

**Age-related changes in the skulls of
Japanese macaques**

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Abstract

Assessment of the age-related changes in skulls of non-human primates is important for understanding evolution of age-related changes in the skull for the life-history evolution and the mechanisms of the aging of the skull, and contributes to give implications for the aging of human skulls. In this study, I investigated age-related changes in the skulls of Japanese macaques (*Macaca fuscata*), using dried skulls.

In chapter 1, I studied age-related skull morphometric changes of Japanese macaques. Some skull dimensions increased from young adulthood (7.0 years) and peaked at 13.3–19.0 years in males and at 19.7–22.6 years in females. Some dimensions stayed at the peak to very old age, and others continued increasing through very old age. Intertemporal distance and biorbital breadths after 16.0 years of age decreased significantly in males, and cranial and posterior basicranial lengths increased only in males. I suggest that these craniometric changes are associated with the development of insertion area onto which muscles attach (by accumulation of physical stress).

In chapter 2, I investigated age-related changes in the craniofacial thickness of Japanese macaques, using computed tomography scans. The cranial thickness at many sites in neurocranium showed a pattern of increasing from young adulthood (7–9 years) to mid-adulthood (14–19 years in males and 19–24 years in females) and decreasing to the oldest age group (24 years or older). However, the thickness at the two facial sites (MFZ and MLZ) showed an exceptionally distinctive pattern of decreasing from young adulthood to very old age in both sexes. The thickness at sites on the mid-sagittal plane significantly increased in males from young adulthood to mid-adulthood, though they did not show any significant change from mid-adulthood through very old age. This sex difference may be associated with the differences in the size of projecting face and canines between males and females. This leads to the development of masticatory and postural muscles, and stimulates the increase in the cranial thickness at these sites in males.

In chapter 3, I investigated age-related changes in the sulcus imprint on the endocranium of Japanese macaques from juveniles to old age, using virtual endocasts generated by computed tomography scans. The details of the sulcal imprints showed a slight decrease from the juvenile period (age group 2–4 years) to the adolescent period (group 4–6 years), and then, remained unchanged to mid-adulthood (age group 15–16 years). After that, the definiteness of the sulcal imprints significantly decreased to old age (age group >20

years). The decrease in the definiteness of the sulcal imprints between the younger age classes and the older age class may be associated both with the shrinkage of the brain and an increase of the endocranial volume with age.

These findings indicate that the continued increase of cranial size in adulthood of macaques has also been observed in humans, but the magnitude of change was greater in macaques. Facial and mandibular dimensions showed larger and more significant increases than neurocranial dimensions in macaques, as in humans, including facial height, bizygomatic breadth, mandibular body height, and ramus breadth in both sexes. The face and mandible are greatly influenced by tooth loss and/or dental disorders, both of which are evident in humans. In the present study large changes were also found in skulls with the loss of several teeth. Cranial thickness significantly decreases with age in Japanese women, but not in men. It may be that the cranial thickness might be greatly affected by lower bone metabolism. In the present study, the cranial thickness at many sites in macaques showed a significant decrease from mid-adulthood to very old age in both sexes, although females revealed more sites with decreasing thickness than males. This sex difference may be associated postmenopausal estrogen depletion in female macaques.

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General Introduction

The head and face show significant physiognomic changes in adult life with advancing age. In humans, age-related changes in the skull have received increasing attention in forensic research (Lynnerup, 2001; Lynnerup et al., 2005; Albert et al., 2007), esthetic surgery (Bartlett et al., 1992; Pessa et al., 1999; Pessa, 2000; Shaw and Kahn, 2007; Mendelson and Wong, 2012), and the risk of skull fracture with aging (Torimitsu et al., 2014). However, there are no substantial studies revealing age-related changes in the skull. On the other hand, age-related changes in postcranial skeletons have been extensively studied on aspects of morphology, density (osteoporosis), and osteoarthritis; because they are of importance for positional behavior and quality of life in the elderly.

Aging is a deterioration process that occurs in most animals after maturity, which includes weakening, increased susceptibility to disease, the loss of mobility and agility, and adverse changes in physiology (Godsmith, 2010, 2012). Aging is commonly understood to bring about a progressive decline in the function of various physiological systems including cardiorespiratory, musculoskeletal, neuroendocrine, immune, gastrointestinal, auditory, visual, and reproductive systems (Arking, 1998; Godsmith, 2012).

In the life history, post-reproductive life is important in human evolution, and “elderly” period is terminally added. Reproductive senescence, such as increase of interbirth intervals, reduction of fertility or cessation of ovulation or menopause (Walker and Herndon, 2008; Godsmith, 2012), is considered to relate to the evolution of an extended post-reproductive life for a large proportion of human females (Pavelka and Fedigan, 1991; Fedigan and Pavelka, 2001; Thompson et al., 2007). On the other hand, post-reproductive life in non-human primates is either absent or short (or variable with individuals), and thus, non-human animals are considered to have no substantial elderly period, if aging is demarcated by menopause in

females (women). However, they show significant physical aging in almost all tissues and organs like those in humans.

Bones are organs that show deterioration with age in both humans and non-human primates (Mazess, 1982; DeRousseau, 1985; Pope et al., 1989; Champ et al., 1996; Colman et al., 1999; Black et al., 2001). Age-related changes in bones indicate smaller inter-individual variation than other organs. Therefore bones are considered to be a representative of physical aging. The postcranial bone morphology, density, and prevalence of osteoarthritis, have been studied on non-human primates, and compared with those in humans.

Bones are highly dynamic tissue undergoing continuous remodeling through the absorption and deposition of bone minerals, even after epiphyseal union. In adulthood, by the remodeling process, bones are maintained to perform their function, to be stiff to resist deformation in response to both internal (primarily muscular) and external forces (Currey, 2002). With advancing age, bones show deterioration in both quantitative and qualitative aspects. Bone mass decreases from young adulthood to very old age in humans (Colman and Binkley, 2002). Cortical loss starts in middle age and is accelerated in postmenopausal life (Colman and Binkley, 2002; Riggs, 2004), while trabecular bone loss, which starts before or after menopause, is still debatable (Riggs, 2004; Genant et al., 1982).

The factors which influence bones are physical stress, hormones, and whole body aging, which correlate with each other. Age-related changes occur more or less in all body tissues, which affect biochemical mechanisms involved in bone absorption and deposition of bone minerals. Physical stress plays an essential role in the regulation of bone modelling. Mechanical loading from muscle action is considered to play an important role to bone morphology (size and shape), bone mass maintenance and distribution. Physical activity (stress) has been shown to slow the rate of age-related bone loss in humans (Forwood and Burr, 1993; Kannus et al., 1995; Nguyen et al., 1998; Sigurdsson et al., 2006). Cortices of

long bones in humans have been demonstrated to be thicker at muscle attachment sites compared with non-attachment sites (Niinimäki et al., 2013).

Hormones are systemic regulators that control the remodeling process, and whose homeostasis is greatly influenced by increasing age (Jóhannesdóttir, 2012). Gonadal hormone, particularly estrogen, is crucial for the attainment and maintenance of bone mass in both sexes. The loss of ovary-derived estrogen following menopause or in the elderly shows a phase of rapid bone loss in both humans (Riggs et al., 2008) and non-human primates (macaques; Champ et al., 1996; Stevens and Lowe, 1997; Cowin, 2009; Lindsay et al., 1978; Khosla et al., 1998; Colman and Binkley, 2002). Similarly, testosterone levels in men diminish with advancing age (andropause) and are related to male osteoporosis. Although the role of testosterone on the bone loss in aged male macaques remains unclear, male rhesus macaques sustain aging-related bone loss in the absence of hypogonadism (Colman et al., 1999).

The skull is structurally similar to long bones, cortical bone encloses the inner space to carry brain, nasopharyngeal organs, and bone marrows or adipose tissues. The skulls receive physical stress from masticatory and postural muscles. The long bones (e.g., rib, femur, tibia, humerus, and metacarpals) show a generalized remodeling, which increases bone cross-sectional diameter through the continued periosteal apposition and endosteal resorption, on the other hand, decreases cortical thickness both in humans (Smith and Walker, 1964; Epker and Frost, 1965; Garn et al., 1967; Ericksen, 1976; Pfeifer, 1980; Ruff and Hayes, 1982; Riggs et al., 2004) and non-human primates (macaques; Bowden et al., 1979; Kimura, 1994; chimpanzees; Morbeck et al., 2002). The enlargement of diameter is considered to be the mechanical compensation for the decrease of cortex, that is, bone loss.

As for the skull, a question arises whether similar changes, including the three aspects, outer surface morphology (morphometry), cortical thickness, and inner table bone surface

occur with advancing age. Although age-related changes in the skull have been studied in humans (e.g., Albert et al., 2007), there is still a controversy with respect to age related changes of the outer surface morphology and cortical thickness of the skull in adult humans. With sexual maturation, cranial growth in humans has been considered to cease or be insignificant (Tallgren, 1974). However, it has been recognized that cranial growth continues at a slow rate throughout adulthood (Hrdlicka, 1936; Israel, 1977; Ruff, 1980; Forsberg et al., 1991). Some studies (Israel, 1973a; Adeloje et al., 1975) suggested a small increase in the thickness of the skull during adult life. However, others found no correlation between age and cranial thickness during adult life (Tallgren, 1974; Lynnerup, 2001). Given that the facial cranium and mandible are under masticatory stress, tooth loss is of primary importance, and leads to a decrease in facial dimensions (Goldstein, 1936; Bartlett et al., 1992). Also the position of the skull holds postures and is moved by nuchal muscles, sterno-cleido mastoideus, and digastric muscles, and so on. This muscular force may influence on the skull.

Little is known on the internal table bone of the skull. Exceptionally, it has been suggested that the endocranial cavity tends to enlarge during adult life in humans (Israel, 1973a). The cranial vault generally changes in size primarily by pressure from the brain, while it varies in shape under the constraints of tensions generated by connective layers such as the falx cerebri and the tentorium cerebelli (Moss and Yung, 1960). In contrast, the internal surface of the skull is influenced by a complex interplay between bone, meninx, and the brain (Moss and Young, 1960; Richtsmeier et al., 2006), combining with the interaction of cranial base dynamics and the morphology of the face (Lieberman et al., 2000; McCarthy, 2001; Ross et al., 2004). Thus the size of the brain is an important factor on the inner surface of the skull. The cortical structure of the brain contacts the surface of the endocranium through layers of dura mater and leaves its impression on the surface of the bone (i.e., sulcus

and gyrus). Thus, the surface of the endocast may produce information about the sulcus which demarcates the gyri and larger convolutions of the cerebral cortex.

The degree of definiteness of sulcal patterns which are reproduced on non-human primate endocasts, have been considered to depend mostly on species (brain size) and age of the individual (Radinsky, 1974; Holloway, 1974; Falk, 1978, 2014; Kobayashi et al., 2014; Bruner, 2015). The degree of definiteness negatively relates with brain size. The detailed imprints of the sulcal pattern on the surface of the endocast are difficult to be recognized in primate species with larger brains, including great apes, humans, and fossil hominids. Meanwhile, species with smaller brains tend to record the detailed imprints of the sulcus (Le Gros Clark et al., 1936; Radinsky, 1972, 1974; Holloway, 1974; Falk, 1978, 2014; Kobayashi et al., 2014; Bruner, 2015). In addition, it has been suggested that juveniles reproduce brain details on the surface of the endocast better than adults in primates (Falk, 1978, 2014). Ontogenetically, after maturation (by the myelinization), the brain shrinks in elderly humans and non-human primates (Metter, 1956; David and Wright, 1977; Millerand Corsellis, 1977; Matsumae et al., 1996; Dekaban and Sadowsky, 1978; Svennerholmet al., 1997; Kumakura, 1994; Shamy et al., 2006; Picq et al., 2012). Therefore, age-related brain shrinkage may be a potential factor affecting the internal surface of the endocranium together with bone loss in the whole skull. However, there are no studies on age-related changes in the sulcal imprints on the endocranium of primates.

Age-related changes in the skull have been studied in humans (e.g., Albert et al., 2007), however, studies are restricted by legal, ethical, and practical issues in human experiments (Colman and Binkley, 2002). On the other hand, factors such as physical activity or lifestyle may influence the changes of bones in general, however, it is difficult to control them in humans (Lieberman, 1996; Turner, 2001) to evaluate influence. Therefore, using other species is appropriate in order to model human skeletal changes with advancing age,

and especially non-human primates, are excellent candidates to be used as substitute models (Walker, 1995; Colman and Binkley, 2002).

Non-human primates are frequently used for studies concerning bone biology because they share similarities with humans in most aspects of reproductive functions, anatomy, skeletal physiology, neurology, immunology, and behavior more closely than other mammals commonly used in biomedical research (Bowden, 1979; Pritzker and Kessler, 1998). For example, like humans, but unlike rodents, the epiphyses of nonhuman primates close toward the end of adolescence (Bowden et al., 1979). In nonhuman primates, the micro-structure of compact bones and bone remodeling with a formation of osteons in the cortical bones, resembles those of humans (Haversian system) (Kimura, 1994; Pritzker and Kessler, 1998). In contrast, rodents lack the osteonal bone remodeling (Jilka, 2013) and the cortex is composed mostly of the circumferential lamellae (Enlow and Brown, 1958). Moreover, increases in bone loss associated with estrogen depletion in the elderly are found in humans and nonhuman primates (Black et al., 2001; Roth et al., 2004). Therefore, non-human primates are considered a useful model for studies associated with the aging of the skeleton (Havill et al., 2003).

Macaques are considered to be a good model of human skeletal aging. It is because, they share 95% of their genome with humans (Magness et al., 2005), and they are commonly comparable to the biological system of humans. The life cycle of macaques is the same as that of humans (Hamada and Yamamoto, 2010), including the elderly stage. Macaques are long-lived and their average life span is around 25 years of age, however, the maximum life-span of macaques in captivity is reported at about 40 years of age (Walker and Herndon, 2008). Macaques generally age at a rate of approximately 2.5 to 3.5 times that of humans (King et al., 1988; Tiggs et al., 1988; Duncan et al., 2011). Like all nonhuman primates (the exception of prosimians), macaques have larger brains than other mammals with comparable

body size (‘Roth and Dicke, 2005, 2012). They also share other similarities with humans, including posture (Black et al., 2001), the musculoskeletal system (Pritzker and Kessler, 1998), and decreases in the cortical thickness and bone loss due to estrogen depletion in postcranial skeletons with advancing age (Bowden et al., 1979; Colman and Binkley, 2002). Their postcranial skeletons have been and are studied on aging. However, there have been no studies on age-related changes in the skulls of macaques.

In the present study, I investigate age-related changes in the skulls of Japanese macaques (*Macaca fuscata*), and compare them with those in human skulls. Based on the results of the present study, I will discuss aspects of three age-related changes of the skull: outer surface morphology (morphometry), cortical thickness, and internal surface. The implications of these changes for the evolution of life-history will be presented.

The aim of the first chapter is to investigate age-related morphometric changes in the skull and compare them with those in humans. Describing the age-related changes of craniofacial skeletal sizes, I examined whether the skull of non-human primates increases or decreases in size and/or changes shape with age in comparison with the femur morphometric changes to understand the whole body bone change (bone loss in older-aged individuals), whether changes in the skull differ with age between the sexes (especially in relation to reproductive activity age-related changes), whether magnitude of size or shape changes in macaques is influenced by physical stresses, and whether facial dimensions are influenced by tooth loss in macaques.

In the second chapter, I investigate age-related changes in craniofacial cortical thickness using computed tomography scans. I examine whether age-related changes in cranial thickness are associated with physical stress (muscular forces) and with whole body bone turnover by comparison with cortical thickness of the femur. Based on these results, I will give implications for interpreting age-related changes of cranial thickness in humans.

In the third chapter, I investigate age-related changes in the sulcal imprint on the endocranium from juvenile to adulthood to elderly, using virtual endocasts generated by computed tomography scans. I also examine whether the endocranial volume shows age-related changes (increase/decrease), and discuss the age-related changes in the definiteness of sulcal patterns of the endocast in macaques.

Finally I discuss the whole aspects of age-related changes in the skull of Japanese macaques (*Macaca fuscata*), taking whole body bone age-related changes into consideration and the influential factors on bones; physical stress, hormones, and aging as a whole. I will also discuss the life-history of macaques in comparison with that of humans, that is, the age-related changes in adulthood and the demarcation of “elderly” from the viewpoint of age-related changes in the skull.

Chapter 1

Age-related changes in the skulls of Japanese macaques (*Macaca fuscata*)

Introduction

Aging involves a decline in the ability to adapt to environmental stress (Bogin, 2001). Senescence is defined by changes that occur primarily in the postreproductive period in human (around 50 years of age or more; Fedigan and Pavelka, 2001), which as a whole reduce the functional capacities of the organism and tissues in the decades after menopause (Bokan, 1982; Bogin, 2001). Although nonhuman primates maintain reproductive activity until ages near death (Pavelka and Fedigan, 1999), physical aging advances from young adulthood throughout adult life (Hamada and Yamamoto, 2010).

Elderly adults can be characterized by the extent of aging in the musculoskeletal system, such as by a decrease in stature or kyphosis in the vertebral column (Hamada and Yamamoto, 2010). Bone is an organ that shows deterioration with age in both humans and nonhuman primates (Mazess, 1982; Black et al., 2001). Age-related changes in human skulls are receiving increased attention and have been studied, for example in forensic research (Albert et al., 2007) and esthetic surgery (Bartlett et al., 1992). Postcranial skeletal changes in aging are obvious in both humans and nonhuman primates. Continuous expansion in the human postcranial skeleton (transverse diameters) has been documented for various bones (Smith and Walker, 1964; Garn et al., 1967; Pfeiffer, 1980; Ruff and Hayes, 1982). It is also seen in nonhuman primates, as shown by macaques (Bowden et al., 1979; Kimura, 1994), and chimpanzees (Morbeck et al., 2002).

There is controversy with respect to age change of the skull in adult humans. With sexual maturation, cranial growth in humans has been considered to cease or be insignificant (Tallgren, 1974). However, it has been recognized that cranial growth does not end at the adolescent stage in humans, but continues at a slow rate throughout adulthood (Hrdlička, 1936; Israel, 1977; Ruff, 1980; Forsberg et al., 1991), and it has been suggested that the cranium decreases in size in very old people (Hrdlička, 1936; Israel, 1973b). A small increase in the thickness of the skull during adult life has been suggested (Adeloye et al., 1975), but no statistically significant increase was found (Lynnerup, 2001). However, these conclusions are subject to sampling bias (small sample sizes, samples limited to certain historic anatomical collections or homogeneous populations), different age ranges, the confounding effects of pathology, methodologies in data collection, and statistical methods adopted (Lynnerup, 2001; Albert et al., 2007).

The phylogenetic closeness and marked biological similarities to humans make macaques and baboons a good model of human skeletal aging (Walker, 1995; Colman and Binkley, 2002). The similarities include posture (Black et al., 2001), reproductive endocrinology (Pavelka and Fedigan, 1999), bone loss following estrogen depletion (Colman and Binkley, 2002), and histomorphometry (Jerome et al., 1994). In contrast, mice lack the osteonal bone remodeling (Jilka, 2013) and cortex is composed mostly of the circumferential lamellae (Enlow and Brown, 1958). The life cycle of macaques is the same as that of humans (Hamada and Yamamoto, 2010), including the elderly stage. In general, macaques age at a rate of 2.5–3.5 times that of humans (Colman and Anderson, 2011; Duncan et al., 2011). Although being a good model of human aging, macaques have not been well studied on the effect of aging on the skull.

Little is known about age-related changes in the skulls of nonhuman primates. Exceptionally, some studies noticed a decrease of intertemporal distance in male Japanese

macaques in wild populations (Mouri et al., 2004), an enlargement of the medullary cavity of bones that border the facial suture in pig-tailed macaques (Kokich et al., 1979), and endocranial suture closure in rhesus macaques (Wang et al., 2006).

In the present study I investigated age-related changes in skulls of Japanese macaques (*Macaca fuscata*), and compare them with those in human skulls. The objectives of this study were to determine whether skulls of nonhuman primates increase or decrease in size and/or change shape with age, whether changes in skull with age differ between sexes, whether magnitude of size or shape change in macaques is comparable to those in humans, and whether facial dimensions are influenced by tooth loss in macaques.

Materials and Methods

Materials

A total of 145 skulls from adult Japanese macaques (*Macaca fuscata*) of known age, comprising 70 males and 75 females (Table 1), were used. In general, the adult stage is considered to start at the age when body growth stops, represented by such whole-body dimensions as stature (or its proxy, trunk length in nonhuman primates) (Hamada and Yamamoto, 2010). However, the age at which body growth stops has not been determined for Japanese macaques because body weight fluctuates within a given individual and shows great variation among individuals after adolescence (Hamada and Yamamoto, 2010); and the growth in linear dimension is too slow to identify exact age of growth cessation (Hamada and Yamamoto, 2010). Thus 7 years of age at which the permanent teeth fully erupt (Iwamoto et al., 1987) has been regarded as the age demarcating the adult stage.

All the subject macaques were being fed, at least in the period before their death, at the Primate Research Institute of Kyoto University. All skulls were stored at the institute. No macaques suffered from serious disease or received serious experimental treatment. The ages of the macaques are expressed in centesimal age. Although the dates of birth of 11 individuals were not known, their years of birth and dates of death were recorded. For the ages of these macaques we used the date June 15 for their birth, as Japanese macaques have a definite birth season, from March to August (Nozaki and Oshima, 1987). The years of birth of the individuals ranged from 1966 to 2001, and the years of death ranged from 1976 to 2009. Ages of male and female subjects ranged from 7.0 to 26.9 years and from 7.0 to 30.7 years, respectively (Table 1.1).

Twenty linear dimensions and two angles of skull and mandible were measured on each specimen (Table 1.2; Figure 1.1; Mouri, 1994; Mouri et al., 2004); 7 neurocranial

dimensions (cranial length, cranial base length, cranial height, cranial breadth at nuchal line, posterior basicranial length, postorbital breadth, and inter-temporal distance), 10 facial dimensions (facial length, facial height, bizygomatic breadth, zygomatic height at temporo-zygomatic suture, orbital breadth, orbital height, biorbital breadth, nasal length, maxillo-alveolar breadth, and maxillary angle), and 5 mandibular dimensions (ramus breadth, mandibular body height, mandibular body thickness, bicondylar breadth, and mandibular angle). Measurements were made in triplicate, and a median value was used for analysis. All measurements of the skull were made to the nearest 0.01 mm using a digital sliding caliper (Mitutoyo Corp., Japan). Maxillary angle was calculated using the cosine rule of a triangle with three linear sizes of sides. Since the three linear sizes (ZO–ZR, ZR–EAM, and EAM–ZO; Table 2) of the triangle are not on the sagittal plane, maxillary angle is also oblique to the sagittal plane. Mandibular angle was photogrammetrically obtained (Figure 1e). The photographic setup consisted of a tripod (Dolly pod DP-3D, Velbon Tripod Co., Ltd.) holding a digital camera (FinePix HS20EXR, Fujifilm Co., Ltd.). The tripod height was adjusted for the optical axis of the lens to be maintained horizontal and at the subject height. The lateral view of the left side of the mandible was positioned approximately 3 m from the objective lens of the camera and was adjusted so that the sagittal plane of the mandible was perpendicular to the optical axis of the lens. After the optical axis of the lens was raised to the alveolar process at the middle of the second mandibular molar, photographs were taken. The mandibular angle was measured from images with Image J 1.46 (Ferreira and Rasband, 2013). The items measured are showed in Tables 1.2 and Figure 1.1. The status of dentition and of the alveolar bone were recorded for each specimen.

