VBDcra	VBDcau	VBWcra	VBWcau	OST	vBMD	TbBMD
Age	**VBHd**	Correlation		.745**	.553**	.560**	.440**	.424*	.253	.171	.045
		Sig. (2-tailed)		.000	.001	.001	.009	.012	.149	.334	.801
		df		32	32	32	32	32	32	32	32
	VBHv	Correlation			.296	.283	.177	.073	.126	.180	.099
		Sig. (2-tailed)			.089	.105	.316	.680	.479	.307	.577
		df			32	32	32	32	32	32	32
	VBDcra	Correlation				.930**	.153	.505**	.154	-.091	-.168
		Sig. (2-tailed)				.000	.386	.002	.386	.611	.342
		df				32	32	32	32	32	32
	VBDcau	Correlation					.171	.550**	.287	-.042	-.146
		Sig. (2-tailed)					.334	.001	.100	.813	.409
		df					32	32	32	32	32
	VBWcra	Correlation						.565**	-.159	-.015	-.022
		Sig. (2-tailed)						.001	.370	.932	.900
		df						32	32	32	32
	VBWcau	Correlation							.067	-.045	-.148
		Sig. (2-tailed)							.708	.799	.403
		df							32	32	32
	OST	Correlation								.182	.079
		Sig. (2-tailed)								.303	.656
		df								32	32
	vBMD	Correlation									.944**
		Sig. (2-tailed)									.000
		df									32
	TbBMD	Correlation									
		Sig. (2-tailed)									
		df									

Table 1.4 (continued)

Control variable		L7	VBHd	VBHv	VBDcra	VBDcau	VBWcra	VBWcau	OST	vBMD	TbBMD
Age	**VBHd**	Correlation		.717**	.462**	.565**	.434**	.769**	-.147	.029	.029
		Sig. (2-tailed)		.000	.005	.000	.009	.000	.400	.867	.867
		df		33	33	33	33	33	33	33	33
	VBHv	Correlation			.234	.098	.377*	.478**	-.254	-.034	-.047
		Sig. (2-tailed)			.177	.575	.026	.004	.141	.847	.791
		df			33	33	33	33	33	33	33
	VBDcra	Correlation				.846**	.155	.614**	.247	-.273	-.317
		Sig. (2-tailed)				.000	.375	.000	.152	.113	.063
		df				33	33	33	33	33	33
	VBDcau	Correlation					.192	.741**	.213	-.078	-.115
		Sig. (2-tailed)					.270	.000	.219	.655	.512
		df					33	33	33	33	33
	VBWcra	Correlation						.530**	-.200	-.032	-.036
		Sig. (2-tailed)						.001	.248	.855	.836
		df						33	33	33	33
	VBWcau	Correlation							-.176	-.188	-.171
		Sig. (2-tailed)							.312	.281	.325
		df							33	33	33
	OST	Correlation								.101	-.042
		Sig. (2-tailed)								.564	.809
		df								33	33
	vBMD	Correlation									.961**
		Sig. (2-tailed)									.000
		df									33
	TbBMD	Correlation									
		Sig. (2-tailed)									
		df									

** Correlation is significant at the <0.01 level (2-tailed)

* Correlation is significant at the <0.05 level (2-tailed)

VBHd = dorsal vertebral body height; VBHv = ventral vertebral body height; VBDcra = cranial vertebral body depth; VBDcau = caudal vertebral body depth; VBWcra = cranial vertebral body width; VBWcau = caudal vertebral body width; OST = osteophytosis; vBMD = total bone mineral density; TbBMD = trabecular bone mineral density

Table 2.1 Independent *t* test of all variables between female Japanese macaques from captive group (CG) and free-ranging group (FRG)