Table 1.1 Sample sizes of the skull by sex and age

Age (years)	Male		Female	
	Total	Tooth loss subject	Total	Tooth loss subject
7.0–9.9	17	0	15	0
10.0–3.9	19	0	20	0
14.0–17.9	14	0	17	0
18.0–21.9	9	0	8	0
>22	11	4	15	5
Total	70	4	75	5

Table 1.2 Measurements taken on the skull

Abbreviation	Variable and definition
CL	Cranial length: maximum length of neurocranium in the midsagittal plane measured from nasion
CBL	Cranial base length: from basion to nasion
CH	Cranial height: from basion to bregma
CBN	Cranial breadth at nuchal line: maximum breadth at lateral nuchal line
PBL	Posterior basicranial length: from basion to inion
POB	Postorbital breadth: horizontally minimum and vertically maximum breadth of postorbital region
ITD	Inter-temporal distance: distance between the right and left inferior temporal lines at the coronal suture
FL	Facial length: from basion to prosthion
FH	Facial height: from nasion to prosthion
BZB	Bizygomatic breadth: from zygion to zygion
ZH	Zygomatic height at temporo-zygomatic suture: minimum distance measured from superior zygomatic process to inferior temporal process across middle temporo-zygomatic suture
OB	Orbital breadth: from right maxillofrontale to right frontomale orbitale.
OH	Orbital height: height of right orbit in the parasagittal plane measured from zygomaticomaxillary suture at inferior orbital margin.
BOB	Biorbital breadth: from frontomale temporale to frontomale temporale
NL	Nasal length: from nasion to rhinion
MAB	Maxillo-alveolar breadth: maximum breadth across the alveolar borders of the maxilla measured on the lateral surfaces at the middle of the second maxillary molars
MXA	Maxillary angle: the angle taken between the length from zygomaticomaxillary suture at inferior orbital margin (ZO) to zygomaticomaxillary suture at root of zygomatic arch (ZR) and the length from zygomaticomaxillary suture at inferior orbital margin (ZO) to the middle of the external auditory meatus at the upper margin of the meatus (EAM)
RB	Ramus breadth: minimum breadth of the mandibular ramus
MBH	Mandibular body height: direct distance from alveolar process at middle of second mandibular molar to the inferior border of the mandible measured perpendicular to the mandibular occlusal plane following the buccal cusps of the molars
MBT	Mandibular body thickness: maximum breadth in lateral mandibular body at the height of mandibular body measured perpendicular to mid-sagittal plane of mandible
BCB	Bicondylar breadth: direct distance between the most lateral points on the two condyles
MA	Mandibular angle: angle formed by the mandibular occlusal plane following the buccal cusps of molars and the posterior border of the ramus

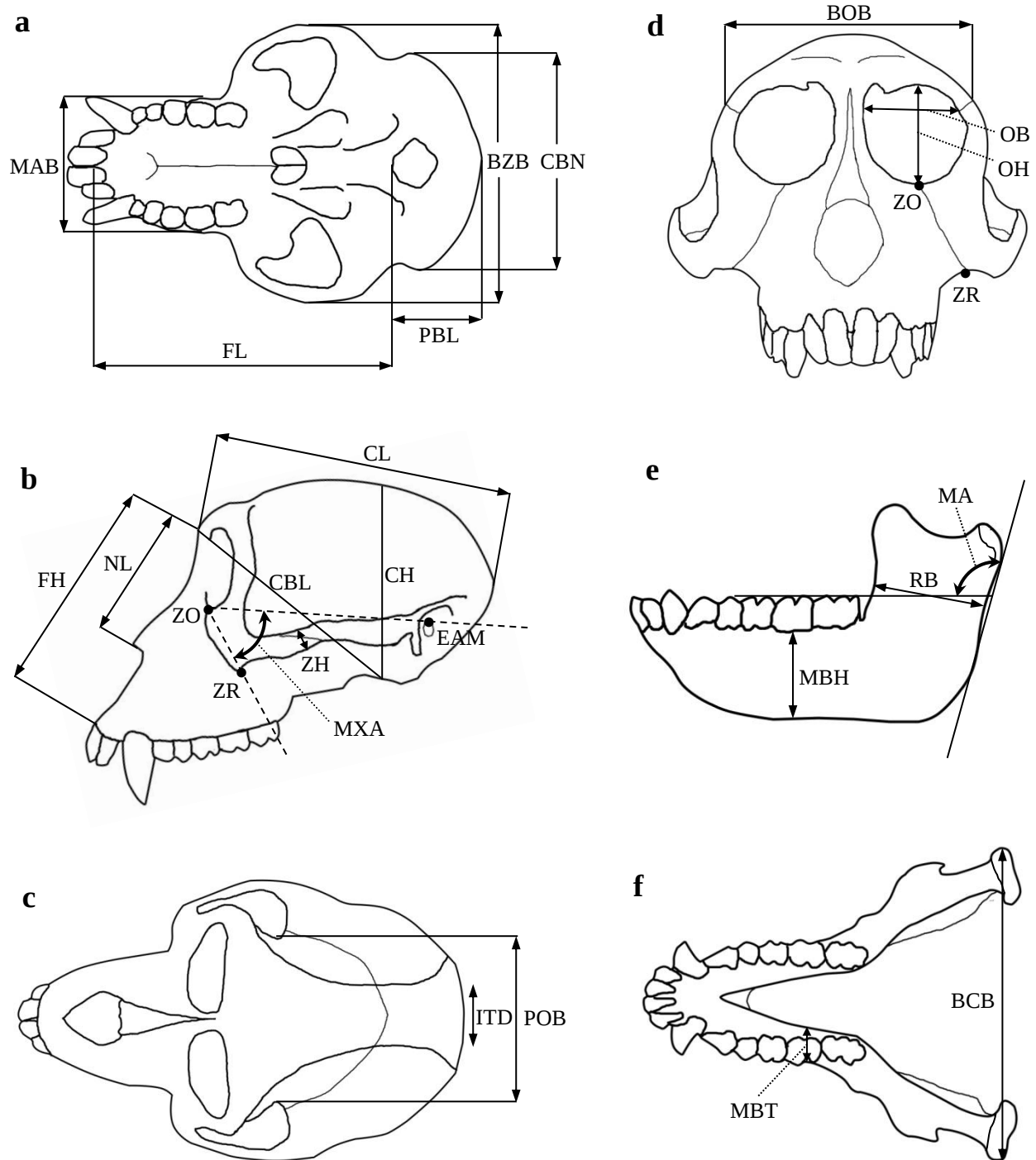


Figure 1.1 Schematic illustration of most of the measurements taken on the skull (for abbreviations see Table 2). a. skull in ventral view; b. skull in lateral view; c. skull in dorsal view; d. skull in anterior view; e. mandible in lateral view; f. mandible in transversal view.

Statistical analysis

Teeth in subjects were almost all retained until death, but 9 subjects had lost several teeth (Table 1.1): 4 males (ages from 23.7 to 26.0 years) and 5 females (ages from 25.2 to 30.0 years) had lost 8–11 teeth and 6–18 teeth, respectively. Tooth loss is an important factor of change in facial dimensions (Goldstein, 1936; Bartlett et al., 1992). Therefore, subjects which lost many teeth were separately analyzed (4 males and 5 females) and then were compared with those subjects of comparable ages with all teeth retained, which comprised of 6 males (ages from 23.3–26.9 years) and 6 females (ages from 25.3–30.7 years). The effect of tooth loss on facial dimensions was thus evaluated.

Statistical analyses were performed using functions of Excel (Microsoft Co. Ltd.). To describe age change patterns, Loess smoothing, locally weighted scatterplot smoothing (Past version 2.17 software; Hammer et al., 2001), was used. I compared craniometric dimensions and angles at start, peak size, and end of the adult stage. The age at peak size was derived from Loess smoothing function. For measurements that did not show increase–peak–decrease age-change pattern (continuous increase or decrease), sizes at start and end were compared. The sizes at start (young adult, 7.0 years) and peak in each measurement were represented by medians of data of 7.0–9.0 years in both sexes, and those of peak age ± 1 year in both sexes, respectively. Because of the small sample size of data from very old subjects, end size was represented by the median of the data of the five oldest subjects; that is, 24.0–26.9 years for males and 25.5–30.7 years for females. Percent differences (median) between sizes at start, peak, and end were obtained and differences of angle measurements were calculated by subtracting the two values to create an absolute value. Differences between start, peak, and end sizes (medians) and between sexes were tested by Mann–Whitney *U* test using Past version 2.17 software (Hammer et al., 2001). Differences between lost tooth and retained tooth subjects and between sexes were also tested by Mann–Whitney *U* test.

As cranial variables are not independent of one another, principal component analysis (PCA) was applied to both males and females using Past (Hammer et al., 2001). The relationship between scores of principal components (PCs) and ages was determined by Loess smoothing function.

Results

Some dimensions and angles showed age-related pattern. Skull measurements at start (young adulthood), peak, and end (very old age) are presented in Tables 1.3, 1.4, and 1.5, and Figure 1.2. Changes with age were significant ($p < 0.05$) in fourteen and seven cranial dimensions in males (Tables 1.3, 1.5) and females (Tables 1.4, 1.5), respectively.

Neurocranial dimensions

The four neurocranial dimensions, i.e., CL, PBL, CBL, and ITD, changed significantly with age in males but not in females (Table 4). CL, PBL, and CBL in males peaked at 16.1 years with an increase of 4.55% ($p < 0.01$), at 16.0 years with 5.19% ($p < 0.05$), and at 14.5 years with 6.25% ($p < 0.05$), respectively. After the peaks these dimensions did not decrease at all. PBL also showed a significant increase between the start age and the end age in males by 4.66% ($p < 0.05$). ITD decreased greatly with age in males by 68.89% from the start to the end ($p < 0.001$, Figure 1.2). Other dimensions, such as CBN and POB in males, remained stable or changed slightly, but not statistically significantly (Table 1.3).

Facial dimensions

Facial cranial dimensions tended to show statistically significant changes with age, but several facial dimensions showed no or slight age changes, including OB, OH, NL, and MXA in both sexes. FH continuously increased through very old age in both sexes with increases of 13.83% ($p < 0.001$) in males and 12.10% ($p < 0.01$) in females. FL increased significantly with age in males and peaked at approximately 16.0 years of age with an increase of 13.44% ($p < 0.001$). After the peak, FL in males decreased slightly. On the other hand, this dimension increased only slightly in females. BZB and BOB also increased

significantly with age in both sexes. After the peaks, these dimensions decreased slightly or not at all in both sexes, however, exceptionally BOB in males decreased significantly ($p < 0.01$). MAB increased significantly with age in both sexes, peaked at 13.3 years of age in males with an increase of 4.24% ($p < 0.05$), and increased 3.96% from the start (7.0 years) to the end (30.7 years) in females ($p < 0.01$). ZH increased significantly with age only in males, and reached the peak at 19.0 years of age with the increase of 16.06% ($p < 0.05$), whereas this dimension changed slightly in females, but not statistically significantly.

Mandibular dimensions

Mandibular dimensions showed significant changes with age in general (Tables 1.3, 1.4, 1.5). Exceptionally, however, BCB changed slightly but not significantly. MBH and RB increased significantly with age in both sexes, and then tended to remain stable or decrease not at all. MBT in males increased continuously with age by 9.67% from the start to the end ($p < 0.01$), whereas this dimension changed in females, but not statistically significantly. MA showed a significant increase with age in both sexes by 8.07 degrees in males ($p < 0.05$) and 5.50 degrees in females ($p < 0.01$).

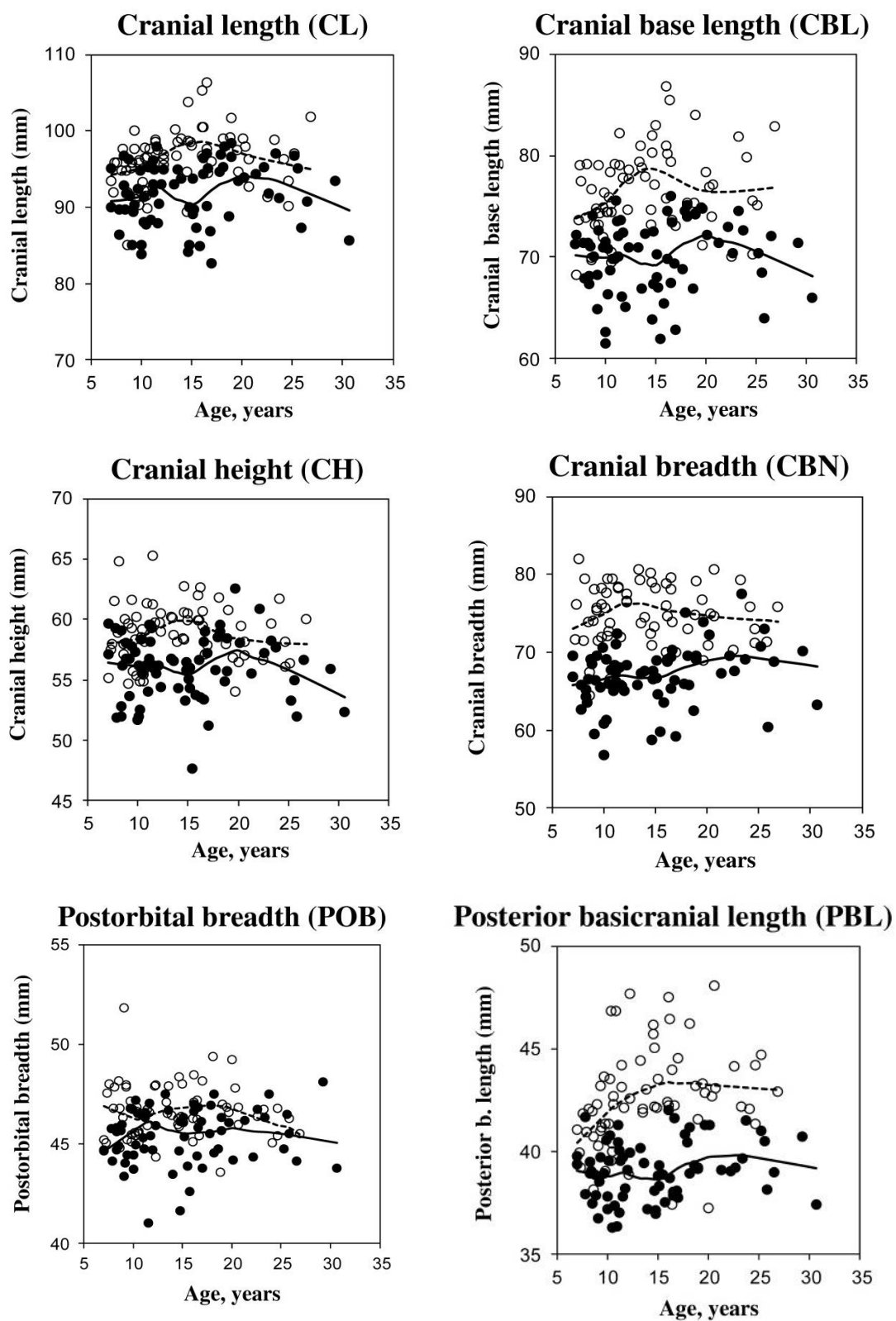


Figure 1.2 Scatter plots showing age change in all skulls (without tooth loss subjects) with Loess smoothing. Male, ○ and dashed line; female, ● and solid line.

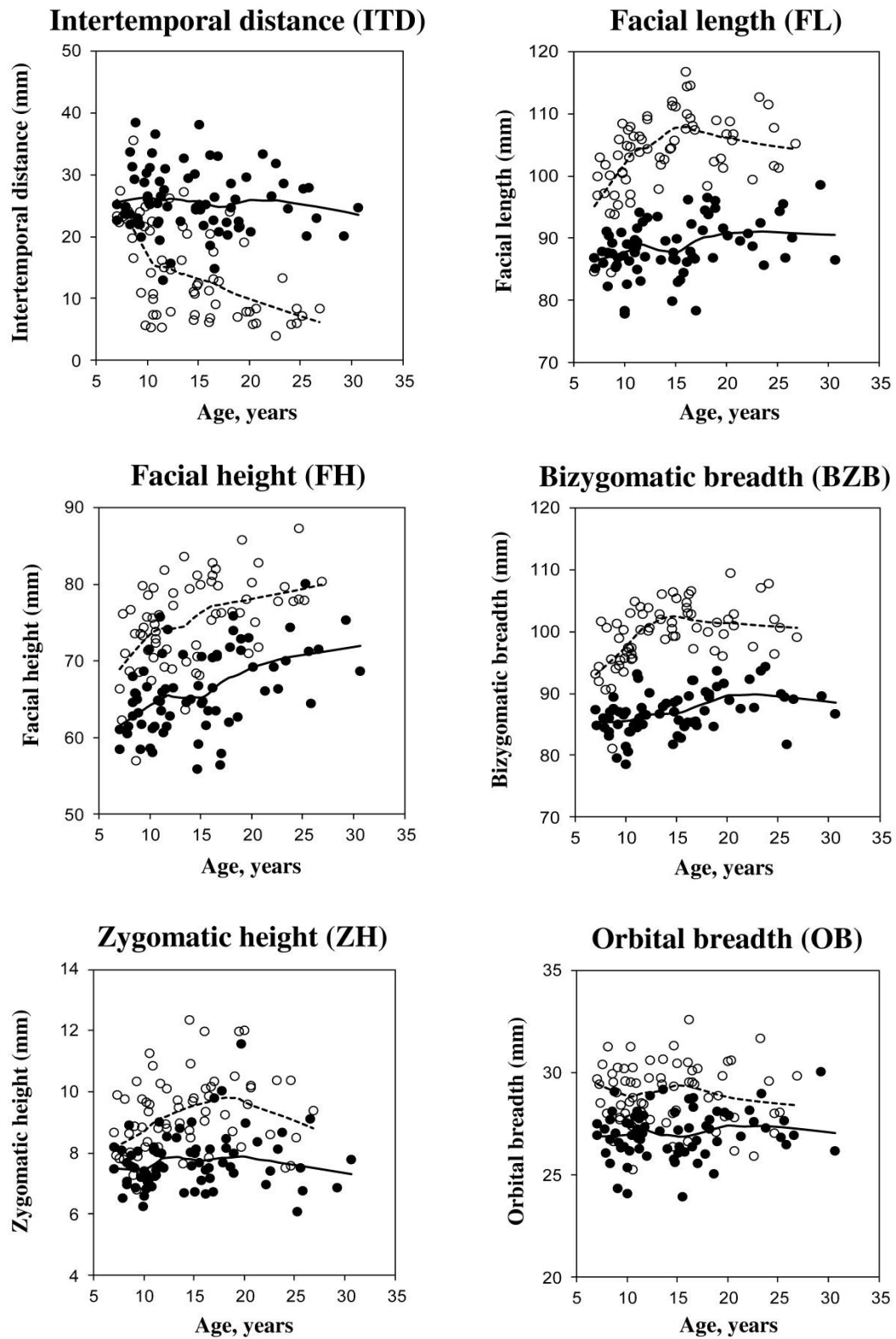


Figure 1.2 (Continued)

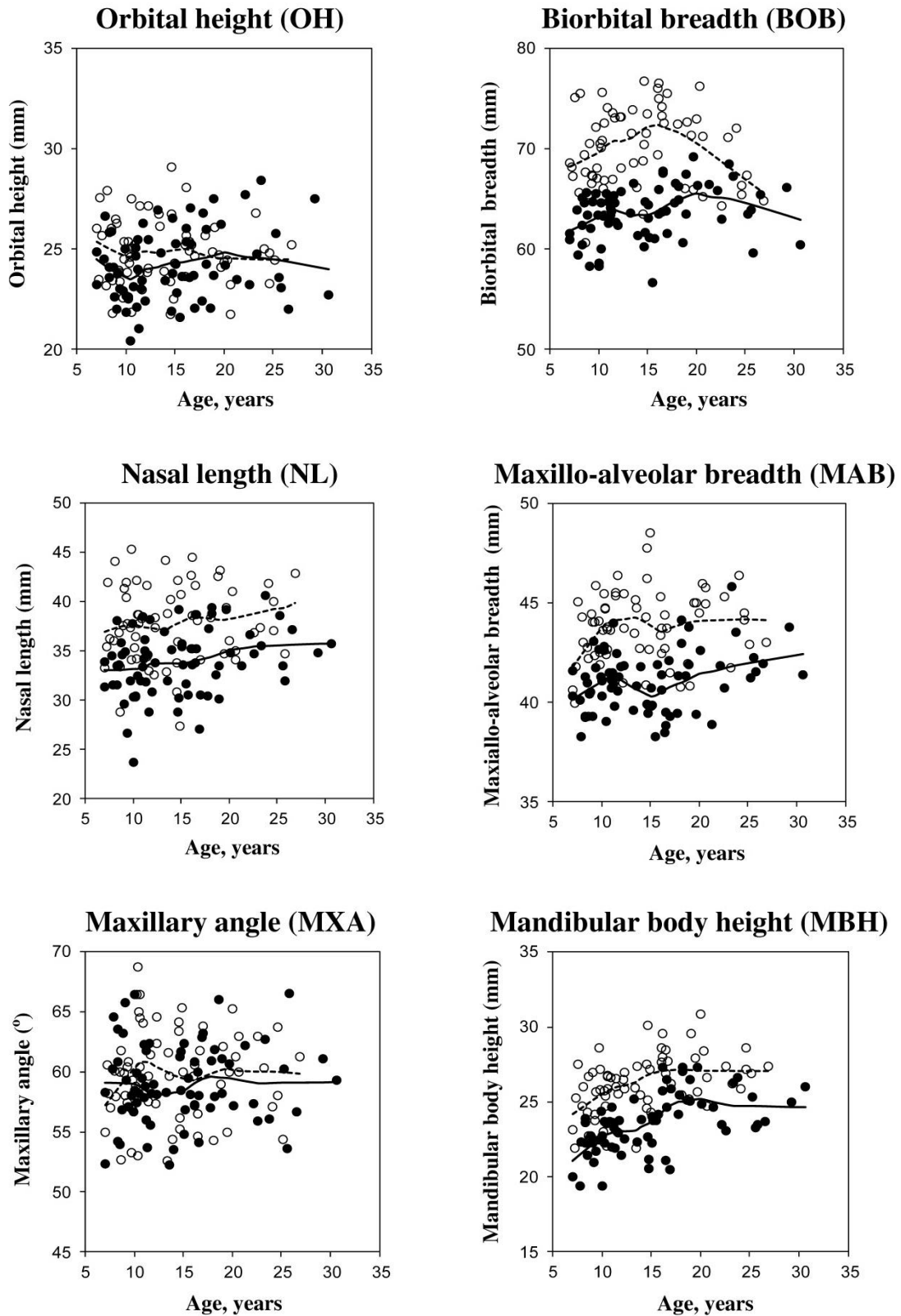


Figure 1.2 (Continued)

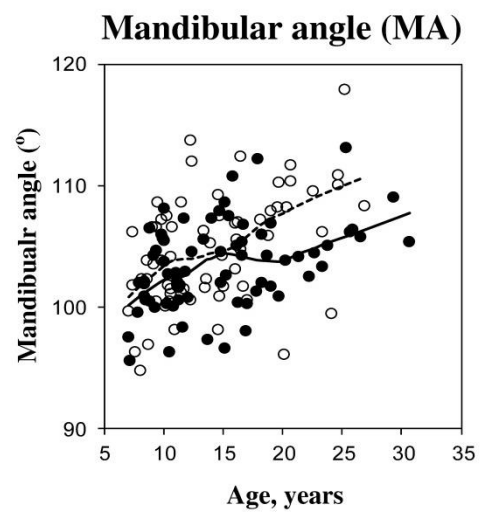
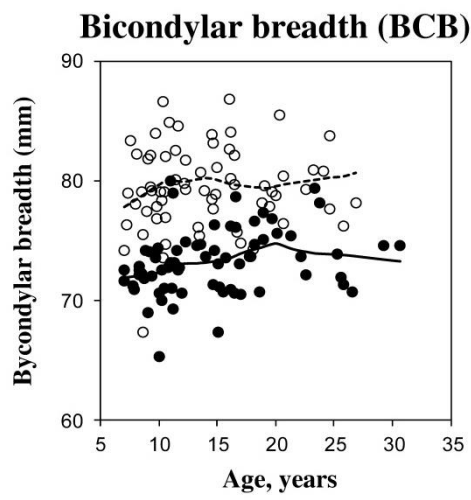
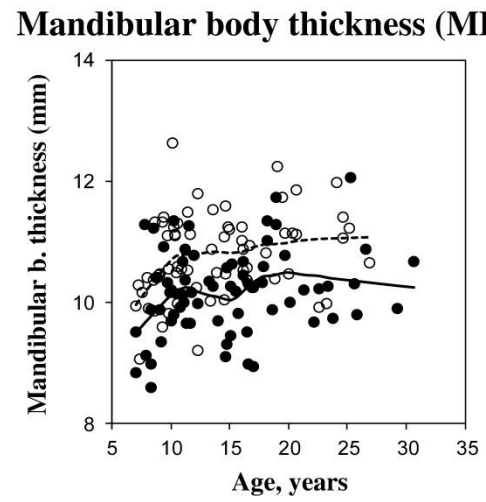
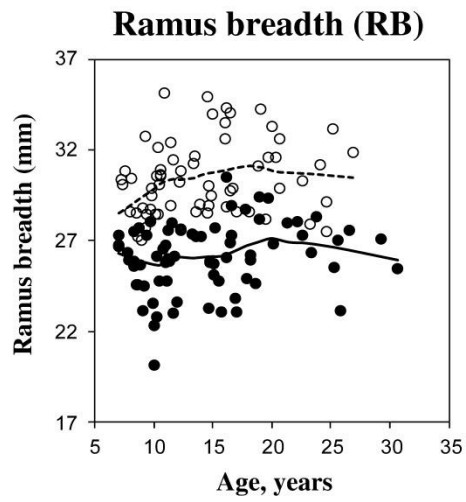


Figure 1.2 (Continued)

Table 1.3 Medians in start, peak, and end and age change (%) in males

Dimension	Start size (7.0–9.0 years)	Peak size (peak age $\pm$ 1.0 years)	Age at peak	End size (24.0–26.9 years)	Age change of Start–Peak (%)	Age change of Peak–End (%)	Age change of Start–End (%)
CL (mm)	94.79	99.10	16.1	96.37	4.55**	–2.75	1.67
CBL (mm)	73.79	78.40	14.5	75.55	6.25*	–3.64	2.39
CH (mm)	57.99	59.90	13.9	58.13	3.29	–2.95	–0.24
CBN (mm)	72.81	74.50	13.5	72.95	2.32	–2.08	0.19
PBL (mm)	41.01	43.14	16.0	42.92	5.19*	–0.51	4.66*
POB (mm)	46.80	47.01	18.1	45.42	0.45	–3.38	–2.95
ITD (mm)	23.05	–	–	7.17	–	–	–68.89***
FL (mm)	96.99	110.03	16.0	105.16	13.44***	–4.43	8.42*
FH (mm)	68.54	–	–	78.02	–	–	13.83***
BZB (mm)	93.59	101.00	14.6	100.62	7.92**	–0.38	7.51*
ZH (mm)	7.97	9.25	19.0	8.50	16.06*	–8.11	6.65
OB (mm)	29.49	–	–	28.06	–	–	–4.85
OH (mm)	25.87	–	–	24.80	–	–	–4.14
BOB (mm)	67.93	74.66	16.0	66.37	9.91**	–11.10**	–2.30
NL (mm)	36.15	–	–	40.00	–	–	10.65
MAB (mm)	42.49	44.29	13.3	44.14	4.24*	–0.34	3.88
RB (mm)	28.57	31.26	17.0	31.20	9.42*	–0.19	9.21
MBH (mm)	24.50	26.95	18.1	27.13	10.00*	0.67	10.73*
MBT (mm)	10.23	–	–	11.22	–	–	9.68**
BCB (mm)	77.76	–	–	80.88	–	–	4.01

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ statistically significant difference using Mann–Whitney U test between start size vs. peak size; peak size vs. end size, and start size vs. end size.

Table 1.4 Medians in start, peak, and end and age change (%) in females

Dimension	Start size (7.0–9.0 years)	Peak size (peak age $\pm$ 1.0 years)	Age at peak	End size (25.5–30.7 years)	Age change of Start–Peak (%)	Age change of Peak–End (%)	Age change of Start–End (%)
CL (mm)	91.68	94.90	20.2	90.75	3.51	–4.37	–1.01
CBL (mm)	71.17	73.23	19.7	70.35	2.89	–3.93	–1.15
CH (mm)	56.91	58.10	19.7	54.95	2.09	–5.42	–3.44
CBN (mm)	66.16	69.18	23.3	68.79	4.56	–0.56	3.98
PBL (mm)	38.98	39.20	22.6	39.00	0.56	–0.51	0.05
POB (mm)	45.26	46.13	11.6	45.54	1.92	–1.27	0.62
ITD (mm)	25.00	26.25	10.0	23.00	5.00	–12.38	–8.00
FL (mm)	87.61	90.80	23.5	90.00	3.64	–0.88	2.73
FH (mm)	63.00	–	–	70.62	–	–	12.10**
BZB (mm)	86.02	92.25	22.6	89.01	7.24**	–3.51	3.48
ZH (mm)	7.62	8.00	19.7	7.50	4.99	–6.25	–1.57
OB (mm)	27.15	27.90	20.2	26.93	2.76	–3.48	–0.81
OH (mm)	24.31	24.17	20.1	23.07	–0.58	–4.55	–5.10
BOB (mm)	62.84	66.45	20.2	63.89	5.74**	–3.85	1.67
NL (mm)	33.71	–	–	34.78	–	–	3.17
MAB (mm)	40.36	–	–	41.95	–	–	3.96**
RB (mm)	26.13	28.11	20.2	27.03	7.58*	–3.84	3.44
MBH (mm)	22.26	25.05	19.7	23.65	12.67***	–5.70	6.24*
MBT (mm)	9.70	10.77	19.7	10.12	11.03	–6.04	4.33
BCB (mm)	72.21	75.53	20.2	71.98	4.60	–4.70	–0.32

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ statistically significant difference using Mann–Whitney U test between start size vs. peak size; peak size vs. end size, and start size vs. end size.

Table 1.5 Angle measures and age change (°) in males and females

Measure	Start	Peak (peak age ±1.0 years)	Age at peak	End	Age change of Start–Peak (°)	Age change of Peak–End (°)	Age change of Start–End (°)
Male (7.0–26.9 years)							
MXA (°)	59.08	61.42	11.0	57.99	3.96	–5.58	–1.84
MA (°)	102.10	–	–	110.17	–	–	8.07*
Female (7.0–30.7 years)							
MXA (°)	59.29	60.99	–	59.27	2.87	–2.82	–0.03
MA (°)	100.78	–	–	106.28	–	–	5.50**

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ statistically significant difference using Mann–Whitney U test between start size vs. end size

Size and shape change of skull with age

In a PCA using 20 linear cranial measurements and based on a correlation matrix, the first three principal components explained 58.98%, 8.18%, and 5.05% of total variation, respectively (Table 1.6). The first principal component (PC1), representing size change, showed that all dimensions but ITD had positive loadings. The PC1 scores showed age change patterns similar to many dimensions (Figure 1.3a). Males had higher PC1 scores than females. Age at the peak PC1 score was 16.0 and 20.2 years in males and females, respectively.

The second principal component (PC2), describing shape change with age, showed that females had higher scores than males (Figure 1.3b). OH (0.476), ITD (0.429), POB (0.360), and OB (0.298) were positively loaded, whereas PBL (−0.237), MAB (−0.208), and MBT (−0.250) were negatively loaded (Table 1.6). The PC2 scores showed a rather rapid decrease from 7.0 to 11.0 years, remained stable from 11.0 to 17.0 years, and then decreased again in males, although remained almost unchanged from 7.0 to 30.7 years in females (Figure 1.3b).

The third principal component (PC3) represented shape change, with NL (−0.511), OH (−0.372), and FH (−0.366) negatively loaded and CH (0.395) positively loaded (Figure 1.3c; Table 1.6). PC3 showed gradual decreases in both males and females (Figure 1.3c).

Table 1.6 Factor loadings of PC1, PC2, and PC3 of craniometric variables obtained from the principal component analysis using both sexes

Variable	PC1	PC2	PC3
CL	0.236	0.195	0.013
CBL	0.260	0.130	0.079
CH	0.200	0.145	0.395
CBN	0.257	0.092	0.122
PBL	0.213	-0.237	0.048
POB	0.156	0.360	0.304
ITD	-0.193	0.429	0.202
FL	0.269	-0.119	-0.020
FH	0.248	-0.013	-0.366
BZB	0.278	-0.087	0.033
ZH	0.188	-0.171	0.096
OB	0.227	0.298	0.086
OH	0.138	0.476	-0.372
BOB	0.261	0.099	0.205
NL	0.203	0.170	-0.511
MAB	0.205	-0.208	0.089
RB	0.250	-0.120	0.026
MBH	0.216	-0.107	-0.242
MBT	0.154	-0.250	0.162
BCB	0.250	-0.094	0.001
Eigenvalue	11.80	1.64	1.01
Proportion	58.98	8.18	5.05

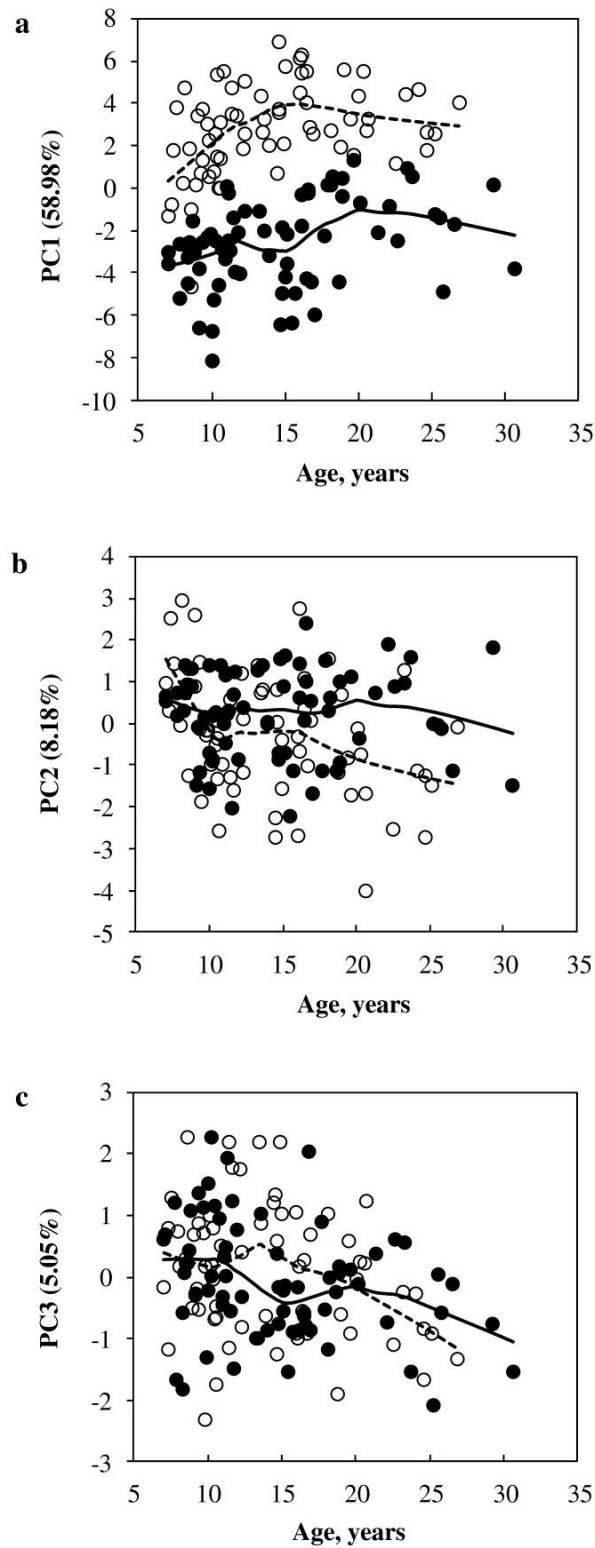


Figure 1.3 Plots of (a) first principal component scores, (b) second principal component scores, and (c) third principal component scores against age with Loess smoothing. Male, ○ and dashed regression line; female, ● and solid regression line.

The effect of tooth loss on facial and mandibular dimensions

Thirteen facial and mandibular dimensions of subjects lost several teeth were compared with those subjects of comparable ages with all teeth retained (Table 1.7). The results of the comparison in this study showed that two dimensions of lost tooth subjects differed significantly with retained tooth subjects in both sexes. FH and MBH in males were 16.75% ($p < 0.01$) and 17.29% ($p < 0.01$) smaller than the medians for male macaques with all teeth retained, respectively, whereas these measurements in females were 11.16% ($p < 0.05$) and 15.45% ($p < 0.01$) smaller than female subjects with all teeth retained, respectively.

Table 1.7 Comparison of facial and mandibular dimensions between retained and lost tooth subjects

Dimension	Retained tooth subjects		Lost tooth subjects		Percentage change between retained and lost tooth subjects
	Median	Range	Median	Range	
FL (mm)					
Male	106.50	101.39–112.68	98.36	94.28–107.37	–7.74
Female	92.13	86.50–98.51	87.96	79.33–94.52	–4.53
FH (mm)					
Male	78.88	77.77–87.32	65.67	63.22–71.64	–16.75**
Female	71.40	64.42–80.05	63.12	56.01–70.66	–11.60*
BZB (mm)					
Male	101.30	96.41–107.85	99.03	96.34–105.49	–2.24
Female	89.24	81.70–89.84	89.70	87.60–91.70	0.51
ZH (mm)					
Male	8.94	7.50–10.38	9.30	7.11–11.34	4.03
Female	7.18	6.09–9.10	6.91	6.28–10.80	–3.20
OB (mm)					
Male	28.83	27.04–31.69	27.94	27.05–30.19	–3.09
Female	26.90	26.19–30.06	26.69	25.42–27.32	–0.78
OH (mm)					
Male	24.91	23.27–26.80	24.85	23.39–26.51	–0.24
Female	23.33	22.00–27.50	24.50	24.12–27.39	5.02
BOB (mm)					
Male	66.86	64.81–72.09	69.51	66.08–71.75	3.96
Female	63.68	59.65–66.18	63.22	62.85–65.96	–0.72
NL (mm)					
Male	38.70	34.75–42.92	37.08	34.75–40.51	–4.19
Female	35.28	31.97–38.63	34.79	31.19–36.86	–1.39
MAB (mm)					
Male	43.57	41.76–46.37	43.47	40.00–44.72	0.00
Female	41.74	41.25–43.78	41.41	37.30–43.69	–0.79
RB (mm)					
Male	30.15	27.48–33.17	31.04	27.95–32.68	2.95
Female	26.29	23.11–27.59	26.23	25.64–27.30	–0.23
MBH (mm)					
Male	27.01	25.89–28.62	22.34	20.10–25.88	–17.29**
Female	24.33	23.24–26.00	20.57	18.78–21.41	–15.45**
MBT (mm)					
Male	11.14	9.99–11.98	11.08	9.99–11.18	–0.54
Female	10.49	9.80–12.06	10.60	10.16–10.95	1.05
BCB (mm)					
Male	79.52	76.29–83.84	78.14	76.20–81.27	–1.74
Female	72.96	70.71–74.65	76.02	70.77–76.39	4.19

* $p < 0.05$ and ** $p < 0.01$ statistically significant difference using Mann–Whitney U test for retained tooth males ($N = 6$, ages from 23.3–26.9 years) vs. lost tooth males ($N = 4$, ages from 23.7–26.0 years) and retained tooth females ($N = 6$, ages from 25.3–30.7 years) vs. lost tooth females ($N = 5$, ages from 25.2–30.0 years)

Discussion

Cross-sectional aging studies suffer from secular trends or environmental variability that masks ontogeny (Baer, 1956). Although we studied macaque skulls cross-sectionally, all of them originated from captive monkeys fed under the same conditions during their life spans. Thus, secular trends or environmental factors may not have had any effect on cranial dimensions in this study.

Results of the present study indicate that part of cranial dimensions showed age-related changes, with a pattern increasing from young adulthood (7.0 years) to mid-adulthood (13.3–19.0 years in males and 19.7–22.6 years in females) and unchanged from mid-adulthood to very old age (26.9 years or more) or increasing from young adulthood to very old age. It is probable that the increase from 7.0 years to mid-adulthood or very old age is both in total physical growth, which ends at 10.0 or 15.0 years of age (Hamada, 1994; Hamada and Yamamoto, 2010) and in specific increases in the head and face. Maximal stages of skull size in some measurements, including bizygomatic, biorbital, and ramus breadths and mandibular body height, tended to be attained later in females than in males. This corresponds with the data that trunk length and epiphyseal unions in postcranial skeletons tended to occur later in females than in males in Japanese macaques (Kimura, 1994; Hamada, 2008). Intertemporal distance and biorbital breadth (after 16.0 years) decreased significantly in males. Facial and mandibular dimensions tended to show greater and more significant change with age than neurocranial dimensions, including facial height, bizygomatic breadth, mandibular body height, and ramus breadth in both sexes. Furthermore, as for proportional changes in the face, facial height increased more than bizygomatic breadth in both sexes, making the face relatively longer with age in macaques. In addition, proportions changed; that is, orbital height to orbital or biorbital breadth.

Continued increase of cranial size in adulthood has been observed in humans (Goldstein, 1936; Garn et al., 1967; Nasjeleti and Kowalski, 1975; Israel, 1973a, 1977; Susanne, 1977; Ruff, 1980; Bartlett et al., 1992). Human skulls attained maximal size at about 70 years of age (Israel, 1973b). In macaques, ages at maximal skull size are estimated (by principal component analysis) to be 16.0 ± 3 years and 20.2 ± 3 years in males and females, respectively; that is, 48.0 and 60.6 year equivalents in human age.

The magnitude of extensive development after completion of tooth eruption in skull dimensions is much greater in macaques than in humans. For example, facial height increases by 3.66% in men from 20.0 to ≥ 60.0 years (Nasjeleti and Kowalski, 1975), or 1.49% in men and 1.28% in women aged from 18.0 to 42.0 years of age (Formby et al., 1994). In contrast, facial height in male macaques increased by 13.83% from young adulthood to 26.9 years and by 12.10% in females from 7.0 to 30.7 years. Similarly, facial length in male macaques is larger than that of men (Ruff, 1980) and bizygomatic breadth is also larger in both male and female macaques than that of men and women (Susanne, 1977; Bartlett et al., 1992). Neurocranial dimensions, such as cranial length, show smaller contrasts between humans (Israel, 1977) and macaques than facial dimensions.

The magnitude of age change in skull dimensions, which differs between humans and macaques, may be associated with mechanical stress from mastication, which is likely to stimulate the increase in facial dimensions and change in neurocranial dimensions. Previous studies demonstrated that hard, tough and unprocessed diets play an important role in an increase in the general sizes of the skull and face (Carlson and Van Gerven, 1977; Sardi et al., 2006) and thus the softer and more processed foods in human diets are considered to reduce masticatory stress in the craniofacial skeleton (Carlson and Van Gerven, 1977; Lieberman et al., 2004; Sardi et al., 2006; Paschetta et al., 2010), so that it is possible that human faces are

subjected to smaller masticatory stress, resulting in a smaller increase in facial size than in macaques.

Sex differences in age-related changes in cranial dimensions are not large in humans (Ruff, 1980). However, sex differences in change in macaques are rather large. Various degrees of sex difference were found in macaques, large in facial and small in neurocranial dimensions. Human–macaque differences in sex difference and sex differences in macaques may be associated with the stress of mastication (Wang et al., 2006). Larger masticatory forces are applied to the face in male macaques (Dechow and Carlson, 1990).

Expansion of the cranium may be associated with the development of bones as a response to physical stress from masticatory and/or postural muscles. The increase in cranial and posterior basicranial lengths in male macaques may be associated with the development of the nuchal crest or insertion processes (tubercles) on which nuchal muscles attach. The large decrease in intertemporal distance and postorbital breadth may be the result of development or stress from temporalis (Figure 1.4).

Facial crania and mandibles are greatly influenced by tooth loss and/or dental disorders in humans (Israel, 1973c; Bartlett et al., 1992; Merrot et al., 2005). In the present study subject macaques that had lost many teeth exhibited reduction of the alveolar bone and also large changes in the face and mandible. These changes were especially true for mandibular body height and facial height compared with those of subjects of comparable ages with all teeth retained (Figures 1.5, 1.6). Similar findings have been reported for humans (Israel, 1973c; Bartlett, 1992) and for other nonhuman primates; alveolar resorption occurred in captive rhesus macaques (Lapin et al., 1979), and wild chimpanzees (Kilgore, 1989; Morbeck et al., 2002).



Figure 1.4 The differences in temporal line and bone around inion between old males (a, c) and females (b) in Japanese macaques.

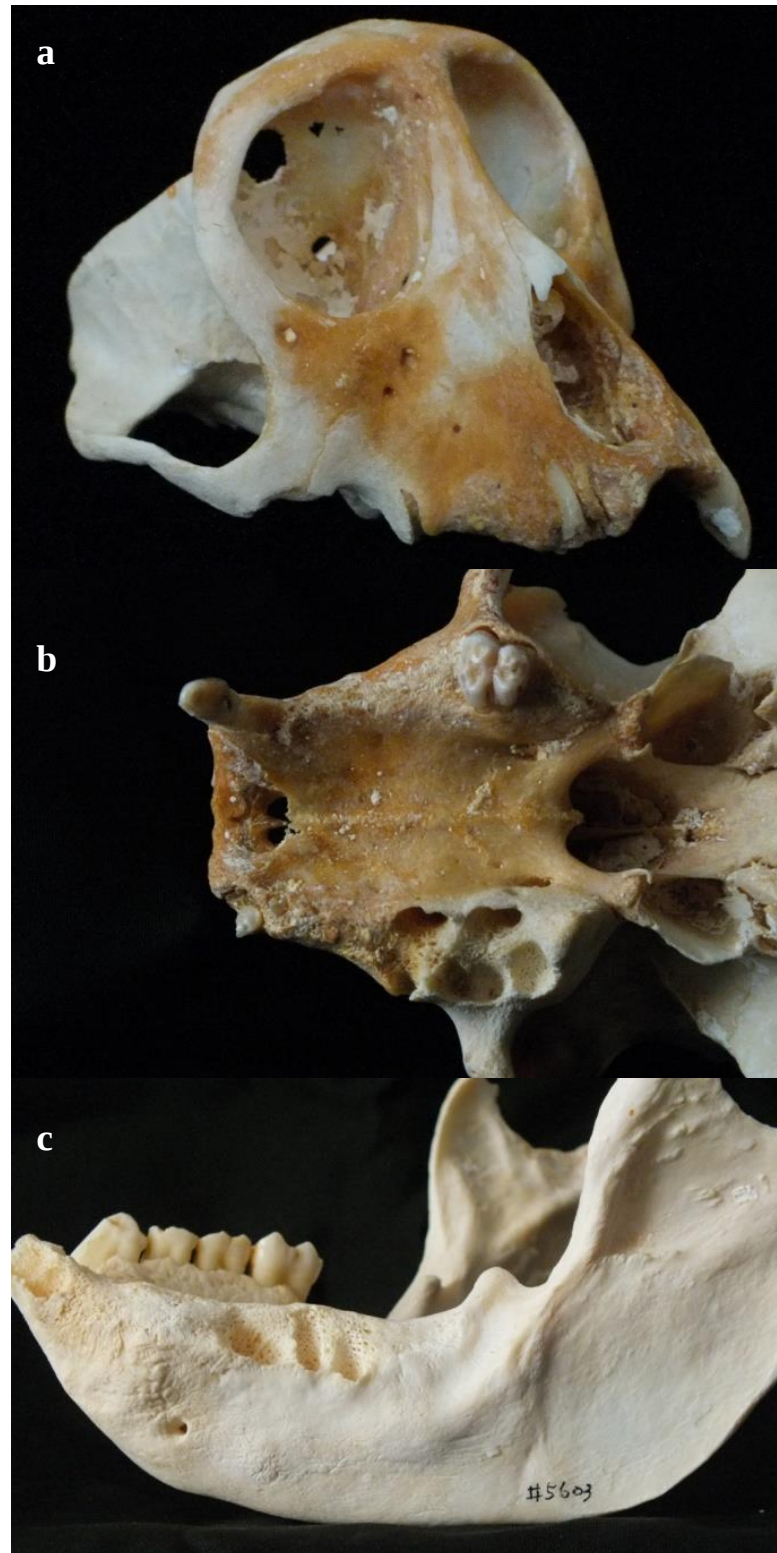


Figure 1.5 Atrophy of alveolar process of maxilla with lateral and ventral views (a and b) and mandible with lateral view (c) in some Japanese macaques having lost teeth

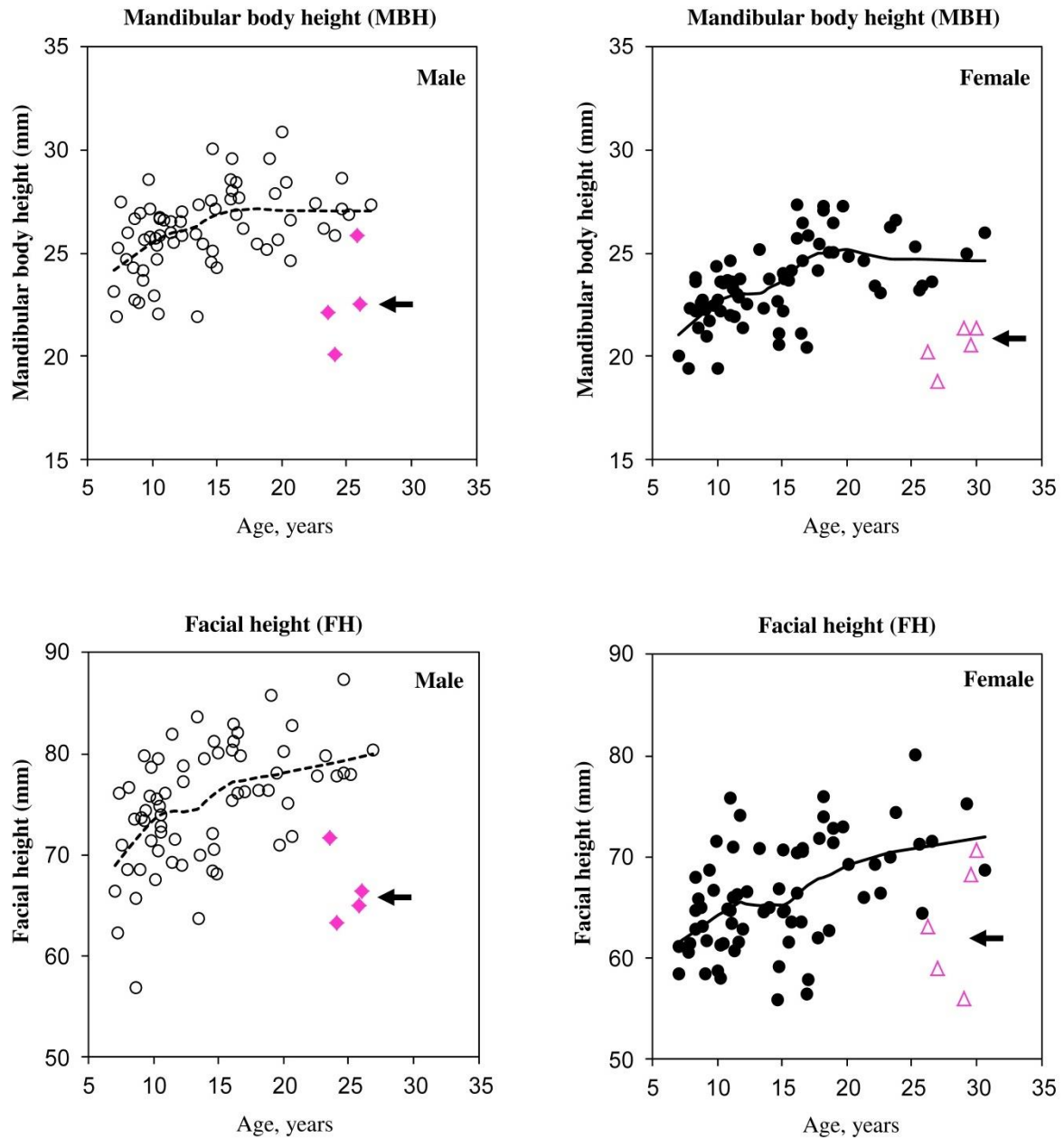


Figure 1.6 Scatter plots showing the effect of tooth loss on large decreases of facial and mandibular body height in tooth loss individuals of Japanese macaques. *Open circle* male, *filled rectangular* male with tooth loss, *filled circle* female, *open triangle* female with tooth loss.