		Levene's test for equality of variances		*t* test for equality of means						
									95% confidence interval of the difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Std. error difference	Lower	Upper
Age	Equal variances assumed	.710	.403	5.026	50	.000	7.92316	1.57647	4.75673	11.08960
	Equal variances not assumed			5.027	43.137	.000	7.92316	1.57599	4.74518	11.10115
BM	Equal variances assumed	12.988	.001	7.003	50	.000	3.38631	.48354	2.41509	4.35752
	Equal variances not assumed			5.989	23.230	.000	3.38631	.56542	2.21729	4.55532
BMI	Equal variances assumed	20.498	.000	4.322	50	.000	7.03827	1.62836	3.76762	10.30893
	Equal variances not assumed			3.597	21.093	.002	7.03827	1.95670	2.97017	11.10637
DSN	Equal variances assumed	25.147	.000	4.664	50	.000	.69375	.14875	.39497	.99253
	Equal variances not assumed			3.857	20.655	.001	.69375	.17985	.31935	1.06816
OST-S	Equal variances assumed	35.508	.000	5.821	50	.000	.91997	.15804	.60254	1.23740
	Equal variances not assumed			5.002	23.642	.000	.91997	.18392	.54008	1.29986
OST-V	Equal variances assumed	32.221	.000	6.774	50	.000	1.02699	.15161	.72247	1.33152
	Equal variances not assumed			5.765	22.829	.000	1.02699	.17814	.65833	1.39566

BM = body mass; BMI = body mass index like-index; DSN = disc space narrowing; OST = osteophytosis

Table 2.2 Independent *t* test of all variables between male Japanese macaques from captive group (CG) and free-ranging group (FRG)

		Levene's test for equality of variances		*t* test for equality of means						
									95% confidence interval of the difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Std. error difference	Lower	Upper
Age	Equal variances assumed	4.864	.032	1.288	47	.204	1.98126	1.53773	-1.11225	5.07477
	Equal variances not assumed			1.321	46.939	.193	1.98126	1.49966	-1.03576	4.99828
BM	Equal variances assumed	1.991	.165	7.391	47	.000	4.06695	.55027	2.95996	5.17395
	Equal variances not assumed			7.486	46.660	.000	4.06695	.54325	2.97387	5.16003
BMI	Equal variances assumed	1.738	.194	3.258	47	.002	3.18225	.97681	1.21716	5.14734
	Equal variances not assumed			3.164	38.166	.003	3.18225	1.00582	1.14636	5.21814
DSN	Equal variances assumed	10.172	.003	2.335	47	.024	.25679	.10996	.03559	.47799
	Equal variances not assumed			2.167	26.612	.039	.25679	.11853	.01343	.50015
OST-S	Equal variances assumed	29.042	.000	3.291	47	.002	.52761	.16032	.20510	.85012
	Equal variances not assumed			3.044	25.961	.005	.52761	.17334	.17128	.88394
OST-V	Equal variances assumed	33.887	.000	3.831	47	.000	.63111	.16474	.29971	.96252
	Equal variances not assumed			3.533	25.368	.002	.63111	.17863	.26349	.99873

BM = body mass; BMI = body mass index like-index; DSN = disc space narrowing; OST = osteophytosis

Table 2.3 Analysis of covariance (ANCOVA) of disc space narrowing (DSN) and osteophytosis (OST-S and OST-V) between female Japanese macaques from captive group (CG) and free-ranging group (FRG) after controlling for body mass (BM)

Tests of between-subjects effects

Dependent variable: DSN

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	6.767[a]	3	2.256	8.259	.000
Intercept	.006	1	.006	.024	.878
Group	.003	1	.003	.011	.917
BM	.091	1	.091	.331	.568
Group * BM	.135	1	.135	.493	.486
Error	13.109	48	.273		
Total	25.901	52			
Corrected total	19.876	51			

a. R squared = .340 (Adjusted R squared = .299)

Tests of between-subjects effects

Dependent variable: DSN

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	6.633[a]	2	3.316	12.270	.000
Intercept	.021	1	.021	.078	.781
BM	.607	1	.607	2.246	.140
Group	1.430	1	1.430	5.290	.026
Error	13.244	49	.270		
Total	25.901	52			
Corrected total	19.876	51			

a. R squared = .334 (Adjusted R squared = .306)

Table 2.3 (continued)

Tests of between-subjects effects

Dependent variable: OST-S

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	10.793[a]	3	3.598	11.188	.000
Intercept	.306	1	.306	.951	.334
Group	.124	1	.124	.386	.538
BM	.034	1	.034	.105	.747
Group * BM	.026	1	.026	.081	.778
Error	15.436	48	.322		
Total	41.777	52			
Corrected total	26.229	51			

a. R squared = .411 (Adjusted R squared = .375)