Chapter 2

Age-related changes in the cranial thickness of Japanese macaques

(*Macaca fuscata*)

Introduction

Aging is a deterioration process that occurs in animals and is characterized by weakness, increased susceptibility to disease, loss of mobility and agility, and adverse changes in physiology (Goldsmith, 2010, 2012). Senescence is defined as the changes that primarily occur in the postmenopausal period of human life, in which all functional capacities of the organs and tissues are diminished (Borkan, 1982; Bogin, 2001). Although senescence in nonhuman primates is unclear, physical aging advances from young adulthood and throughout adult life (Hamada and Yamamoto, 2010). The bone is an organ that shows deterioration with age in both humans and nonhuman primates (Mazess, 1982; Black et al., 2001), and changes in bones are therefore considered to be representative of physical aging (Pomchote, 2015).

Bone tissue exhibits active metabolism, remodeling through the absorption and deposition of bone minerals. Hormonal secretion and physical stresses on the bone can affect this metabolism. Aging also has an influence on the mechanism of metabolism, since gonadal hormones (particularly estrogen), are crucial for the attainment and maintenance of bone mass in both sexes. The loss of estrogen in postmenopausal life plays a central role in osteoporosis aggravation, as its deficiency is associated with accelerated bone loss in both humans and macaques (Lindsay et al., 1978; Khosla et al., 1998; Colman and Binkley, 2002). Recent investigations have strongly suggested that estrogen deficiency along with declining

testosterone levels play a dominant role in bone loss in aging men (Khosla et al., 2001; Clarke and Khosla, 2010), though the roles of these hormones in bone loss in aging male macaques are still unclear. However, male rhesus macaques sustain aging-related bone loss in the absence of hypogonadism (Colman et al., 1999). Alternatively, the wider but thinner structure of long bones in older individuals has been explained as a mechanical compensation (Garn, 1967; Kimura, 1994).

Age-related changes in postcranial skeletons are evident in both human and nonhuman primates. Continuous expansion of the medullary cavity in the postcranial skeletons and age-related thinning of the cortex of long bones have also been documented in various bones in both humans and nonhuman primates (Smith and Walker, 1964; Garn et al., 1967; Pfeiffer, 1980; Ruff and Hayes, 1982; Bowden et al., 1979; Kimura, 1994; Morbeck et al., 2002). Age-related expansions in postcranial skeletons and decreasing cortical thickness are thought to be the result of absorption-biased bone turnover with aging (Smith and Walker, 1964; Garn, 1967; Ruff and Hayes, 1982; Kimura, 1994; Riggs et al., 2004).

Age-related changes in human cranial thickness have been previously studied, and are receiving an increasing amount of attention. For example, forensic research has focused on the relationship between cranial thickness and sex, age, and general body build (Lynnerup, 2001; Lynnerup et al., 2005), and the relationship between age and risk of skull fracture (Torimitsu et al., 2014). Cranial dimension increase slightly in male macaques (Minh et al., 2015), that is, slight expansion. There is still controversy with respect to age-related changes in the cranial thickness of adult humans. A small increase in the thickness of the skull during adult life has been suggested in some studies (Israel, 1973a; Adeloje et al., 1975), but others have reported no correlation between age and cranial thickness (Tallgren 1974; Lynnerup, 2001). Potential reasons for these contradictory conclusions might include sampling bias, small sample size, confounding effects of pathology, and methodologies in data collection

(Roos, 1998; Lynnerup, 2001).

To delineate definite age-related changes in cranial thickness in humans there are some issues regarding legal, ethical, and practical restrictions in human experiments (Colman and Binkley, 2002). Studies involving other mammals are therefore a more appropriate approach to modeling human skeletal changes with advancing age, and nonhuman primates have been commonly used as substitute models (Walker, 1995; Colman and Binkley, 2002). Macaques are considered to be a good model of human aging in the postcranial skeleton, because they age at a rate of approximately 2.5–3.5 times that of humans (King et al., 1988; Tigges et al., 1988; Duncan et al., 2011) and show an age-related decrease in cortical thickness and bone loss in the postcranial skeleton similar to that of humans (Bowden et al., 1979; Colman and Binkley, 2002). However, there have been no studies on age-related changes in the cranial thickness of macaques.

The aim of the present study is to investigate age-related changes in the craniofacial thickness of Japanese macaques (*Macaca fuscata*) of known age, reared in the same condition, and known origins. Age changes in craniometry showed several sizes developed in response to muscular force, reproductive cessation, and whole-body aging in macaques (Minh et al., 2015). I therefore examined whether age-related changes in cranial thickness are associated with those factors. Since the cortical thicknesses of long bones generally decrease with age, I examined whether the craniofacial cortical thickness of macaques decreased or not. Here I try to provide the implications for age-related changes in cranial thickness in humans, based on the results of the present study.

Materials and Methods

Materials

I examined the cranial thicknesses of 140 crania from adult Japanese macaques of known age (67 males, 73 females) stored at the Primate Research Institute (PRI) at Kyoto University, Japan. All macaque subjects were reared in corral cages and were fed monkey chow and sweet potatoes. They originated from the Arashiyama, Takahama, and Wakasa areas. Subjects died when they were healthy and showed no effect of disorders on their cranial bones. No macaques suffered from serious disease nor received serious experimental treatment. The ages of the macaques were expressed as centesimal ages. Although the dates of birth of 11 individuals were not known, their years of birth and dates of death were recorded. The ages of these macaques were calculated using June 15 as their date of birth, since Japanese macaques have a definite birth season from March to August (Nozaki and Oshima, 1987). The subjects were divided into the five age groups: 7.0–8.9 years (10 males, 10 females), 9.0–13.9 years (25 males, 25 females), 14.0–18.9 years (17 males, 23 years), 19.0–23.9 years (9 males, 7 females), and 24.0 years or more (6 males, 8 females).

Data collection

Computed tomography (CT) scans of each cranium were performed using the helical scanner (Asteion Premium 4 Editions; Toshiba Medical Systems, Japan) at PRI, with a pixel size of 0.25 mm x 0.25 mm and an interval between slices of 0.5 mm. The orientation of the slices was parallel to the axial or coronal plane. Because the cortical thickness of long bones showed a decrease with age in humans and nonhuman primates, CT scans of each femur were also taken to examine their thickness in relation to changes in the patterns of cranial thickness caused by aging.

Twelve craniofacial thickness measurements were taken from the neurocranium and

face of each individual. I examined 10 sites on the Neurocranium (cranial vault bones) (Figure 2.1): (MBN) midpoint between bregma and nasion; (B) bregma; (AB) 1 cm anterior to bregma; (PB) 1 cm posterior to bregma; (LB) 1 cm left of bregma; (RB) 1 cm right of bregma; (MBI) midpoint between bregma and inion; (I) inion; (LTL) left temporal line; and (RTL) right temporal line (the cranial thickness at the temporal line was measured at the site of intersection between the frontal plane and temporal line). All thickness measurements at the neurocranium were taken perpendicular to the external cranial surface, except for thickness at inion, which was taken perpendicular to the internal cranial surface. 2 sites were taken from the face on the left side (Figure 2.2): (MFZ) the midpoint between the frontomale orbitale and the zygomatico-maxillary suture at the root of the zygomatic arch; and (MLZ) the midpoint between the zygomatico-maxillary suture at the root of the zygomatic arch and the lowest point of orbital margin. Thickness measurements of the face were taken perpendicular to the front of the external facial surface in the parasagittal plane. Because two cortical bones (front and back) at MFZ on maxilla were fused together in each individual, a total thickness value was used for this site. I also measured the cortical thickness from 3 sites at the midlength of the femur for each individual (Figure 2.3): AT for anterior thickness; MT for medial thickness; and LT for lateral thickness. All measurements were done using three-dimensional reconstruction images on a computer screen using the AZE Virtual Place software (AZE Co., Ltd., Tokyo, Japan). The thickness of each specimen was measured without knowledge of sex or age (Lynnerup, 2001). Each measurement was made three times and the average value was used for analysis.

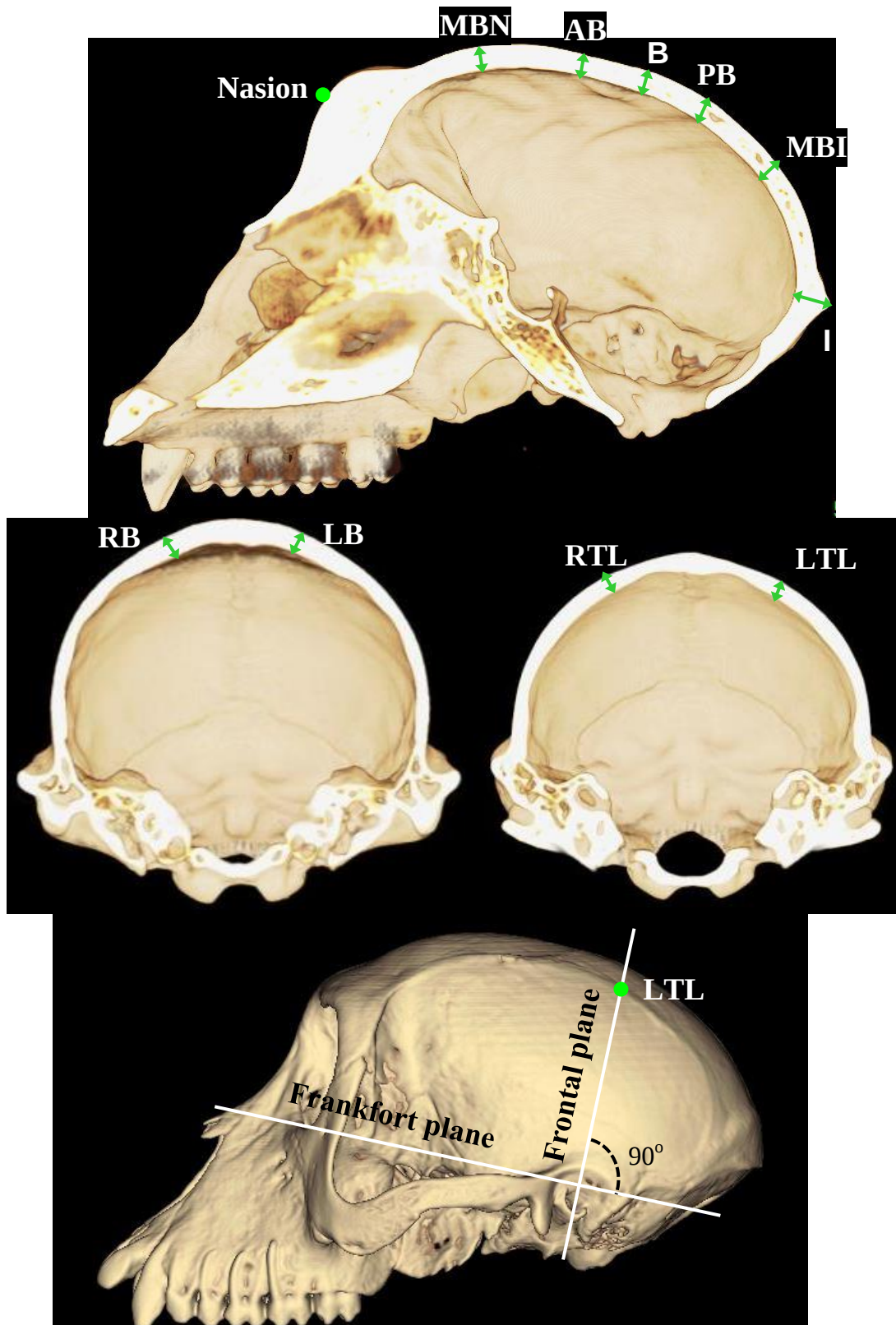


Figure 2.1 The sites on the neurocranium for measurements of thickness. MBN: midpoint between the bregma and nasion; B: bregma; AB: 1cm anterior to bregma; PB: 1 cm posterior to bregma; LB: 1 cm left of bregma; RB: 1 cm right of bregma; MBI: midpoint between bregma and inion; I: inion; LTL: left temporal line; and RTL: right temporal line.

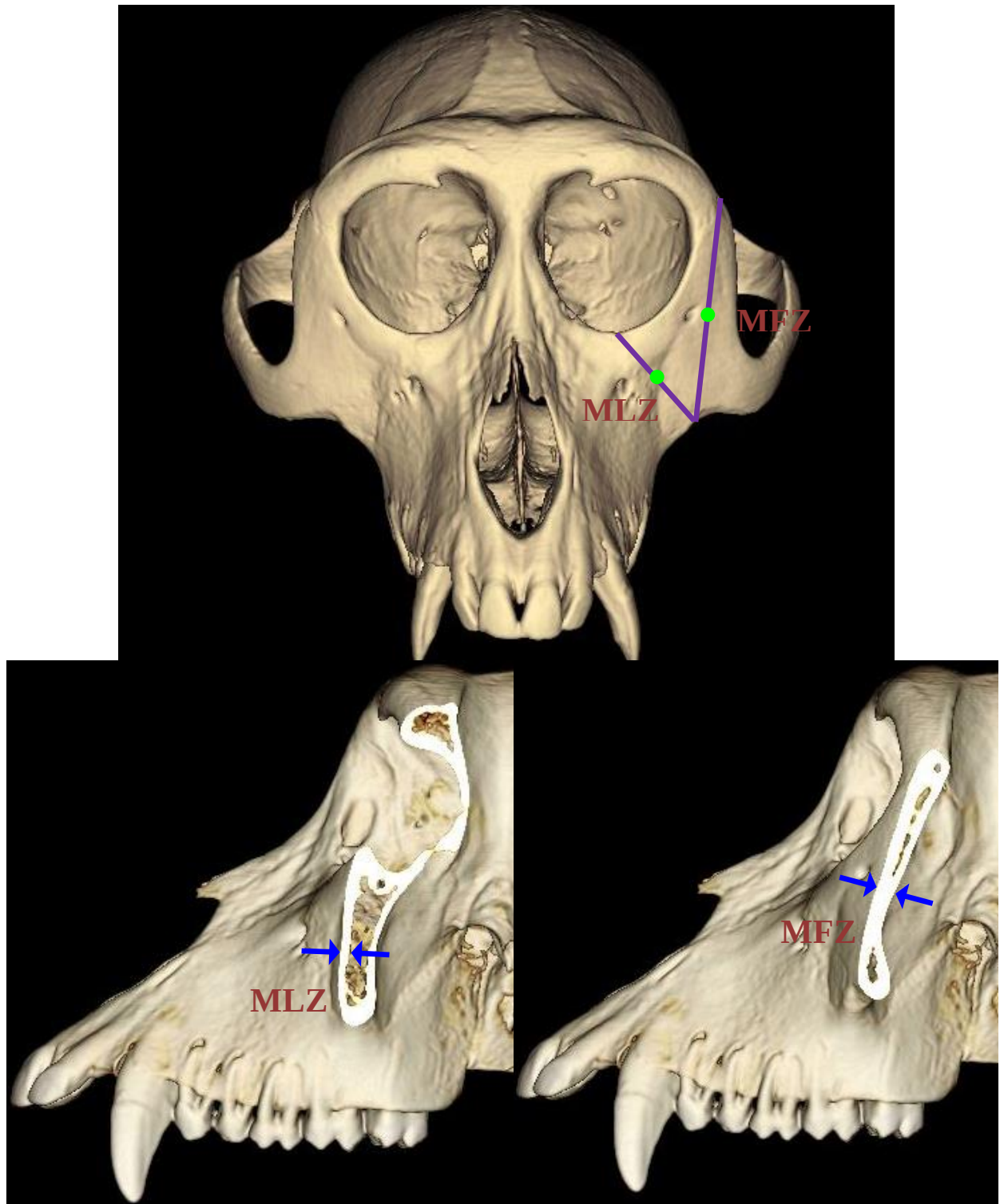


Figure 2.2 The sites of thickness measurements in the mid-face. MFZ: midpoint between the frontomale orbitale and zygomatico-maxillary suture at the root of the zygomatic arch, and MLZ: midpoint between the zygomatico-maxillary suture at the root of the zygomatic arch and the lowest point of orbital margin.

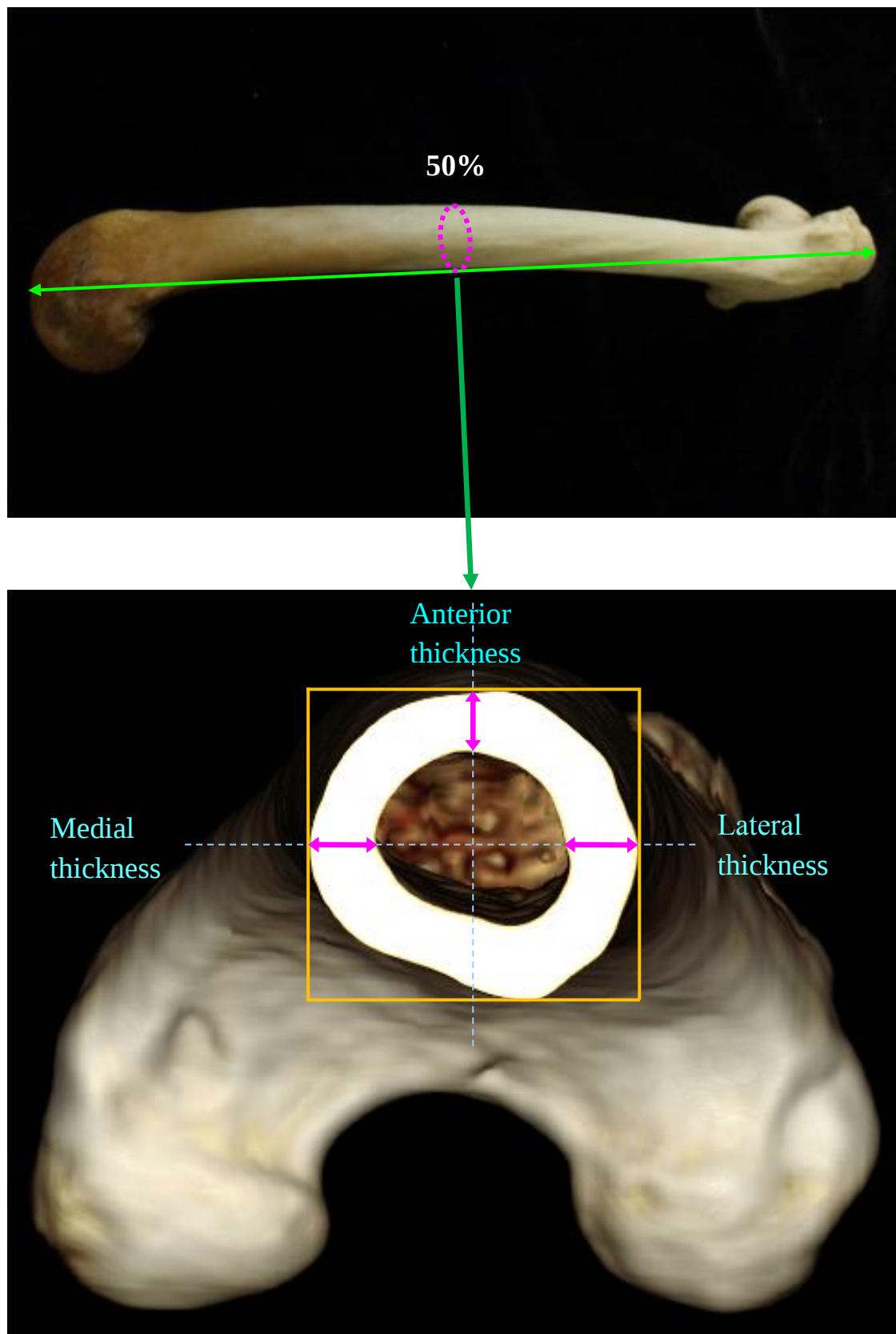


Figure 2.3 The sites of thickness measurements on the cortex on the midlength of femur.

Statistical analysis

All statistical analyses were performed using functions of Excel (Microsoft Co. Ltd.) or Past version 2.17 software (Hammer et al., 2001) with significance determined as $p < 0.05$. To describe age-related pattern changes in the cranial thickness and the cortical thickness of the femur, Loess (locally weighted scatterplot smoothing) was used. Loess was chosen because it is local fitting and allows greater flexibility than some other smoothers (Cohen, 1999; Jacoby, 2000). The smoothing parameter value (α) selected was 0.8, which was applied identically for each function of Loess in all variables (Y: measurements and X: ages). After Loess had been analyzed by Past software, the smoothed values which were relevant with the data were created to describe trends of age-related changes. The cranial thickness measurements and the cortical thickness measurements of the femur between age groups were analyzed by ANOVA with Turkey-Kramer HSD post-hoc testing separately males and females. Analysis of covariance (ANCOVA) was used to determine whether there were sex differences in cranial thickness. The relationships between cranial thickness measurements and cortical thickness measurements of the femur were examined.

Principal component analysis (PCA) was applied to the cranial thickness measurements at the twelve sites (neurocranium and face) in both males and females using Past (Hammer et al., 2001). The relationship between the scores of principal components (PCs) and age was determined using the Loess function. The relationship between the scores of principal components (PCs) and the cortical thickness measurements of the femur was analyzed in terms of Pearson correlation.

Results

Table 2.1 shows the means and standard deviations of cranial thickness measurements at the ten sites of the neurocranium and the two sites of the face. Cranial thickness was significantly greater in males than in females ($p < 0.05$) (at MBN, AB, B, PB, MBI, LTL, RTL, I, MFZ, and MLZ), except those at LB and RB. There were no significant differences in the cranial thickness between left and right bregma and between the left and right temporal line in each sex.

Age-related change in cranial thickness

The cranial thickness at many sites showed an age-related change pattern of increase-peak-decrease (Figure 2.4). The cranial thickness at the cross-sectional age groups is presented in Tables 2.2 and 2.4, and Figures 2.6. Changes in neurocranial and facial thickness with age were significant ($p < 0.05$) at the eleven sites in both males and females (Table 2.2), respectively.

The common pattern of age-related changes in cranial thickness at the sites of the neurocranium was an increase from young adulthood (7–9 years) to mid-adulthood (14–19 years in males and 19–24 years in females) followed by a decrease with age (Tables 2.2 and 2.4). However, the pattern of age-related change was somewhat different between males and females. The thickness at the MBN, PB, and MBI increased significantly in males, with peaks at 14-19 years with an increase of 23.35% ($p < 0.05$), 24.62% ($p < 0.05$), and 24.34% ($p < 0.05$), respectively. We observed a decrease in thickness at these sites following each of the peaks, but it was not statistically significant. On the other hand, the cranial thickness in females at these respective sites only increased slightly, peaking at 19–24 years, followed by a significant decrease in thickness of 24.11% ($p < 0.001$), 24.69% ($p < 0.001$), and 19.36% ($p < 0.01$), respectively.

The thickness at B and AB showed a significant decrease of 30.33% and 28.50% , respectively, from mid-adulthood (19-24 years) to the oldest age group (>24 years) ($p < 0.001$) in females, whereas these thicknesses only changed slightly in males. At sites such as LB and RB, the thickness increased slightly with age from young adulthood (7–9 years) to mid-adulthood (14–19 years) and subsequently decreased significantly in both sexes.

The thickness at other sites, such as at LTL and RTL also showed the same trend in both sexes, with a significant increase from young adulthood (7–9 years) to mid-adulthood (19-24 years) followed by an unchanging period from mid-adulthood to the oldest age group (>24 years).

The cranial thickness at I (inion) showed a different pattern compared with those at other sites in the neurocranium in males, with a significant increase ($p < 0.05$) in the oldest age group (>24 years) of 32.37%. In contrast, the thickness at this site changed only slightly in females.

Age-related change patterns of the thickness at facial sites were characterized by a decrease from young adulthood (7–9 years) to the oldest age group (>24 years) (Tables 2.2 and 2.4). The thickness at MFZ and MLZ showed an age-related decrease of 28.27% ($p < 0.001$) and 23.52% ($p < 0.001$) in males, respectively; and 29.42% ($p < 0.05$) and 23.16% ($p < 0.001$) in females, respectively.

Size and shape change in cranial thickness with age

Principal component analysis was based on a correlation matrix and applied using the cranial thicknesses at the twelve sites in the neurocranium and face. The first three principal components accounted for 50.73%, 16.33%, and 9.38% of the total variation, respectively (Table 2.6). The first principal component (PC1) represented size change, and showed that the thickness at all sites had positive loadings, except for those in the face, MFZ (0.145), and MLZ (0.122). The PC1 scores showed age-related change patterns similar to those of cranial

thickness at many sites (Figure 2.8a). Males had higher PC1 scores than females, and ages at the peak PC1 score were 17.0 and 20.2 years in males and females, respectively.

The second principal component (PC2) represented age-related shape changes in cranial thickness. The thickness at LB (0.523), RB (0.496), MFZ (0.434), and MLZ (0.205) had higher positive loadings, whereas the thickness at LTL (−0.271), RTL (−0.262), and I (−0.234) had high negative loadings (Table 2.6). PC2 stands for relative decreasing of thickness in adulthood. Females had higher scores than males, especially from mid-adulthood (approximately 17 to 20 years) to very old age (Figure 2.8b). The PC2 scores were stable from 7.0 to 20.0 years and then decreased gradually in females, whereas males showed a gradual decreased from 7.0 to 17.0 years followed by a dramatic decrease from 17.0 to 26.9 years (Figure 2.8b).