Tests of between-subjects effects

Dependent variable: OST-S

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	10.767[a]	2	5.384	17.061	.000
Intercept	.309	1	.309	.980	.327
BM	.172	1	.172	.545	.464
Group	4.085	1	4.085	12.945	.001
Error	15.462	49	.316		
Total	41.777	52			
Corrected total	26.229	51			

a. R squared = .411 (Adjusted R squared = .386)

Table 2.3 (continued)

Tests of between-subjects effects

Dependent variable: OST-V

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	13.439[a]	3	4.480	15.191	.000
Intercept	.295	1	.295	1.000	.322
Group	.189	1	.189	.640	.428
BM	.056	1	.056	.190	.665
Group * BM	.019	1	.019	.063	.802
Error	14.154	48	.295		
Total	44.573	52			
Corrected total	27.593	51			

a. R squared = .487 (Adjusted R squared = .455)

Tests of between-subjects effects

Dependent variable: OST-V

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	13.420[a]	2	6.710	23.198	.000
Intercept	.314	1	.314	1.085	.303
BM	.216	1	.216	.746	.392
Group	5.085	1	5.085	17.580	.000
Error	14.173	49	.289		
Total	44.573	52			
Corrected total	27.593	51			

a. R squared = .486 (Adjusted R squared = .465)

Table 2.4 Analysis of covariance (ANCOVA) of disc space narrowing (DSN) and osteophytosis (OST-S and OST-V) between male Japanese macaques from captive group (CG) and free-ranging group (FRG) after controlling for body mass (BM)

Tests of between-subjects effects

Dependent variable: DSN

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	1.588[a]	3	.529	3.904	.015
Intercept	.338	1	.338	2.491	.121
Group	.059	1	.059	.436	.513
BM	.786	1	.786	5.798	.020
Group * BM	.061	1	.061	.449	.506
Error	6.100	45	.136		
Total	10.172	49			
Corrected total	7.688	48			

a. R squared = .206 (Adjusted R squared = .154)

Tests of between-subjects effects

Dependent variable: DSN

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	1.527[a]	2	.763	5.699	.006
Intercept	.277	1	.277	2.069	.157
BM	.727	1	.727	5.430	.024
Group	.000	1	.000	.002	.963
Error	6.161	46	.134		
Total	10.172	49			
Corrected total	7.688	48			

a. R squared = .199 (Adjusted R squared = .164)

Table 2.4 (continued)

Tests of between-subjects effects

Dependent variable: OST-S

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	4.347[a]	3	1.449	4.769	.006
Intercept	.129	1	.129	.424	.518
Group	.064	1	.064	.210	.649
BM	.856	1	.856	2.818	.100
Group * BM	.015	1	.015	.049	.826
Error	13.671	45	.304		
Total	24.667	49			
Corrected total	18.018	48			

a. R squared = .241 (Adjusted R squared = .191)

Tests of between-subjects effects

Dependent variable: OST-S

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	4.332[a]	2	2.166	7.280	.002
Intercept	.198	1	.198	.664	.419
BM	.957	1	.957	3.217	.079
Group	.283	1	.283	.951	.335
Error	13.686	46	.298		
Total	24.667	49			
Corrected total	18.018	48			

a. R squared = .240 (Adjusted R squared = .207)

Table 2.4 (continued)

Tests of between-subjects effects

Dependent variable: OST-V

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	5.609[a]	3	1.870	5.731	.002
Intercept	.038	1	.038	.115	.736
Group	.139	1	.139	.426	.517
BM	.656	1	.656	2.009	.163
Group * BM	.026	1	.026	.080	.779
Error	14.681	45	.326		
Total	28.347	49			
Corrected total	20.290	48			

a. R squared = .276 (Adjusted R squared = .228)