The third principal component (PC3) represented shape change, with significant loadings at MLZ (0.751) and MFZ (0.427) and RB (−0.305), LB (−0.255), and MBN (−0.250) (Figure 2.8c; Table 6). PC3 scores showed gradual decreases from 7.0 to 17.0 years in males and from 7.0 to 19.0 years in females followed by a stable period in both sexes (Figure 2.8c).

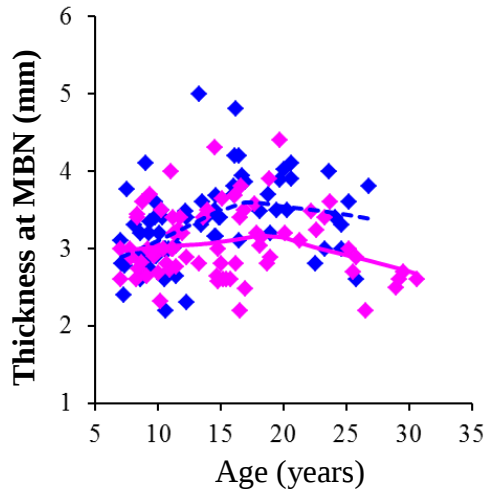
Age-related change in cortical thickness at the midlength of the femur

The cortical thickness at the midlength of the femur showed a pattern of age-related change similar to that of cranial thickness: an increase from young adulthood to the peak in mid-adulthood, followed by a decrease in very old age (Tables 2.3 and 2.5; Figure 2.7). Anterior thickness at the midlength of the femur showed a slight increase and peaked at 14–19 years in females ($p > 0.05$), followed by a significant decrease of 27.00% in the oldest age group (>24 years) ($p < 0.001$), while males only showed a slight change. Medial and lateral thicknesses in both males and females showed slight increases with the peaks at 14–19 years, though not significantly ($p < 0.05$). Following the peaks, males showed significant decreases of 26.44% ($p < 0.05$) and 25.83% ($p < 0.05$), respectively, and females showed

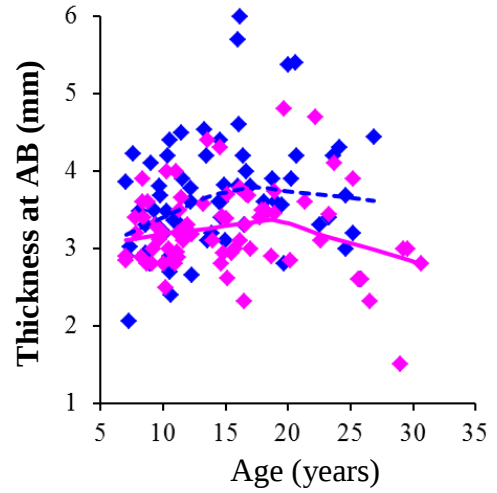
decreases of 30.19% ($p < 0.01$) and 31.26% ($p < 0.01$), respectively.

Medial and lateral thicknesses at the midlength of the femur showed significant age-related changes in both sexes, similar to those observed in cranial thickness. A significant correlation was found between cranial thickness and cortical thickness at the midlength of the femur, however not all correlations were statistically significant. A positive relationship was observed between the cranial thickness at all sites and the medial cortical thickness of the femur in both males ($r_p = 0.036$ to 0.316 , $p < 0.01$ to 0.8) and females ($r_p = 0.124$ to 0.297 , $p < 0.05$ to 0.3). A positive relationship was also found between the cranial thickness at all sites and the lateral cortical thickness of the femur in both males ($r_p = 0.013$ to 0.456 , $p < 0.001$ to 0.9) and females ($r_p = 0.117$ to 0.394 , $p < 0.001$ to 0.3). Additionally, the first principal component scores (size) of the cranial thickness significantly correlated with the cortical thickness at the medial and lateral sides of the midlength of the femur in both males ($r_p = 0.260$, $p < 0.05$; $r_p = 0.346$, $p < 0.01$) and females ($r_p = 0.308$, $p < 0.01$; $r_p = 0.353$, $p < 0.01$) (Figure 2.9).

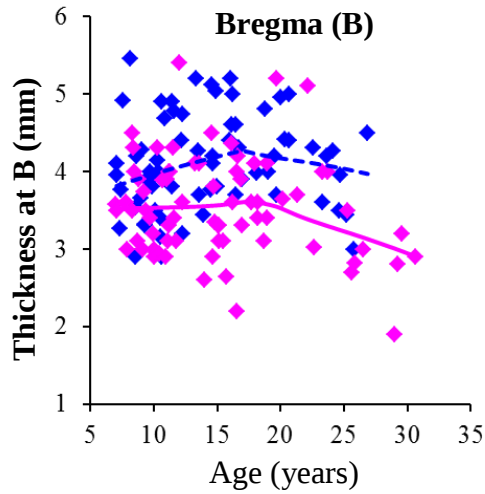
Midpoint of nasion and bregma (MBN)



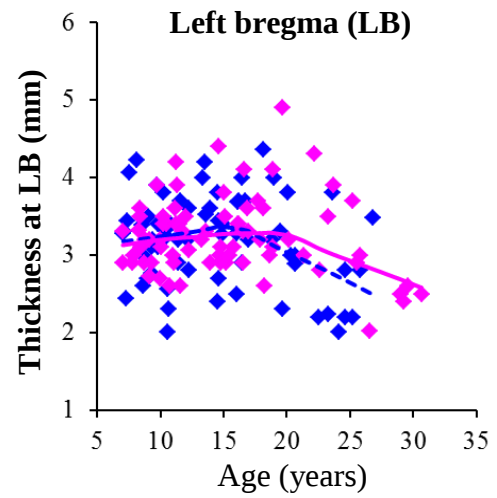
Anterior to bregma (AB)



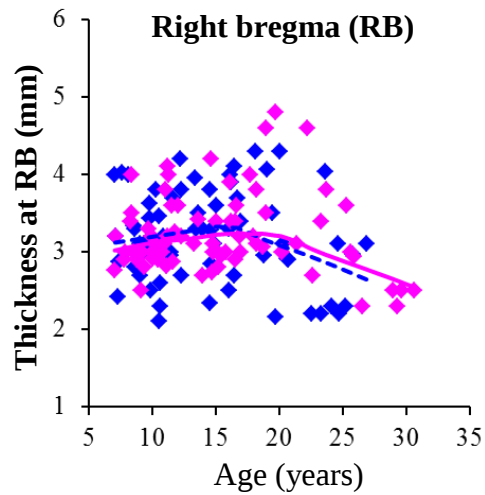
Bregma (B)



Left bregma (LB)



Right bregma (RB)



Posterior to bregma (PB)

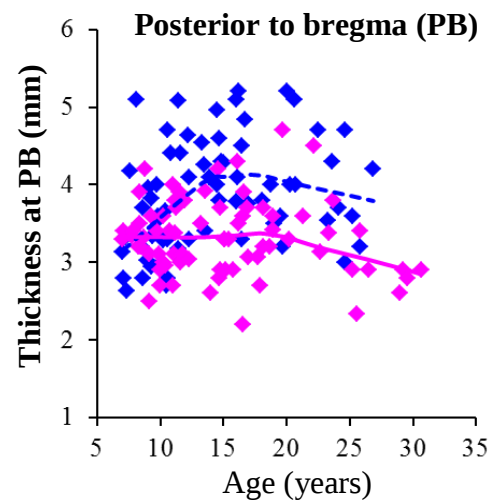
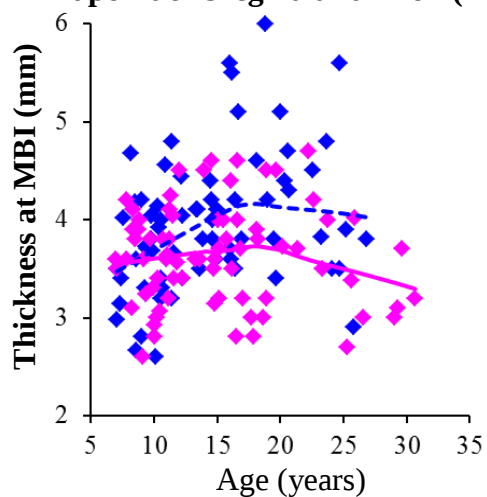
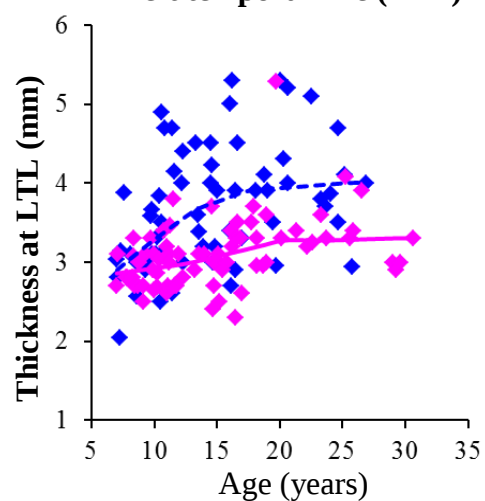


Figure 2.4 Scatter plots showing age-related changes in cranial thickness in all neurocranial and facial sites, with trend lines by Loess. Male: blue diamond and blue dash line; female: pink diamond and pink solid line.

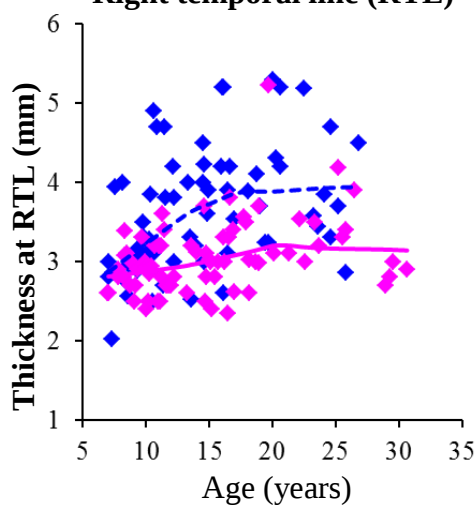
Midpoint of bregma and inion (MBI)



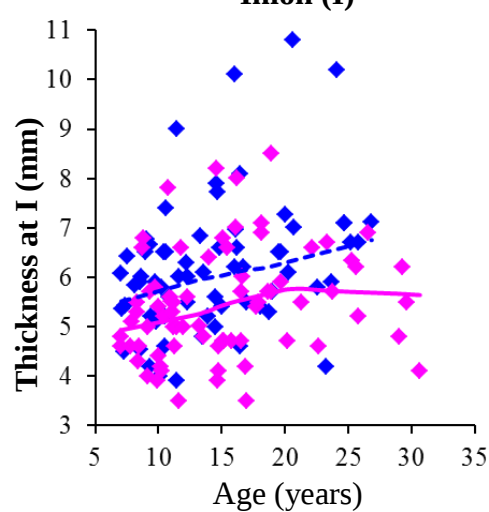
Left temporal line (LTL)



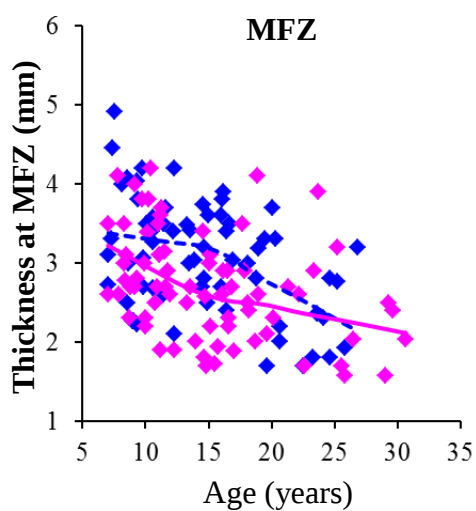
Right temporal line (RTL)



Inion (I)



MFZ



MLZ

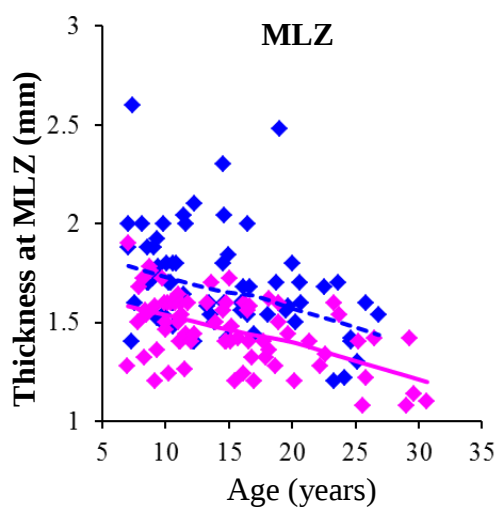


Figure 2.4 (Continued)

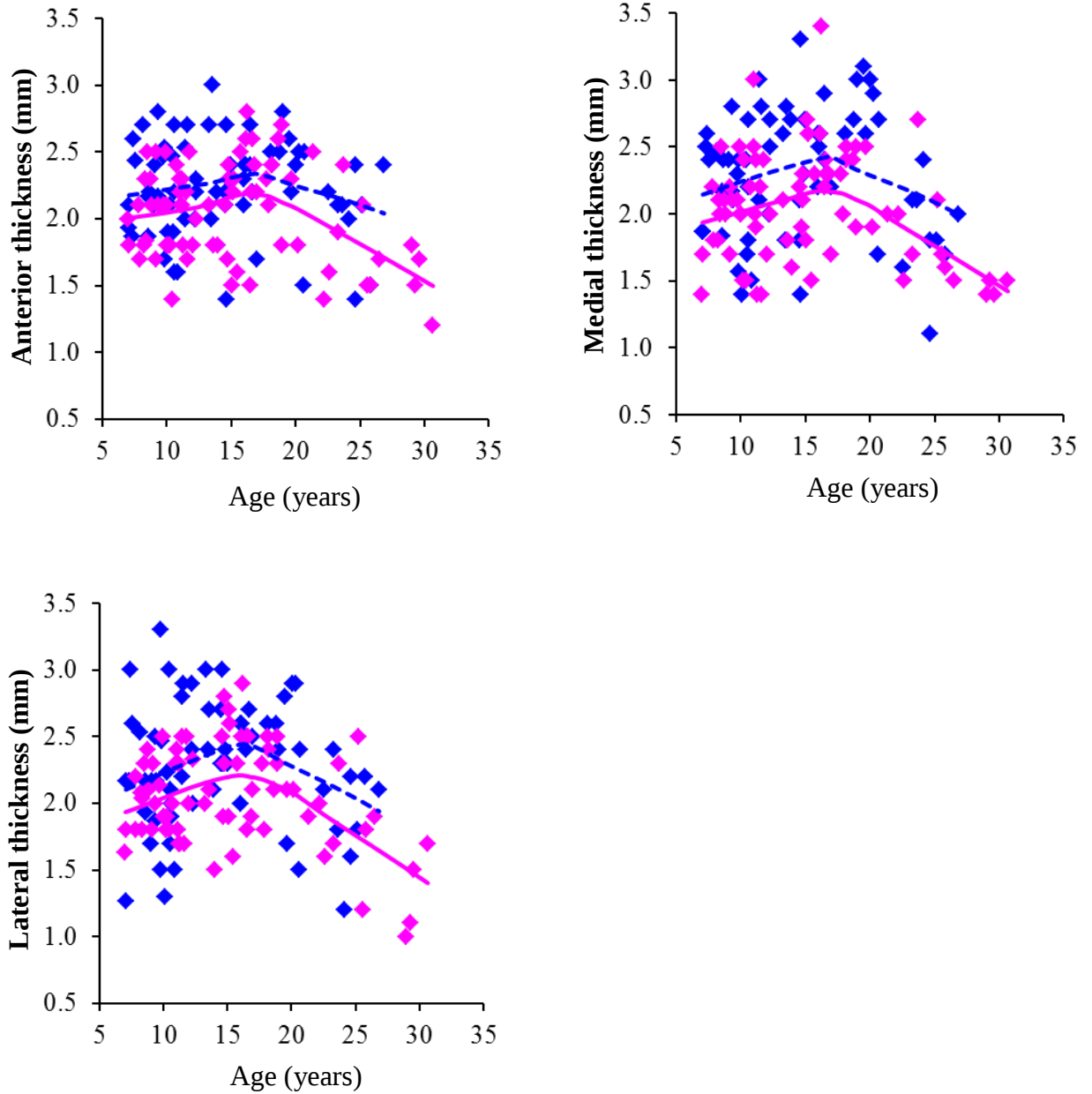


Figure 2.5 Scatter plots showing age-related changes in the cortical thickness at the midlength of the femur with trend lines by Loess. Male: blue diamond and blue dash line; female: pink diamond and pink solid line.

Table 2.1 Means, standard deviations, and differences in cranial thickness at each sample point between male and female samples

	Males	Females	Difference between males and females (%)	P value
MBN	3.36 ± 0.54 ⁺	3.07 ± 0.46 ⁺	9.5	< 0.001***
AB	3.67 ± 0.75 ⁺	3.25 ± 0.55 ⁺	12.7	< 0.001***
B	4.11 ± 0.62 ⁺	3.53 ± 0.65 ⁺	16.3	< 0.001***
LB	3.19 ± 0.58 ⁺	3.24 ± 0.50 ⁺	-1.7	< 0.459
RB	3.19 ± 0.60 ⁺	3.22 ± 0.51 ⁺	-0.9	< 0.687
PB	3.88 ± 0.70 ⁺	3.32 ± 0.49 ⁺	16.9	< 0.001***
MBI	3.96 ± 0.70 ⁺	3.63 ± 0.51 ⁺	9.3	< 0.001***
LTL	3.65 ± 0.76 ⁺	3.09 ± 0.44 ⁺	18.4	< 0.001***
RTL	3.63 ± 0.77 ⁺	3.05 ± 0.46 ⁺	19.0	< 0.001***
I	6.19 ± 1.36 ⁺	5.46 ± 1.10 ⁺	13.4	< 0.001***
MFZ	3.08 ± 0.71 ⁺	2.71 ± 0.67 ⁺	13.7	< 0.01**
MLZ	1.69 ± 0.26 ⁺	1.46 ± 0.18 ⁺	16.3	< 0.001***

⁺ Mean ± standard deviation (SD) mm.

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ statistically significant differences between males and females from analysis of covariance (ANCOVA). I have described the abbreviations in the text.

Table 2.2 Age-related changes of cranial thickness in males and females

	Age group (years)	Male			Female		
		Mean (mm)	SD	Age difference	Mean (mm)	SD	Age difference
MBN	7–9	2.97	0.39	b [*] , c [*]	3.01	0.36	
	9–14	3.22	0.57		3.08	0.38	
	14–19	3.66	0.45	b [*]	3.09	0.54	
	19–24	3.63	0.47	c [*]	3.49	0.44	l ^{***}
	>24	3.28	0.43		2.65	0.24	l ^{***}
AB	7–9	3.15	0.60	b [*] , c [*]	3.25	0.39	
	9–14	3.53	0.58		3.23	0.44	
	14–19	4.03	0.80	b [*]	3.30	0.44	
	19–24	4.02	0.90	c [*]	3.80	0.76	l ^{***}
	>24	3.54	0.73		2.72	0.68	l ^{***}
B	7–9	3.98	0.76		3.70	0.48	d [*]
	9–14	3.98	0.63		3.54	0.63	
	14–19	4.38	0.51		3.51	0.56	
	19–24	4.31	0.48		4.09	0.79	l ^{***}
	>24	3.78	0.56		2.85	0.46	d [*] , l ^{***}
LB	7–9	3.24	0.59		3.17	0.26	
	9–14	3.27	0.52		3.24	0.41	
	14–19	3.38	0.54	k [*]	3.35	0.45	k [*]
	19–24	2.95	0.62		3.57	0.72	l ^{***}
	>24	2.61	0.53	k [*]	2.70	0.50	k [*] , l ^{***}
RB	7–9	3.27	0.58	d [*]	3.17	0.37	
	9–14	3.19	0.51	g [*]	3.18	0.39	
	14–19	3.40	0.60	k ^{**}	3.34	0.48	k [*]
	19–24	3.04	0.79		3.63	0.81	l ^{***}
	>24	2.66	0.44	d [*] , g [*] , k ^{**}	2.71	0.45	k [*] , l ^{***}
PB	7–9	3.39	0.76	b [*]	3.48	0.32	d [*]
	9–14	3.76	0.65		3.27	0.44	
	14–19	4.22	0.57	b [*]	3.32	0.49	
	19–24	4.18	0.70		3.77	0.61	l ^{***}
	>24	3.73	0.63		2.84	0.30	d [*] , l ^{***}
MBI	7–9	3.50	0.64	b [*] , c [*]	3.77	0.33	
	9–14	3.78	0.49		3.55	0.49	
	14–19	4.35	0.76	b [*]	3.65	0.55	
	19–24	4.31	0.55	c [*]	4.05	0.44	l ^{**}
	>24	3.87	0.92		3.26	0.42	l ^{**}
LTL	7–9	2.93	0.47	b [*] , c ^{***} , d [*]	2.90	0.21	c ^{**}
	9–14	3.55	0.68		2.98	0.31	f [*]
	14–19	3.87	0.72	b [*]	3.08	0.40	
	19–24	4.21	0.83	c ^{***}	3.55	0.77	c ^{**} , f [*]
	>24	3.85	0.59	d [*]	3.26	0.52	c ^{**}
RTL	7–9	2.99	0.59	b [*] , c ^{**}	2.90	0.25	c ^{**}
	9–14	3.43	0.66		2.89	0.32	f ^{**}
	14–19	3.93	0.68	b [*]	3.06	0.42	
	19–24	4.19	0.86	c ^{**}	3.52	0.78	c ^{**} , f ^{**}
	>24	3.82	0.70		3.27	0.53	

Table 2.2 (Continued)

	Age group (years)	Male			Female		
		Mean (mm)	SD	Age difference	Mean (mm)	SD	Age difference
I	7–9	5.66	0.70	d [*]	5.22	0.86	
	9–14	5.77	1.13	g [*]	5.16	0.93	
	14–19	6.40	1.40		5.75	1.43	
	19–24	6.67	1.78		5.67	0.83	
	>24	7.49	1.34	d [*] , g [*]	5.66	0.93	
MFZ	7–9	3.43	0.89	c ^{***} , d ^{***}	3.02	0.54	d [*]
	9–14	3.26	0.58	f [*] , g [*]	2.94	0.67	g [*]
	14–19	3.17	0.47	k [*]	2.56	0.62	
	19–24	2.45	0.78	c ^{**} , f [*]	2.60	0.70	
	>24	2.46	0.55	d ^{***} , g [*] , k [*]	2.13	0.56	d [*] , g [*]
MLZ	7–9	1.85	0.32	d ^{***}	1.60	0.20	c [*] , d ^{***}
	9–14	1.70	0.21		1.50	0.14	g ^{**}
	14–19	1.75	0.30	k [*]	1.44	0.15	k [*]
	19–24	1.59	0.17		1.40	0.14	c [*]
	>24	1.41	0.14	d ^{***} , k [*]	1.23	0.16	d ^{***} , g ^{**} , k [*]

^{a–l} Statistically significant differences between age groups (ANOVA) at the * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ level for ^a 7–9 vs. 9–14 years, ^b 7–9 vs. 14–19 years, ^c 7–9 vs. 19–24 years, ^d 7–9 vs. >24 years, ^e 9–14 vs. 14–19 years, ^f 9–14 vs. 19–24 years, ^g 9–14 vs. >24 years, ^h 14–19 vs. 19–24 years, ^k 14–19 vs. >24 years, ^l 19–24 vs. >24 years. I have described the abbreviations in the text.

Table 2.3 Age-related changes of cortical thickness at the midlength of femur in males and females

	Age group (years)	Male			Female		
		Mean (mm)	SD	Age difference	Mean (mm)	SD	Age difference
Anterior thickness	7–9	2.20	0.29		2.04	0.26	d*
	9–14	2.25	0.38		2.04	0.29	g*
	14–19	2.30	0.35		2.23	0.38	k***
	19–24	2.23	0.33		1.99	0.42	
	>24	1.97	0.43		1.63	0.27	d*, g*, k***
Medial thickness	7–9	2.22	0.29		1.96	0.30	
	9–14	2.24	0.48		2.02	0.39	
	14–19	2.45	0.45	k*	2.27	0.39	k**
	19–24	2.42	0.56	l*	2.04	0.42	
	>24	1.80	0.42	k*, l*	1.59	0.23	k**
Lateral thickness	7–9	2.16	0.48		2.01	0.25	d*
	9–14	2.28	0.52		2.03	0.29	g*
	14–19	2.49	0.22	k*	2.29	0.35	k**
	19–24	2.28	0.53		1.96	0.24	
	>24	1.85	0.40	k*	1.58	0.47	d*, g*, k**

^{a-l} Statistically significant differences between age groups (ANOVA) at the * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ level for ^a 7–9 vs. 9–14 years, ^b 7–9 vs. 14–19 years, ^c 7–9 vs. 19–24 years, ^d 7–9 vs. >24 years, ^e 9–14 vs. 14–19 years, ^f 9–14 vs. 19–24 years, ^g 9–14 vs >24 years, ^h 14–19 vs. 19–24 years, ^k 14–19 vs. >24 years, ^l 19–24 vs. >24 years.

Table 2.4 Percentage change in cranial thickness in males and females

	7–9 to 14–19	7–9 to 19–24	7–9 to >24	14–19 to >24	19–24 to >24
Male					
MBN	23.35*	22.33*	10.55	–10.38	–9.63
AB	28.12*	27.64*	12.42	–12.25	–11.93
B	10.22	8.37	–5.01	–13.82	–12.35
LB	4.37	–9.11	–19.39	–22.76*	–11.31
RB	4.25	–7.06	–18.50*	–21.82*	–12.32
PB	24.62*	23.37	10.13	–11.63	–10.73
MBI	24.34*	23.38*	10.60	–11.05	–10.36
LTL	32.03*	43.67***	31.49*	–0.41	–8.48
RTL	31.59*	40.12**	27.73	–2.93	–8.84
I	13.24	17.99	32.37*	16.89	12.19
MFZ	–7.74	–28.62**	–28.27***	–22.25*	0.50
MLZ	–5.08	–13.90	–23.52***	–19.43*	–11.17
Female					
MBN	2.86	15.98	–11.98	–14.43	–24.11***
AB	1.50	16.76	–16.51	–17.75	–28.50***
B	–4.95	10.72	–22.86*	–18.84	–30.33***
LB	5.54	12.59	–14.80	–19.28*	–24.33***
RB	5.55	14.61	–14.56	–19.05*	–25.45***
PB	–4.39	8.58	–18.22*	–14.47	–24.69***
MBI	–3.19	7.31	–13.46	–10.61	–19.36**
LTL	6.40	22.40**	12.49	5.72	–8.10
RTL	5.75	21.65**	13.00	6.85	–7.11
I	10.11	8.65	8.33	–1.61	–0.29
MFZ	–15.12	–13.85	–29.42*	–16.85	–18.08
MLZ	–10.12	–12.72*	–23.16***	–14.51*	–11.96

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ Comparison between age groups by ANOVA with post-hoc testing. I have described the abbreviations in the text.

Table 2.5 Percentage change in cortical thickness at the midlength of femur in males and females

	7–9 to 14–19	7–9 to 19–24	7–9 to >24	14–19 to >24	19–24 to >24
Male					
Anterior thickness	4.70	1.67	-10.47	-14.49	-11.94
Medial thickness	10.06	8.95	-19.04	-26.44*	-25.69*
Lateral thickness	15.29	5.29	-14.48	-25.83*	-18.78
Female					
Anterior thickness	8.94	-2.82	-20.47*	-27.00***	-18.17
Medial thickness	16.02	4.23	-19.01	-30.19**	-22.29
Lateral thickness	13.73	-2.86	-21.82*	-31.26**	-19.53

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ Comparison between age groups by ANOVA with post-hoc testing.

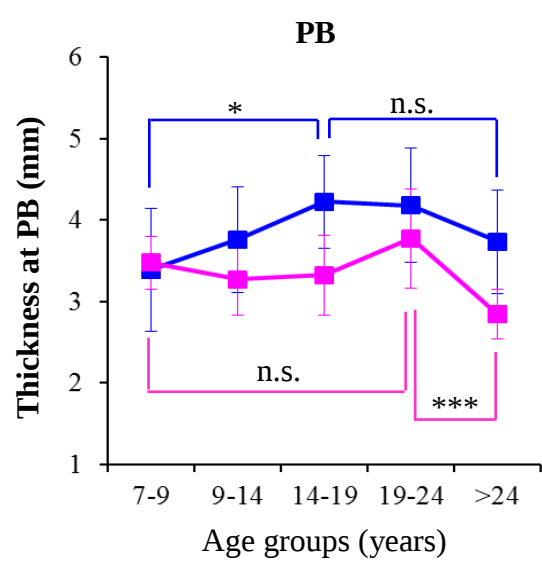
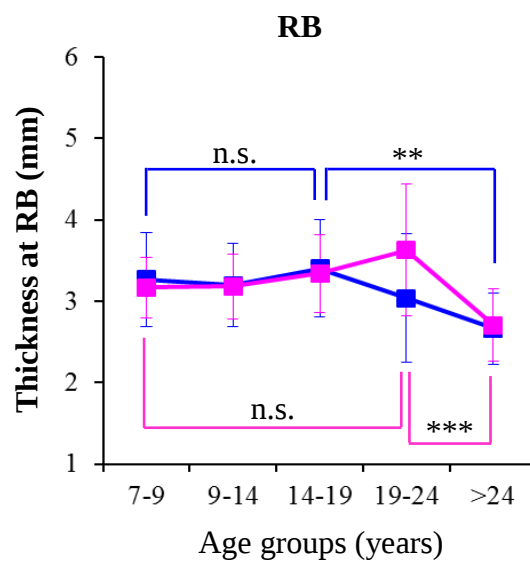
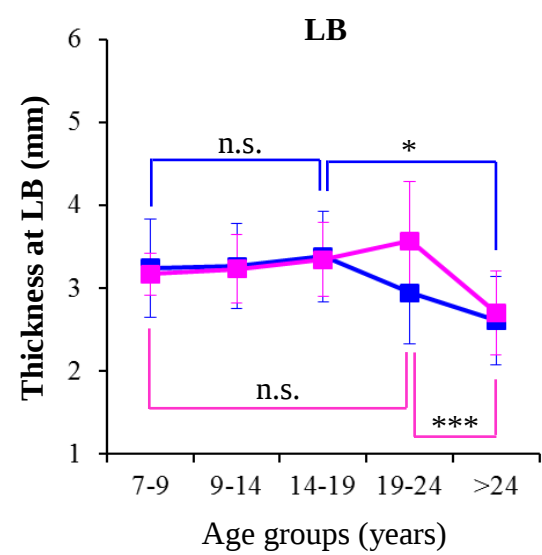
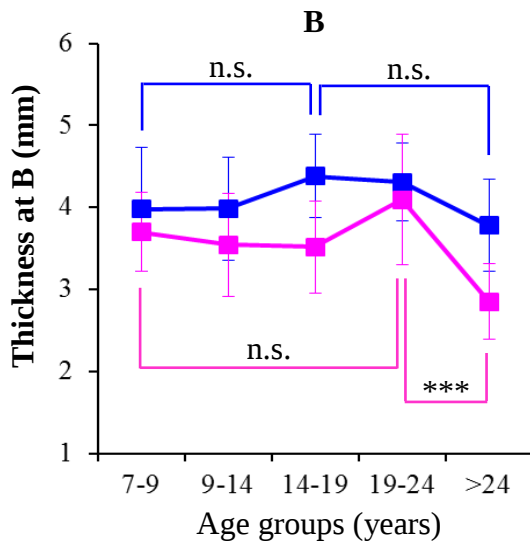
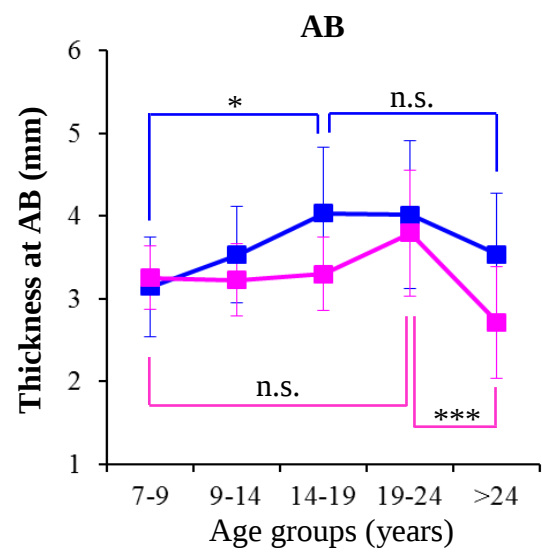
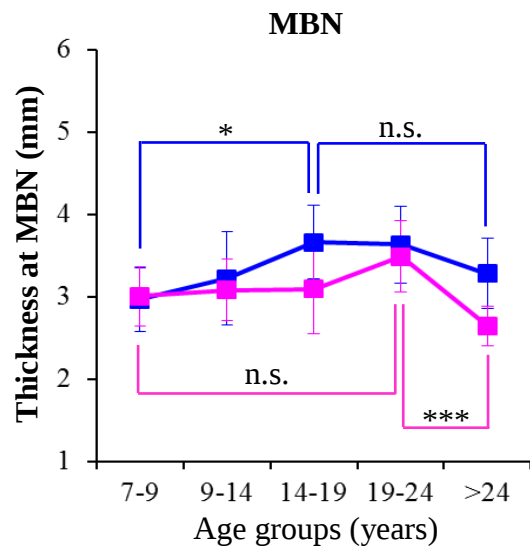


Figure 2.6 Patterns of age-related changes in cranial thickness in all neurocranial and facial sites. Male: blue square and blue solid line; female: pink square and pink solid line. Vertical bars indicate means $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and n.s. (not significant, $p > 0.05$) Comparison between age groups by ANOVA with post-hoc testing. The thickness measurements abbreviated as in Figures 2.1 and 2.2.

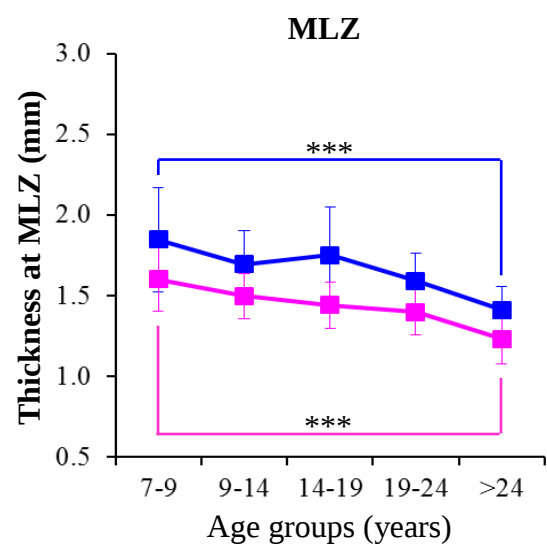
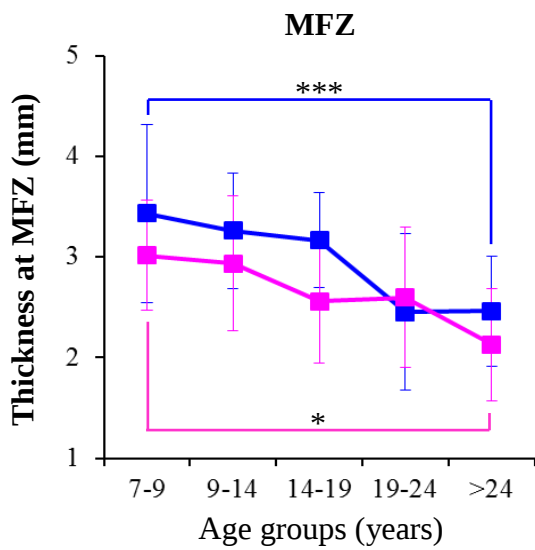
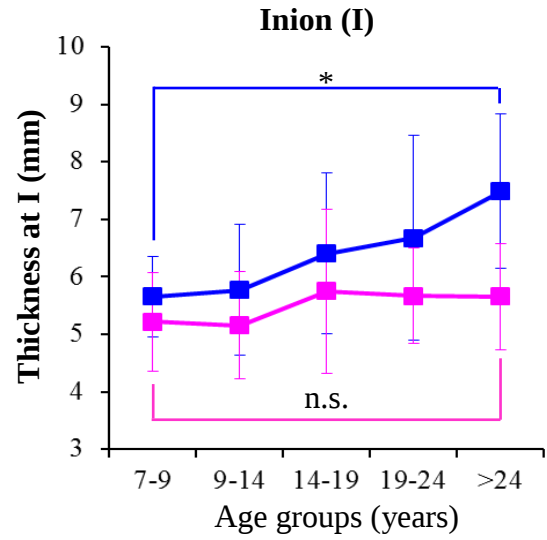
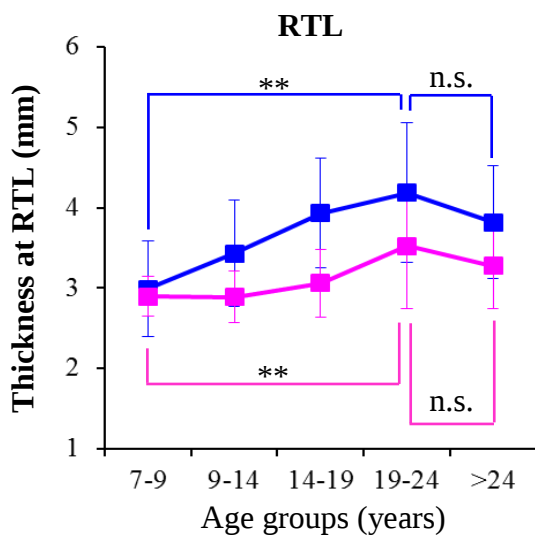
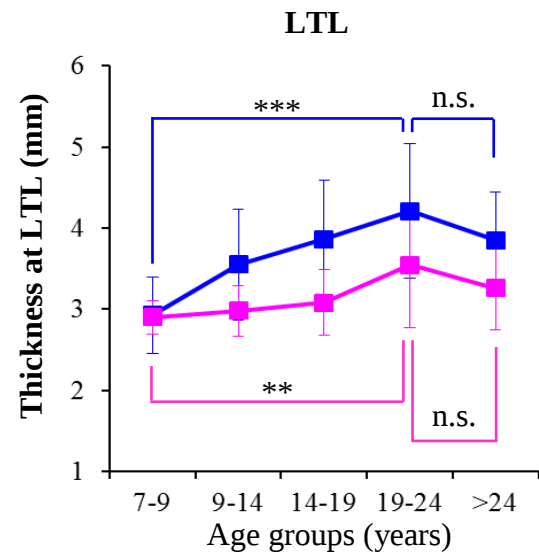
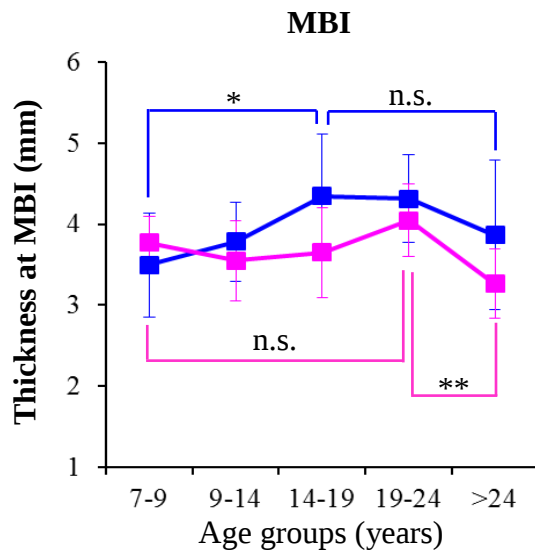


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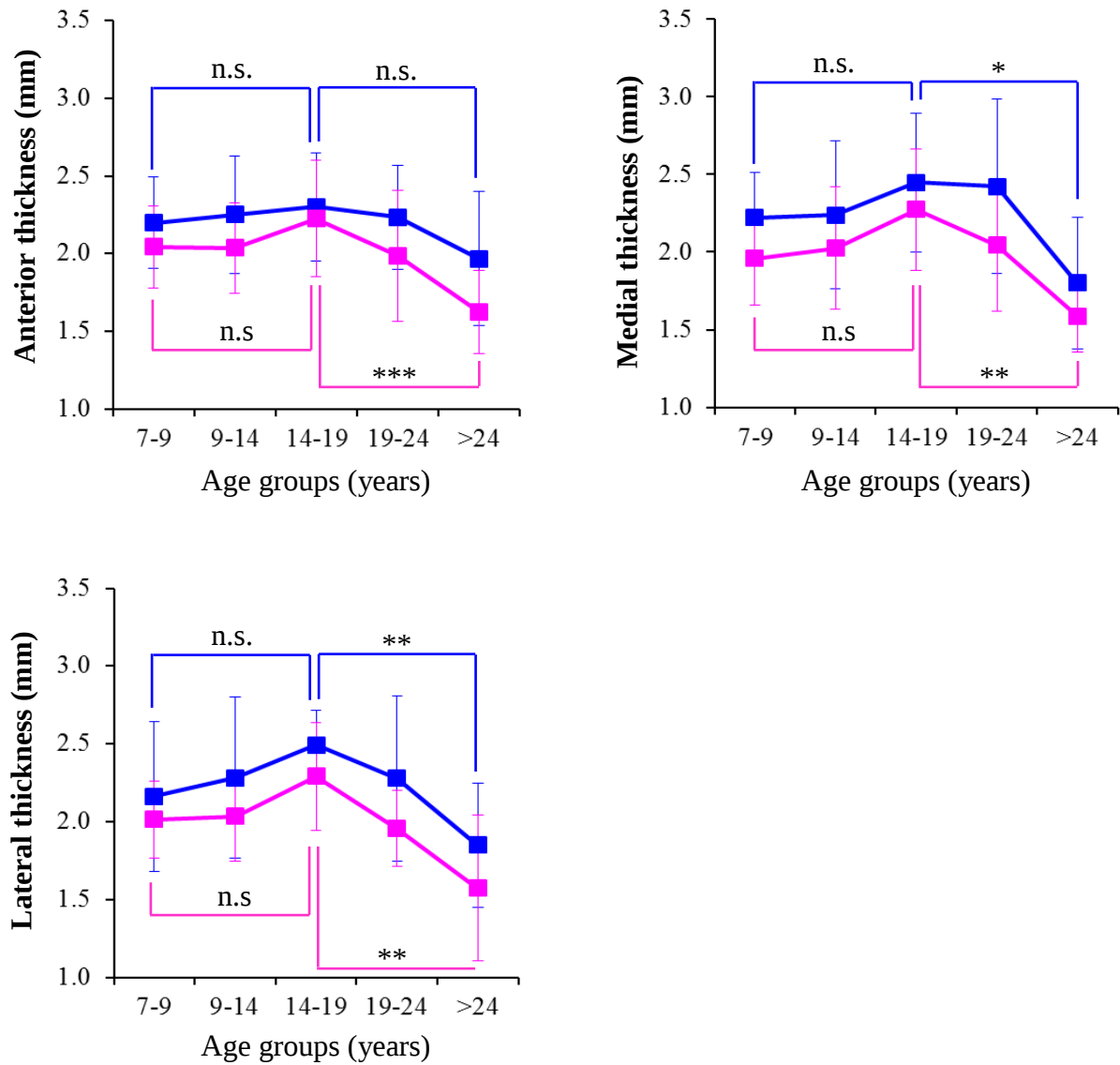


Figure 2.7 Patterns of age-related changes in the cortical thickness at the midlength of the femur. Male: blue square and blue solid line; female: pink square and pink solid line. Vertical bars indicate means $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and n.s. (not significant, $p > 0.05$) Comparison between age groups by ANOVA with post-hoc testing

Table 2.6 Factor loadings of PC1, PC2, and PC3 of the variables of the cranial thickness obtained from the principal component analysis using both sexes.

Variable	PC1	PC2	PC3
MBN	0.315	0.071	−0.250
AB	0.361	−0.043	−0.023
B	0.353	0.031	0.031
LB	0.218	0.523	−0.255
RB	0.221	0.496	−0.305
PB	0.366	−0.096	0.036
MBI	0.320	−0.194	−0.113
LTL	0.331	−0.271	0.096
RTL	0.337	−0.262	0.079
I	0.233	−0.234	0.044
MFZ	0.145	0.434	0.427
MLZ	0.122	0.205	0.751
Eigenvalue	6.09	2.00	1.13
Proportion	50.73	16.33	9.38

I have described the abbreviations in the text.

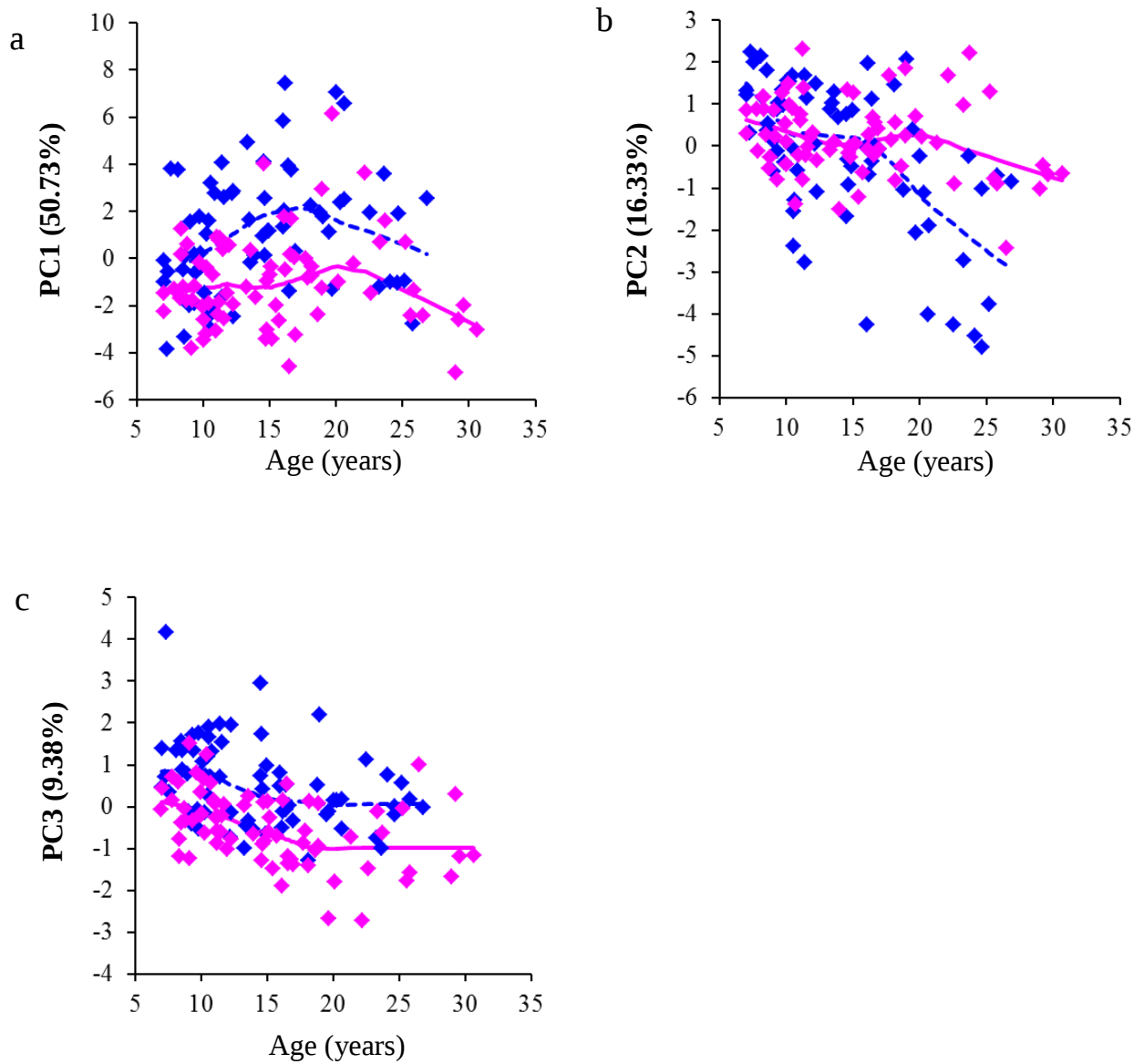


Figure 2.8 Plots of (a) first principal component scores, (b) second principal component scores, and (c) third principal component scores against age with trend lines by Loess. Male: blue diamond and blue dash regression line; female: pink diamond and pink solid regression line.