Tests of between-subjects effects

Dependent variable: OST-V

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	5.583[a]	2	2.792	8.731	.001
Intercept	.079	1	.079	.248	.621
BM	.755	1	.755	2.361	.131
Group	.735	1	.735	2.299	.136
Error	14.707	46	.320		
Total	28.347	49			
Corrected total	20.290	48			

a. R squared = .275 (Adjusted R squared = .244)

Table 3.1 Independent *t* test of all variables between female *M. fuscata* and *M. fascicularis*

		Levene's test for equality of variances		*t* test for equality of means						
									95% confidence interval of the difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Std. error difference	Lower	Upper
Age	Equal variances assumed	4.329	.039	.579	143	.563	.71584	1.23541	-1.72618	3.15786
	Equal variances not assumed			.606	142.832	.546	.71584	1.18136	-1.61936	3.05104
TbBMD	Equal variances assumed	.492	.484	-5.292	141	.000	-35.08538	6.63007	-48.19257	-21.97819
	Equal variances not assumed			-5.373	133.694	.000	-35.08538	6.52951	-47.99989	-22.17087
TbBMC	Equal variances assumed	67.296	.000	-14.024	141	.000	-3.16329	.22556	-3.60921	-2.71737
	Equal variances not assumed			-12.398	70.907	.000	-3.16329	.25515	-3.67206	-2.65452
CorBMD	Equal variances assumed	2.827	.095	5.061	143	.000	25.10443	4.96051	15.29903	34.90983
	Equal variances not assumed			5.250	141.897	.000	25.10443	4.78173	15.65180	34.55706
CorBMC	Equal variances assumed	5.088	.026	-24.644	143	.000	-18.00262	.73052	-19.44662	-16.55861
	Equal variances not assumed			-23.715	109.568	.000	-18.00262	.75914	-19.50711	-16.49812
DSN	Equal variances assumed	.029	.864	.657	139	.513	.07527	.11466	-.15143	.30197
	Equal variances not assumed			.656	128.594	.513	.07527	.11482	-.15191	.30246
OST	Equal variances assumed	.305	.582	-.396	139	.693	-.05513	.13913	-.33021	.21995
	Equal variances not assumed			-.394	126.721	.694	-.05513	.13984	-.33186	.22160
BM	Equal variances assumed	22.811	.000	-25.218	141	.000	-5.74396	.22777	-6.19425	-5.29368
	Equal variances not assumed			-22.532	74.633	.000	-5.74396	.25492	-6.25183	-5.23610
BMI	Equal variances assumed	3.390	.068	-9.762	138	.000	-8.54497	.87534	-10.27578	-6.81417
	Equal variances not assumed			-9.093	90.138	.000	-8.54497	.93978	-10.41197	-6.67798

TbBMD = trabecular bone mineral density; TbBMC = trabecular bone mineral content; CorBMD = cortical bone mineral density; CorBMC = cortical bone mineral content; DSN = disc space narrowing; OST = osteophytosis; BM = body mass; BMI = body mass index like-index

Table 3.2 Mann-Whitney *U* test of all variables between <25 years of age and ≥25 years of age group of female *M. fuscata*

Test Statistics[b]

	Age	TbBMD	TbBMC	CorBMD	CorBMC	DSN	OST	BM	BMI
Mann-Whitney *U*	.000	86.500	140.000	120.000	138.000	43.500	66.000	155.000	134.000
Wilcoxon W	1.540E3	107.500	161.000	1.660E3	159.000	1.584E3	1.606E3	176.000	1.512E3
Z	-3.996	-1.860	-.542	-1.090	-.654	-3.070	-2.426	-.172	-.562
Asymp. sig. (2-tailed)	.000	.063	.588	.276	.513	.002	.015	.863	.574
Exact sig. [2*(1-tailed Sig.)]	.000[a]	.062[a]	.605[a]	.289[a]	.530[a]	.002[a]	.014[a]	.876[a]	.592[a]
Exact sig. (2-tailed)	.000	.062	.601	.289	.530	.001	.012	.873	.590
Exact sig. (1-tailed)	.000	.031	.301	.145	.265	.001	.007	.437	.295
Point probability	.000	.001	.005	.005	.008	.000	.000	.007	.007

a. Not corrected for ties.