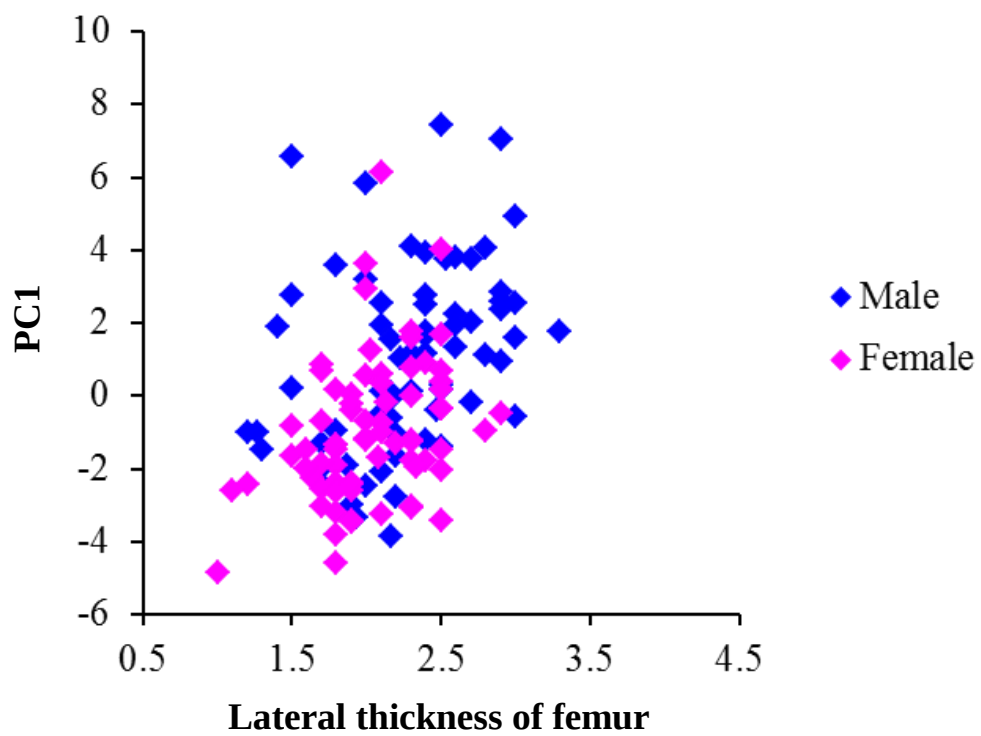
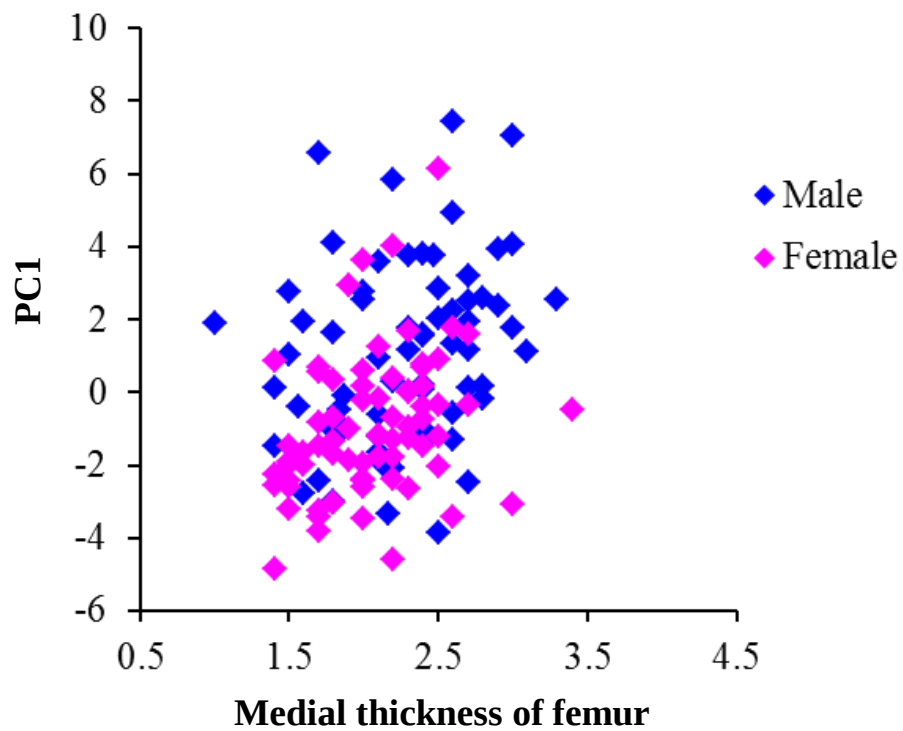


Figure 2.9 Relationship between first principal component scores of the cranial thickness and the cortical thickness at the medial and lateral sides at the midlength of the femur. Male: blue diamond and blue dash regression line; female: pink diamond and pink solid regression line.

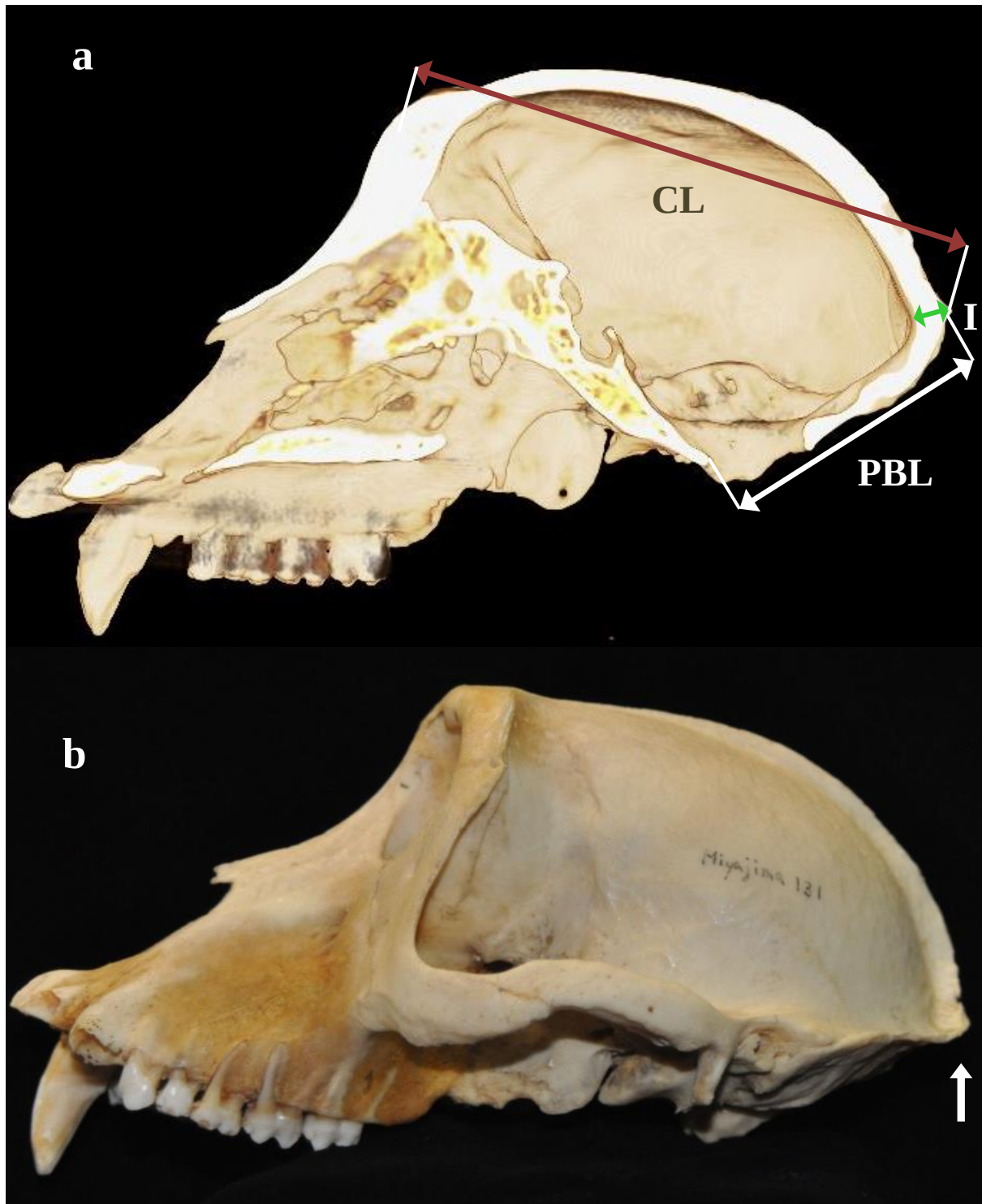


Figure 2.10 (a) Relationship of measurements at inion. Posterior basicranial length (CBL), cranial length (CL), and cranial thickness at inion (I). (b) The bone development (tubercles, white arrow) around inion in old male macaques.

Discussion

Various factors of environment, genetics, and physiological conditions affect bone dimensions, making it difficult to define age-related change. In the present study, we controlled the origin of the subjects, rearing conditions (corral cages), and foods (monkey chow and potato supplements) throughout their life span. Taking these conditions into account, age-related changes in the cranial thickness of Japanese macaques are discussed in this study.

Bone minerals of postcranial skeletons are generally absorbed more than deposited with advancing age. The thinning of the cortex of long bones with advancing age is thought to be the result of absorption of the endosteal bone in the postcranial skeletons of both humans and nonhuman primates (Smith and Walker, 1964; Garn et al., 1967; Ericksen, 1976; Ruff and Hayes, 1982; Bowden et al., 1979; Kimura, 1994). Bone mineral loss starts in middle age and is accelerated by estrogen deficiency (Colman and Binkley, 2000) and the diameter expansion is considered to be mechanical compensation to the thinning. Women display greater bone loss than men, especially in postmenopausal life (Riggs et al., 2008), and age-related changes in the craniofacial skeleton are also expected.

The main findings of the present study demonstrated that cranial thickness at many sites in the neurocranium and face showed significant age-related changes. The cranial thickness at many sites in the neurocranium showed a pattern of increasing from young adulthood (7–9 years) to mid-adulthood (14–19 years in males and 19–24 years in females) followed by a decrease in the oldest age group (>24 years). However, the thickness at the two facial sites (MFZ and MLZ) showed an exceptionally distinctive pattern of continuous decreasing from young adulthood to very old age in both sexes. The thickness at bregma, the temporal line and AB in the neurocranium, and two facial sites (MFZ and MLZ) showed

greater age-related changes compared with other sites in both males and females.

I also observed a sex difference in age-related changes in cranial thickness. The thickness at sites on the mid-sagittal plane significantly increased in males from young adulthood to mid-adulthood, though they did not show any significant change from mid-adulthood to very old age. This was especially evident in the thickness at inion, which increased significantly with age in males. In contrast, the thickness at these sites in females did not increase significantly from young adulthood to mid-adulthood, but did show a significant decrease from mid-adulthood to the oldest age group. This sex difference may be associated with the differences in the sizes of the projecting face and canines between males and females, which leads to the development of masticatory and postural muscles and stimulates the increase in the cranial thickness at these sites in males. The thickness in the part of mid-sagittal plane would be reminiscent of sagittal crest in great apes, which does not necessarily relate with the temporalis muscle development.

The thickness at the temporal line increased significantly from young adulthood to mid-adulthood (19–24 years in both males and females) with a greater increase in males than in females, and they remained stable from mid-adulthood to the oldest age group in both sexes. The greater increase in the thickness at the temporal line in males may be the result of development or stress on the temporal line on which greater forces from the temporalis muscle are applied. The intertemporal distance greatly decreased (Minh et al., 2015).

The thickness at left and right of bregma (which represents thickness of plain cranial vault) showed the same age-related trend between the two sexes: a slight increase from young adulthood to mid-adulthood followed by a significant decrease from mid-adulthood to the oldest age group. This may represent whole cranial vault may become thinner with age, especially the age from midadulthood to elderly. The thickening at the midsagittal plane sites (linearly) may be the mechanical compensation as the buttress (crest) to support

neurocranium from the load to hold face.

The results of studies on the human skull have revealed age-related changes that are contradictory to those observed in macaques. Although a slight increase in the cranial thickness with age has been recorded (Israel, 1973a; Adeloje et al., 1975), Tallgren (1974) and Lynnerup (2001) reported that there was no correlation between age and the cranial thickness during adult life in men and women. Additionally, Lynnerup (2001) has suggested that women over 50–60 years of age display a decrease in cranial thickness. Furthermore, Torimitsu et al. (2014) have reported that cranial thickness significantly decreases with age in Japanese women, but not in men. They suggested that cranial thickness may be greatly affected by lower bone metabolism, which is essentially caused by postmenopausal bone loss in women (Lynnerup, 2001; Torimitsu et al., 2014). In the present study, there were many sites at which the cranial thickness in macaques showed a significant decrease from mid-adulthood to the oldest age group in both sexes, however females displayed thinning at greater number of sites than males.

The differences in patterns of age-related change of cranial thickness between humans and macaques may be associated with differences in the applied statistical analyses. In previous human studies, statistical analysis mostly relied on linear regression to examine significant correlations between measurements and age (Lynnerup, 2001; Torimitsu et al., 2014), however, the present study employed Loess function to describe age-related change patterns in macaques, which may be better at describing complex patterns of change such as increase-peak-decrease.

The peak of the cranial thickness at many sites in the neurocranium was reached later in females than in males. This corresponds with the finding in craniometric age changes (Minh et al., 2015). Similarly, the maximal stages of trunk length and epiphyseal unions in the postcranial skeletons of Japanese macaques were reported to occur later in females than in

males (Kimura, 1994; Hamada, 2008). Vertebral body dimensions in Japanese macaques have also been shown to peak later in females (age group 15–20 years) than in males (age group 10–15 years) (Pomchote, 2015). In the present study, the age at the peak of cranial thickness was estimated to be 18.0 ± 2.0 years and 20.3 ± 2.0 years on average in males and females, respectively. In chapter 1, the age at maximal skull size was estimated to be 16.0 ± 3.0 years and 20.2 ± 3.0 years in males and females, respectively. This sex difference might relate to reproductive activity in Japanese macaques, which differs between the two sexes. Females keep reproducing until the cessation of reproduction in older age (individual variation is wide, but around 18–25 years; Takahata et al., 1995), so adult females may therefore spend more energy in the reproductive costs of pregnancy and the lactation period, which may lead to a slowing of cranial bone remodeling or growth. However, it is also possible for males to start aging earlier than females.

Age-related expansion of the cranium in Japanese macaques is thought to be associated with the development of bones in response to physical stress from the masticatory and/or postural muscles (Chapter 1). Cranial and posterior basicranial lengths, which include inion, increased significantly with age in male Japanese macaques. A relationship was found between age-related changes in the cranial thickness at the inion and cranial and posterior basicranial lengths in male macaques (Figure 2.10). The results of the present study indicate that the thickness at inion (development of tuberosity) increased significantly in males (1.83 mm in an average) and showed a significant positive correlation with cranial length ($r_p = 0.285$, $p < 0.05$) and posterior basicranial length ($r_p = 0.280$, $p < 0.05$) with increments of 1.91 mm and 2.34 mm, respectively. The increases in cranial/posterior basicranial lengths are attributed to the development of the nuchal crest or tubercles, where the nuchal muscles are inserted. Owing to the same mechanical stress to retain the head posture, especially in males that have a longer projecting face and developed canines, the sites on the mid-sagittal plane,

AB, and PB become thicker in males.

The thickness at the temporal line tends to increase with age, whereas the thickness near bregma tends to decrease. Subtraction of these thicknesses yields the relative thickness at the temporal line, and indicates a positive significant correlation with age in both males ($r_p = 0.501$, $p < 0.000$) and females ($r_p = 0.266$, $p < 0.05$) (Figure 2.11). This increase in thickness is in response to physical stress from the temporalis muscle. The location of temporal line also appears higher in the cranial vault with age in males. The thickening was also highlighted by the thinning of plain neurocranium (RB, LB).

Cranial thickness measurements revealed different age-related changes between the neurocranium and facial cranium in Japanese macaques. The thickness at the temporal line and Inion in the neurocranium tends to increase with advancing age, and is considered to be associated with the development of bones responding to physical stress from masticatory (temporalis) and postural muscles, respectively. In contrast, the thickness in the facial cranium showed significant decreases with age in adults. In humans, the area in the mid-face (especially in mid-cheek) has been demonstrated to be more prone to bone resorption with increasing age (Pessa et al., 1999; Pessa, 2000; Shaw and Kahn, 2007; Mendelson and Wong, 2012). It has been suggested that a lack of stress may contribute to bone loss in this area (Mendelson and Wong, 2012). Muscle function is considered to play an important role in the identification of size and shape of the facial bone (Faltin et al., 2003). The cross-sectional area and density of masseter muscles significantly decreases with age in humans (Newton et al., 1993; McComas, 1998). Although it has been suggested that both the masseter and temporalis muscles tend to decrease their activity (measured by sEMG—surface electromyography) with age in humans, masseter muscles displayed lower activation than temporalis muscles in all age periods (Cecílio et al., 2010). The combination of atrophy, loss of density, and lower activation in masseter muscles contributes to lower stress on the mid-

face. The zygomatic height (position of masseter muscle inserts) also tended to decrease from mid-adulthood to old age in both males and females (Minh et al., 2015). Therefore, the age-related decrease in thickness and zygomatic height in the facial areas of macaques may be associated with less stress from muscles, especially masseter muscles. However, further studies examining the effect of aging on the masticatory muscles are needed in macaques.

The results of this study revealed a decrease in cranial thickness from mid-adulthood to the oldest age group. Craniometric measurements generally tended to slightly increase with age in Japanese macaques (Minh et al., 2015), and this phenomenon has also been documented in humans (Goldstein, 1936; Garn et al., 1967; Nasjeletti and Kowalski, 1975; Israel, 1973a, 1977; Susanne, 1977; Ruff, 1980; Bartlett et al., 1992). From these facts it is supposed that the cranial cavity (endocranial volume) enlarge in the older macaques, which has been previously investigated in humans (Israel, 1973a; Lazenby, 1990).

Bone minerals are absorbed more than deposited in adult life, rendering the compact bone physically weak (Yamada, 1970). Age-related decreases in cortical thickness, diameter expansion (outer surface), and medullary cavity expansion (the endosteal bone loss) of the long bones have been recorded in both humans and macaques (Smith and Walker, 1964; Garn et al., 1967; Ericksen, 1976; Ruff and Hayes, 1982; Bowden et al., 1979; Kimura, 1994). In the present study, the cortical thickness at the midlength of the femur (including anterior, medial, and lateral sides) tended to decrease from mid-adulthood to very old age in Japanese macaques. The cortical thickness at the midlength of the femur tends to show a greater decrease in females than in males, especially in the anterior side. In addition, expansions in the postcranial skeletons of older individuals are thought to be a form of mechanical compensation in response to the endosteal bone loss (the medullary cavity expansion) associated with aging (Smith and Walker, 1964; Garn, 1967; Ruff and Hayes, 1982; Kimura, 1994; Riggs et al., 2004).

Age-related changes in thickness at the medial and lateral sides at the midlength of the femur were significant in both sexes, and showed a trend similar to the age-related changes in cranial thickness. Although a correlation was found between these two factors, not all relationships were statistically significant. Age-related changes in bones (including the cranium and postcranial skeletons) are complicated and may therefore exhibit some relations that are not correlated significantly, and may be produced by inter-individual variation or different age change patterns such as age-related monotonous decrease/increase and decrease following increase (Pomchote, 2015).

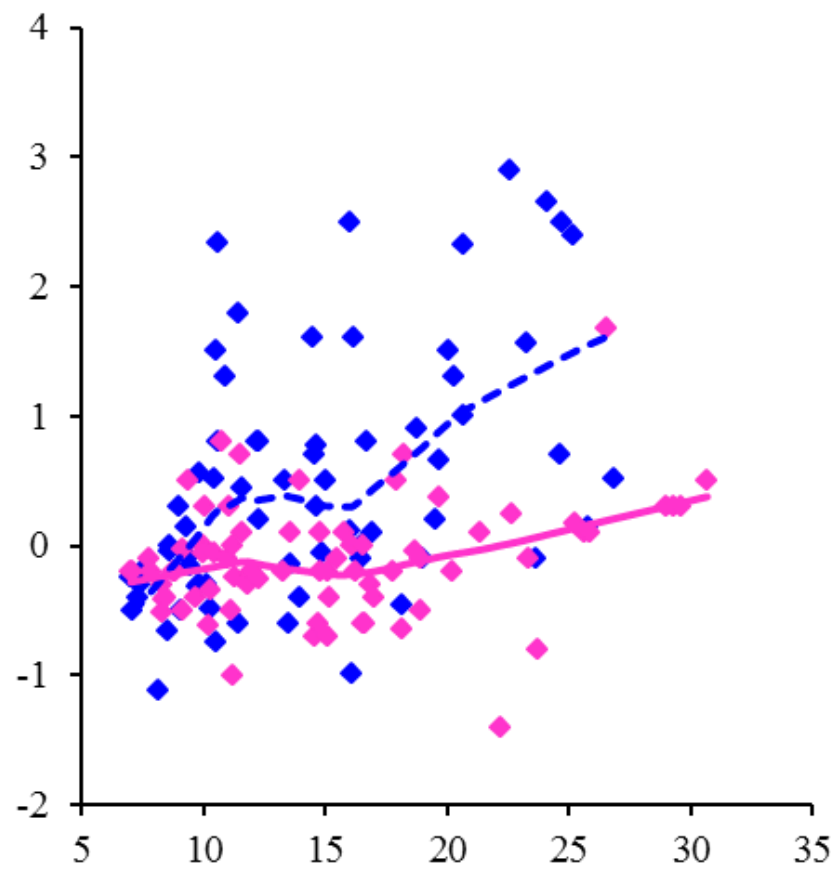


Figure 2.11 Relationship between subtraction of thickness at left temporal line minus left bregma (LTL-LB) and age with trend lines by Loess. Male: blue diamond and blue dash regression line; female: pink diamond and pink solid regression line.

Chapter 3

Age-related changes of sulcal imprints on the endocranium in the Japanese macaque (*Macaca fuscata*)

Introduction

The morphology of the neurocranium is thought to be determined by a combination of genetics, endocrinology, an interaction between hard and soft tissues (Bruner, 2008, 2010), and physical stress. The size of the cranial vault changes in response to pressure exerted by the brain, while it varies in shape due to tension generated by muscles and connective tissues such as the falx cerebri and tentorium cerebelli (Moss and Young, 1960). By contrast, the inferior surface of the endocranium is influenced by a complex interplay between the bone, meninges, and brain (Moss and Young, 1960; Richtsmeier et al., 2006), and further interacts with the cranial base and morphology of the face (Lieberman et al., 2000; McCarthy, 2001; Ross et al., 2004). Cortical brain structures, such as sulci and gyri, contact the endocranial wall through the layers of the dura mater and leave impressions on the bone.

Sulcal imprints on endocasts of fossilized skulls allow paleoneurologists to collect information about the evolution of the size, shape, and surface morphology of the brain (Falk, 2014). Endocasts can provide information about sulci, which demarcate the gyri and larger convolutions of the cerebral cortex. However, the definiteness of sulcal patterns on endocasts of nonhuman primates has been shown to depend primarily on species (brain size) and age of the individual (Radinsky, 1974; Holloway, 1974; Falk, 1978, 2014; Kobayashi et al., 2014; Bruner, 2015). It is difficult to recognize sulcal imprints on endocasts of primate species with larger brains such as great apes, humans, and hominid fossils, but easier to obtain a detailed

imprint of sulci in species with smaller brains (Le Gros Clark et al., 1936; Radinsky, 1972, 1974; Holloway, 1974; Falk, 1978, 2014; Kobayashi et al., 2014; Bruner, 2015). Additionally, it has been suggested that brain details on endocasts are reproduced better in juvenile than adult primates (Falk, 1978, 2014). This may be because juvenile brains are larger relative to endocranial volume. However, as of yet there are no studies of age-related changes in the imprints of the sulcus on the endocranium of primates.

Bone is an adaptive tissue that changes itself in response to physical stress through mineral deposition and resorption. In general, minerals are resorbed from the bone with advancing age (Yamada, 1970). In postcranial skeletons of both humans and nonhuman primates, the cortex of long bones (e.g., femur, tibia, humerus, and metacarpal) appears to become thinner with age. This is a result of resorption along the endosteum and subsequent expansion of the medullary cavity (Smith and Walker, 1964; Garn et al., 1967; Ericksen, 1976; Ruff and Hayes, 1982; Bowden et al., 1979; Kimura, 1994). In contrast, little is known about age-related changes in the internal table of bone of the skull. However, it has been suggested that the endocranial cavity enlarges during adulthood in humans (Israel, 1973a).

The internal table of the skull has been suggested to interact with both the meninges and brain (Moss and Young, 1960; Richtsmeier et al., 2006) and brain changes may therefore influence the surface morphology of the endocranium. Brain shrinkage with age has been documented by many studies in both humans (Mettler, 1956; David and Wright, 1977; Miller and Corsellis, 1977; Matsumae et al., 1996; Dekaban and Sadowsky, 1978; Svennerholm et al., 1997) and nonhuman primates (Kumakura, 1994; Shamy et al., 2006; Picq et al., 2012). Therefore, age-related brain shrinkage may be a potential factor affecting the internal surface of the endocranium.

Craniometry has demonstrated continuous skull expansion in Japanese macaques (*Macaca fuscata*) with age (Minh et al., 2015). Neurocranial thickness has been shown to

increase from young- to mid-adulthood (15-16 years), and decrease from mid-adulthood and old age (>20 years; Minh et al., chapter 2). Thus, endocranial volume may increase with age, especially from mid-adulthood to the older age, reducing the interaction between brain and cranium.

The present study aimed to investigate age-related changes in the imprint of the major sulci on the endocranium of Japanese macaques from the juvenile period to adulthood. The study also examined whether macaque endocranial volume showed age-related changes. Lastly, it discusses changes in sulcal imprints on the surface of endocasts.

Materials and Methods

Materials

The study was conducted on 25 cranial specimens from Japanese macaques of known age and comprised of 12 males and 13 females stored at the Primate Research Institute (PRI), Kyoto University, Japan. All subjects were reared in corral cages and fed monkey chow and sweet potatoes. Subject macaques were healthy and experimentally naive. Subjects died when they were healthy and without cranial bone abnormalities. Cranial vaults of all subjects were intact and unopened, and males and females were equally represented. Subjects were divided into five age groups: juvenile (2.0–3.9 years, 3 males and 3 females), adolescent (4.0–5.9 years, 2 males and 2 females), young adult (7.0–9.9 years, 3 males and 3 females), mid-adult (15.0–16.0 years, 2 males and 2 females), and elderly (>20.0 years, 2 males and 3 females).

Data acquisition

Computed tomography (CT) scans of each cranium was performed using the helical scanner (Asteion Premium 4 Editions; Toshiba Medical Systems, Japan) at the PRI, with a pixel size of 0.25 mm × 0.25 mm and an inter-slice interval of 0.5 mm. Slices were oriented parallel to the axial plane.

Virtual endocasts were produced from scanned crania by a combination of two- and three-dimensional semi-automated segmentation using Avizo 7.1 software package (Visualization Sciences Group) (Figure 3.1). On each coronal CT slice, the endocast cavity was delineated manually by selecting a portion of surrounding bone. This selection was spread to adjacent slices using a snakes algorithm without extrapolation (a filter which fits the contour of a selection to a region of high contrast) (Bienvenu et al., 2011). Gray values corresponding to bone were removed from the selection by threshold segmentation on the complete image stack. Corrections were made manually where the endocast did not fit the

inner table of bone, such as at the cribriform plate or foramina. Finally, a three dimensional surface of the endocast was generated using computed segmented data.