b. Grouping variable: group

TbBMD = trabecular bone mineral density; TbBMC = trabecular bone mineral content; CorBMD = cortical bone mineral density; CorBMC = cortical bone mineral content; DSN = disc space narrowing; OST = osteophytosis; BM = body mass; BMI = body mass index like-index

Table 3.3 Mann-Whitney *U* test of all variables between premenopausal and postmenopausal group of female *M. fascicularis*

Test Statistics[a]

	Age	TbBMD	TbBMC	CorBMD	CorBMC	DSN	OST	BM	BMI
Mann-Whitney *U*	.000	105.500	152.500	313.000	64.000	39.500	42.000	262.500	125.000
Wilcoxon W	2.701E3	150.500	197.500	358.000	109.000	2.668E3	2.670E3	307.500	2.753E3
Z	-4.879	-3.284	-2.578	-.230	-3.924	-3.381	-3.290	-.924	-2.614
Asymp. sig. (2-tailed)	.000	.001	.010	.818	.000	.001	.001	.355	.009
Exact sig. (2-tailed)	.000	.001	.008	.826	.000	.000	.000	.363	.007
Exact sig. (1-tailed)	.000	.000	.004	.413	.000	.000	.000	.182	.004
Point probability	.000	.000	.000	.004	.000	.000	.000	.002	.000

a. Grouping variable: group

TbBMD = trabecular bone mineral density; TbBMC = trabecular bone mineral content; CorBMD = cortical bone mineral density; CorBMC = cortical bone mineral content; DSN = disc space narrowing; OST = osteophytosis; BM = body mass; BMI = body mass index like-index

Table 3.4 Analysis of covariance (ANCOVA) of densitometric variables between *M. fuscata* and *M. fascicularis* after controlling for body mass (BM)

Tests of between-subjects effects

Dependent variable: TbBMD

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	47196.469[a]	3	15732.156	10.353	.000
Intercept	93814.963	1	93814.963	61.738	.000
Species	7358.099	1	7358.099	4.842	.029
BM	.697	1	.697	.000	.983
Species * BM	1200.641	1	1200.641	.790	.376
Error	208180.959	137	1519.569		
Total	2162616.020	141			
Corrected total	255377.428	140			

a. R squared = .185 (Adjusted R squared = .167)

Tests of between-subjects effects

Dependent variable: TbBMD

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	45995.828[a]	2	22997.914	15.158	.000
Intercept	101035.220	1	101035.220	66.591	.000
BM	735.018	1	735.018	.484	.488
Species	13347.933	1	13347.933	8.797	.004
Error	209381.601	138	1517.258		
Total	2162616.020	141			
Corrected total	255377.428	140			

a. R squared = .180 (Adjusted R squared = .168)

Table 3.4 (continued)

Tests of between-subjects effects

Dependent variable: TbBMC

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	355.183[a]	3	118.394	66.753	.000
Intercept	41.732	1	41.732	23.529	.000
Species	15.832	1	15.832	8.926	.003
BM	.001	1	.001	.000	.985
Species * BM	.011	1	.011	.006	.937
Error	242.988	137	1.774		
Total	1420.539	141			
Corrected total	598.171	140			

a. R squared = .594 (Adjusted R squared = .585)

Tests of between-subjects effects

Dependent variable: TbBMC

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	355.172[a]	2	177.586	100.852	.000
Intercept	43.411	1	43.411	24.654	.000
BM	.002	1	.002	.001	.970
Species	65.745	1	65.745	37.337	.000
Error	242.999	138	1.761		
Total	1420.539	141			
Corrected total	598.171	140			

a. R squared = .594 (Adjusted R squared = .588)

Table 3.4 (continued)

Tests of between-subjects effects

Dependent variable: CorBMD

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	22687.928[a]	3	7562.643	8.548	.000
Intercept	8649153.333	1	8649153.333	9.776E3	.000
Species	441.382	1	441.382	.499	.481
BM	139.465	1	139.465	.158	.692
Species * BM	7.275	1	7.275	.008	.928
Error	122976.277	139	884.721		
Total	2.105E8	143			
Corrected total	145664.205	142			

a. R squared = .156 (Adjusted R squared = .138)

Tests of between-subjects effects

Dependent variable: CorBMD

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	22680.654[a]	2	11340.327	12.909	.000
Intercept	8920339.713	1	8920339.713	1.015E4	.000
BM	279.119	1	279.119	.318	.574
Species	2366.325	1	2366.325	2.694	.103
Error	122983.551	140	878.454		
Total	2.105E8	143			
Corrected total	145664.205	142			

a. R squared = .156 (Adjusted R squared = .144)

Table 3.4 (continued)

Tests of between-subjects effects

Dependent variable: CorBMC

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	12007.145[a]	3	4002.382	264.709	.000
Intercept	2693.104	1	2693.104	178.116	.000
Species	304.642	1	304.642	20.148	.000
BM	476.967	1	476.967	31.546	.000
Species * BM	37.710	1	37.710	2.494	.117
Error	2101.669	139	15.120		
Total	138463.721	143			
Corrected total	14108.814	142			

a. R squared = .851 (Adjusted R squared = .848)

Tests of between-subjects effects

Dependent variable: CorBMC

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	11969.435[a]	2	5984.718	391.637	.000
Intercept	2891.824	1	2891.824	189.240	.000
BM	512.508	1	512.508	33.538	.000
Species	630.815	1	630.815	41.280	.000
Error	2139.379	140	15.281		
Total	138463.721	143			
Corrected total	14108.814	142			

a. R squared = .848 (Adjusted R squared = .846)

TbBMD = trabecular bone mineral density; TbBMC = trabecular bone mineral content; CorBMD = cortical bone mineral density; CorBMC = cortical bone mineral content; BM = body mass

Table 3.5 Partial correlation controlling for age and body mass (BM) of all variables of *M. fuscata*

Control variables			TbBMD	TbBMC	CorBMD	CorBMC	DSN	OST
Age & BM	TbBMD	Correlation		.521**	-.239	.344*	.283*	.143
		Significance (2-tailed)		.000	.079	.010	.036	.297
		df		53	53	53	53	53
	TbBMC	Correlation			-.614**	.233	.439**	.139
		Significance (2-tailed)			.000	.087	.001	.311
		df			53	53	53	53
	CorBMD	Correlation				-.331*	-.254	-.018
		Significance (2-tailed)				.014	.061	.895
		df				53	53	53
	CorBMC	Correlation					.043	.040
		Significance (2-tailed)					.757	.772
		df					53	53
	DSN	Correlation						.513**
		Significance (2-tailed)						.000
		df						53
	OST	Correlation						
		Significance (2-tailed)						
		df						

** Correlation is significant at the <0.01 level (2-tailed)

* Correlation is significant at the <0.05 level (2-tailed)

TbBMD = trabecular bone mineral density; TbBMC = trabecular bone mineral content; CorBMD = cortical bone mineral density; CorBMC = cortical bone mineral content; DSN = disc space narrowing; OST = osteophytosis

Table 3.6 Partial correlation controlling for age and body mass (BM) of all variables of *M. fascicularis*

Control variables			TbBMD	TbBMC	CorBMD	CorBMC	DSN	OST
Age & BM	TbBMD	Correlation		.637**	.005	-.036	-.106	-.140
		Significance (2-tailed)		.000	.968	.757	.363	.226
		df		74	74	74	74	74
	TbBMC	Correlation			-.150	-.052	-.039	-.155
		Significance (2-tailed)			.197	.658	.740	.180
		df			74	74	74	74
	CorBMD	Correlation				.119	.067	.193
		Significance (2-tailed)				.304	.567	.094
		df				74	74	74
	CorBMC	Correlation					.360**	.226
		Significance (2-tailed)					.001	.050
		df					74	74
	DSN	Correlation						.584**
		Significance (2-tailed)						.000
		df						74
	OST	Correlation						
		Significance (2-tailed)						
		df						

** Correlation is significant at the <0.01 level (2-tailed)

* Correlation is significant at the <0.05 level (2-tailed)

TbBMD = trabecular bone mineral density; TbBMC = trabecular bone mineral content; CorBMD = cortical bone mineral density; CorBMC = cortical bone mineral content; DSN = disc space narrowing; OST = osteophytosis