Evaluating sulcal imprints on virtual endocasts

Imprints of the sulcus on the virtual endocasts were examined using the Avizo 7.1 software. The virtual endocast of each subject was assessed without knowledge of sex or age by a single observer (NVM). Imprints of the main sulci were well represented on the surface of the endocasts when compared to their corresponding brains (Figure 3.2). Thus, seven sulci were selected for assessment in this study and were defined on the surface of the virtual endocast: the principal (pr), arcuate (ar), sylvian (sy), superior temporal (st), central (ce), intraparietal (ip), and lunate sulci (lu) (Figure 3.3). Definiteness of each sulcal imprint was evaluated with an imprint score: 0, 1, 2, 3 or 4 indicating absent, slight, moderate, marked and very clear, respectively (Table 3.1; Figure 3.4). All endocasts were evaluated twice by the same observer. There were no significant differences between evaluations and thus only scores from the second evaluation were used for analysis. The total average scores of all sulcal imprints were obtained for each specimen.

In order to calculate endocranial volume, all holes such as those at the cribriform plate and foramina, had been manually closed at the inner table of bone during the endocast processing. Endocranial volume in each individual was measured using Avizo 7.1.

Table 3.1 Sulcal imprint scoring

Grade	Definition
0	Sulcal imprint is absent on virtual endocast.
1	Sulcal imprint is slight on virtual endocast. Definiteness of imprint is less than 1/3 length of sulcus or definiteness of imprint is less than 2/3 length of sulcus, but imprint of sulcus can be only observed slightly on virtual endocast.
2	Sulcal imprint is moderate on virtual endocast. Definiteness of imprint is from 1/3 to less than 2/3 length of sulcus.
3	Sulcal imprint is marked on virtual endocast. Definiteness of imprint is from 2/3 to less than entire length of sulcus.
4	Sulcal imprint is very clear on virtual endocast. Definiteness of imprint can be observed on entire length of sulcus.

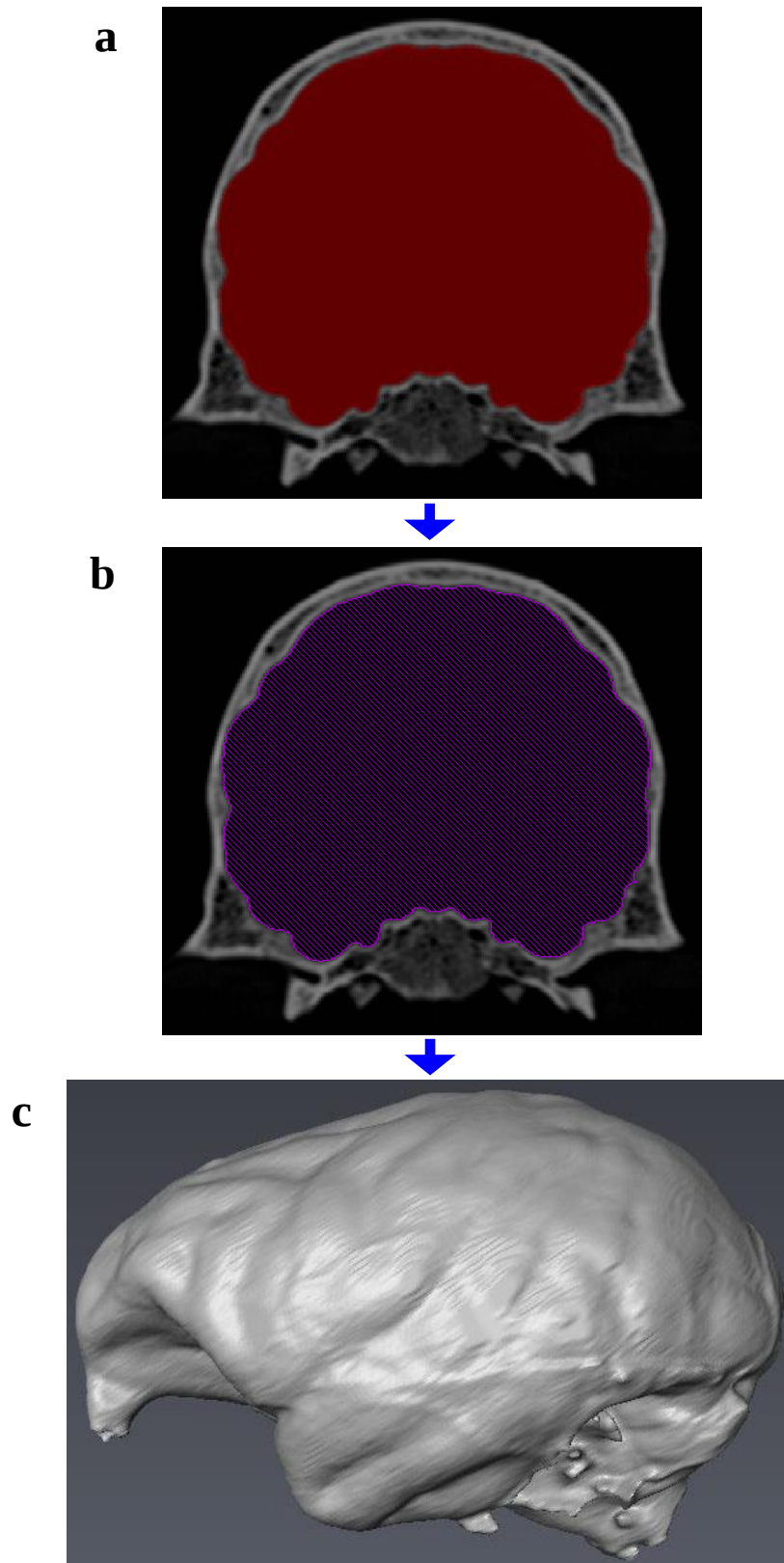


Figure 3.1 Protocol used to extract virtual endocasts. (a) Selection of the endocranial cavity through manual segmentation (coronal slice), (b) removal of the bone by 3D threshold segmentation (coronal slice), and (c) the surface of the endocast computed from the segmentation data (lateral view).

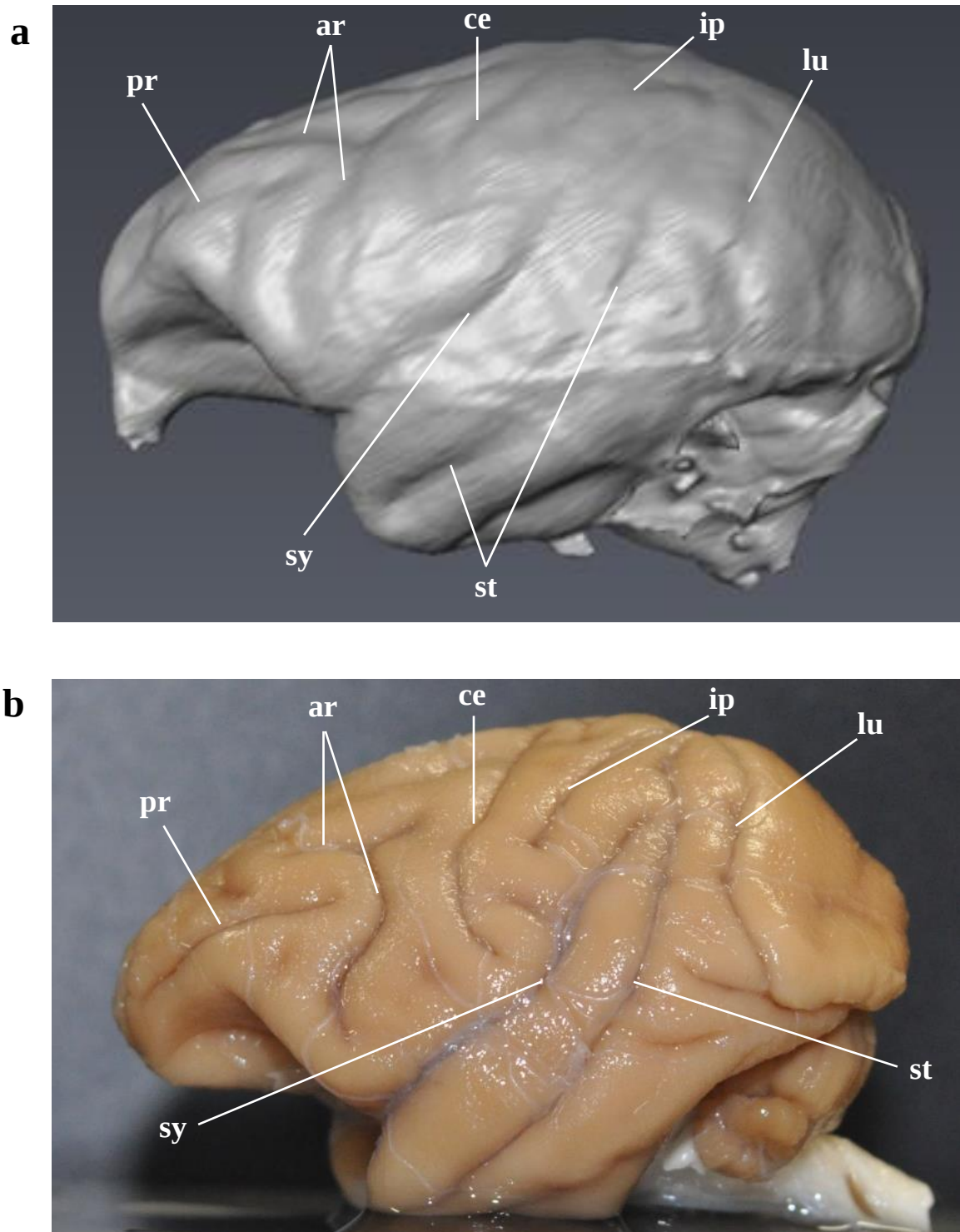


Figure 3.2 Impressions of major cerebral sulci on the surface of the virtual endocast, compared with the real brain. (a) A virtual endocast of Japanese macaques; (b) real brain of an individual Japanese macaque; pr principal, ar arcuate, sy sylvian, st superior temporal, ce central, ip intraparietal, and lu lunate sulci.

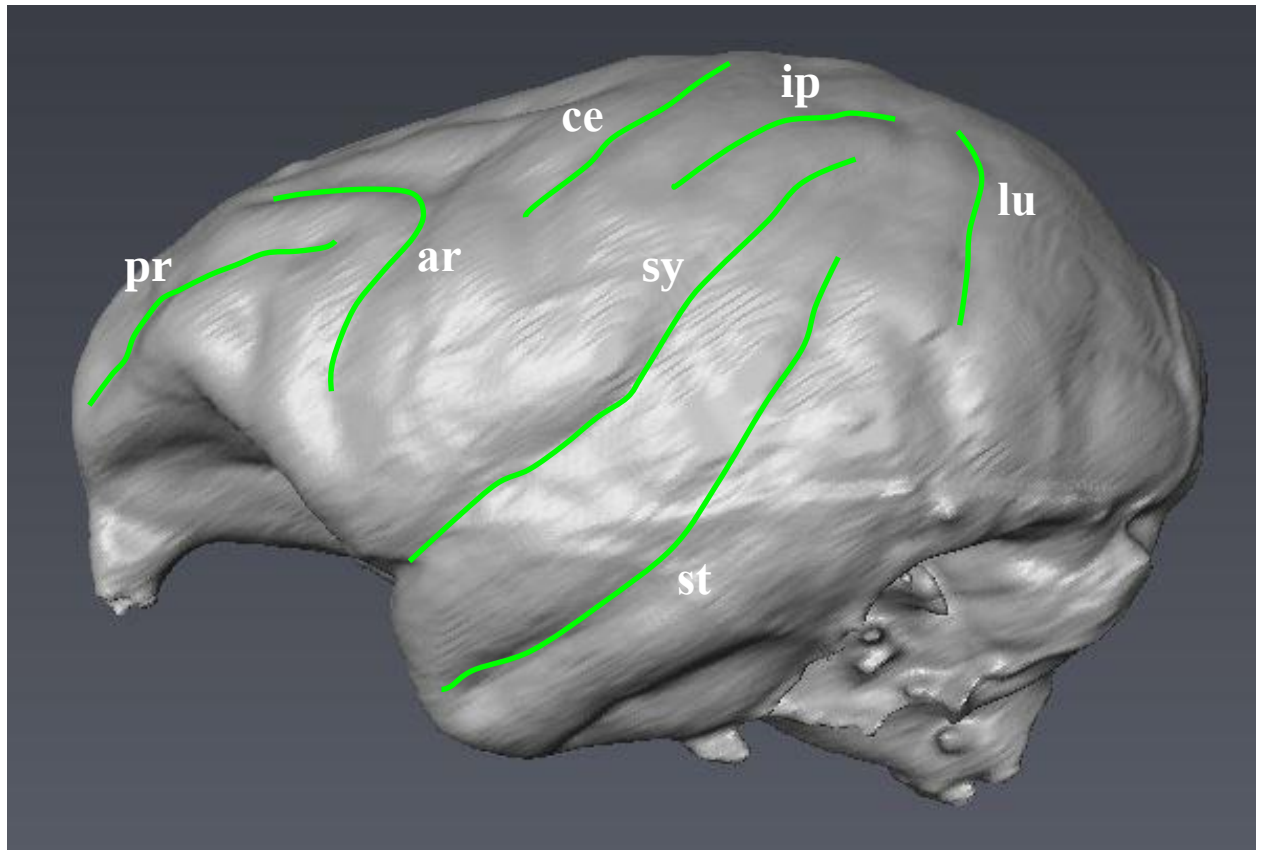


Figure 3.3 Main sulci defined on the surface of the virtual endocast of a Japanese macaque used in this study: sulci abbreviated as in Figure 3.2.

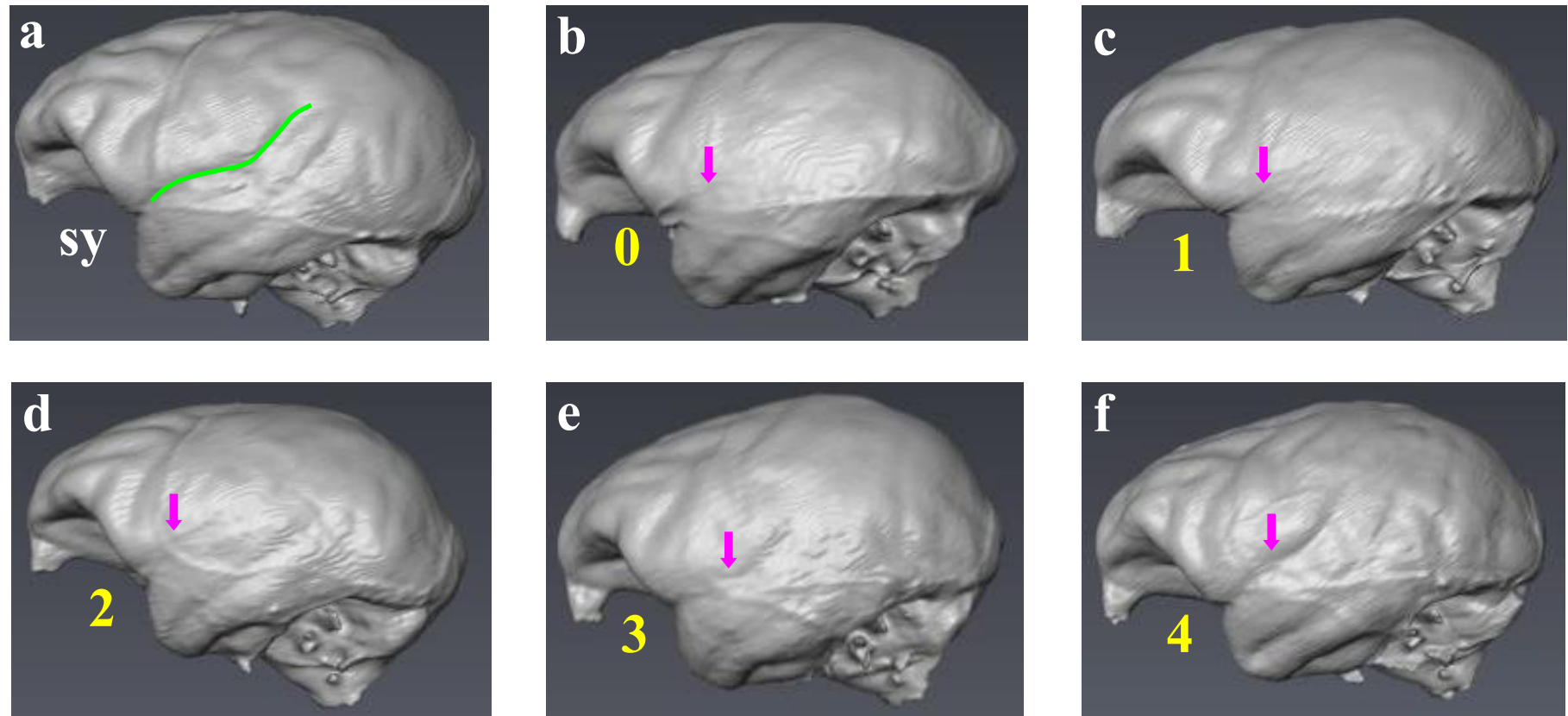


Figure 3.4 The scoring method for sulcal imprints on virtual endocasts of Japanese macaques: example evaluation at Sylvian sulcus. The scores (numbers in yellow) are 0, 1, 2, 3 or 4 for absent, slight, moderate, marked and very clear, respectively. (a–f) Examples of the endocasts evaluated.

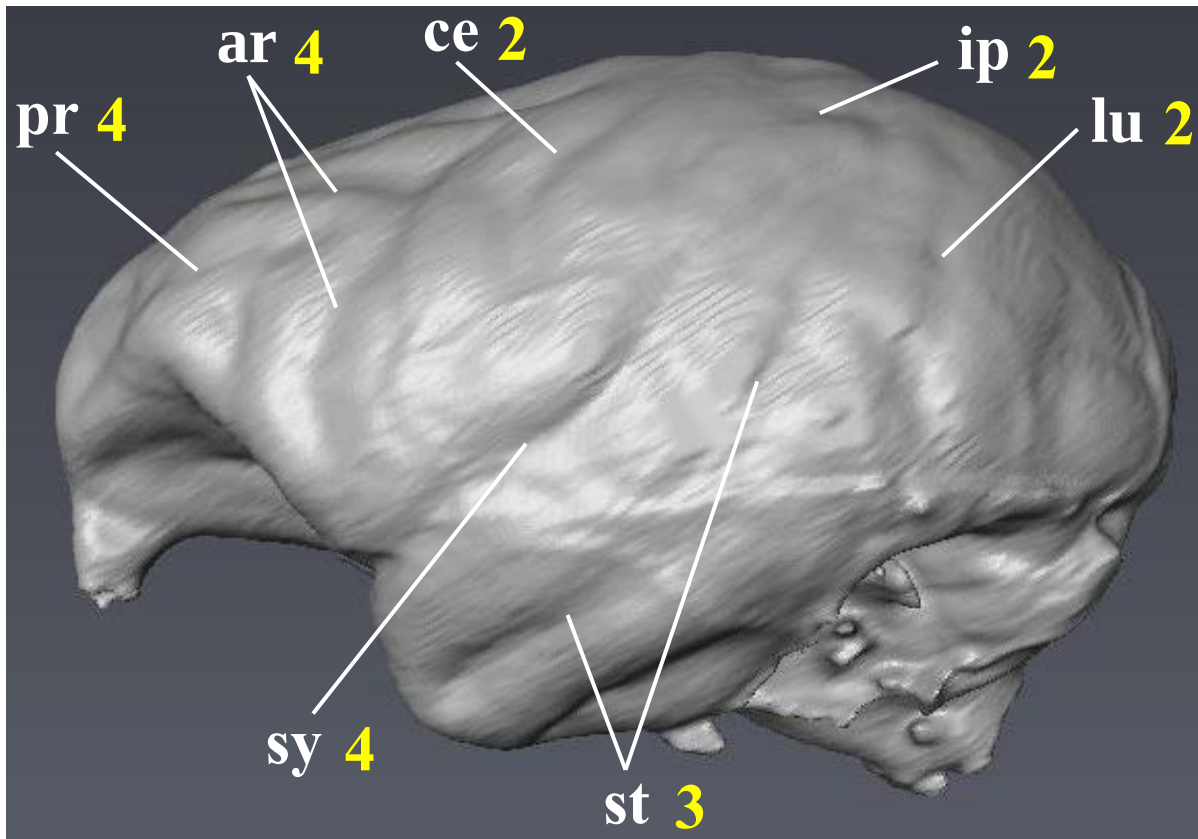


Figure 3.5 The scoring method for the imprints of 7 sulci on the virtual endocast of each individual abbreviation of sulci as Figure 3.2. The scores for each sulcus are indicated by number.

Statistical analysis

All statistical analyses were performed using functions of Excel (Microsoft Co. Ltd.) or Past version 2.17 (Hammer et al., 2001). An analysis of covariance (ANCOVA) was used to determine sex differences in sulcal imprints and endocranial volumes. Kruskal-Wallis tests were used to determine differences in sulcus imprint scores between age groups. An analysis of variance (ANOVA) was used to determine differences in endocranial volume between age groups.

Results

Age-related changes in sulcal imprints differed between sulci. The average scores of definiteness of sulcal imprints did not significantly differ between males and females (ANCOVA, $p > 0.05$). Average definiteness scores were high for all specimens, regardless of age, in the arcuate, superior temporal, and principal sulci: 3.16 ± 0.85 (sd), 2.76 ± 0.88 , and 2.48 ± 0.96 , respectively (Figure 3.6). On the other hand, scores for lunate and intraparietal sulci were significantly lower than them: 1.28 ± 0.61 and 1.28 ± 0.61 , respectively (Figure 3.6). The average definiteness scores in young (7–10 years) and old adulthood (15–16 years) in the arcuate, superior temporal, and principal sulci were 3.38 ± 0.52 , 2.75 ± 0.57 , and 2.38 ± 0.70 , respectively, while those in lunate and intraparietal sulci were 1.25 ± 0.42 and 1.25 ± 0.52 , respectively.

Sulcal imprints showed significant developmental and age-related changes. The average definiteness scores of each age group are listed in Table 3.2. The average definiteness score in juvenile subjects (2–4 years) was 2.74 ± 0.38 , which decreased slightly to 2.29 ± 0.45 in adolescence (4–6 years), and was maintained through mid-adulthood (15–16 years). However, the total average scores of the sulcus imprints in elderly subjects (>20 years) were significantly lower than those in other age groups: 1.14 ± 0.23 (Figure 3.7, 3.10).

Developmental and age-related changes in definiteness differed between sulci (Figure 3.8). Sulcal definiteness scores of the sylvian, arcuate, superior temporal, intraparietal, and central sulci showed slight decreases between the juvenile (2–4 years) to mid-adulthood (15–16 years), and then dramatically decreased in old age (>20 years). However, the scores of principal and lunate sulci gradually decreased from juvenile to old age.

Average endocranial volumes differed between males and females. Males had slightly larger endocranial volumes than females when averaged across all age groups (male mean =

106,200 $\pm$ 6,830 mm³ (sd); female mean = 102,200 $\pm$ 8,490 mm³; ANCOVA, $p < 0.05$). However, average endocranial volumes from young adulthood (7–10 years) to mid-adulthood (15–16 years) were not significantly different between males and females (male mean = 106,600 $\pm$ 5,040 mm³; female mean = 102,700 $\pm$ 4,540 mm³; ANCOVA, $p > 0.05$).

I also examined age-related changes in endocranial volumes. For age-related change analysis, we pooled sexes. Endocranial volume increased by 9.8% from the juvenile period to adolescence (Table 3.3, Figure 3.9). Endocranial volume remained unchanged from adolescence to mid-adulthood. The volume significantly increased again, by 8.8%, from mid-adulthood to old age.

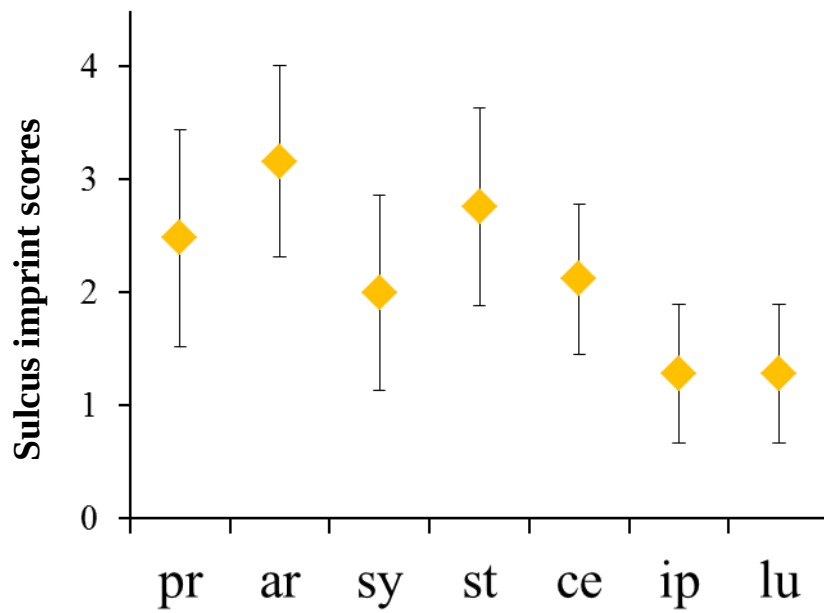


Figure 3.6 Patterns of the imprints of sulci on the surface of the endocasts in Japanese macaques; sulci abbreviated as in Figure 3.2. Vertical bars indicate means $\pm$ SD

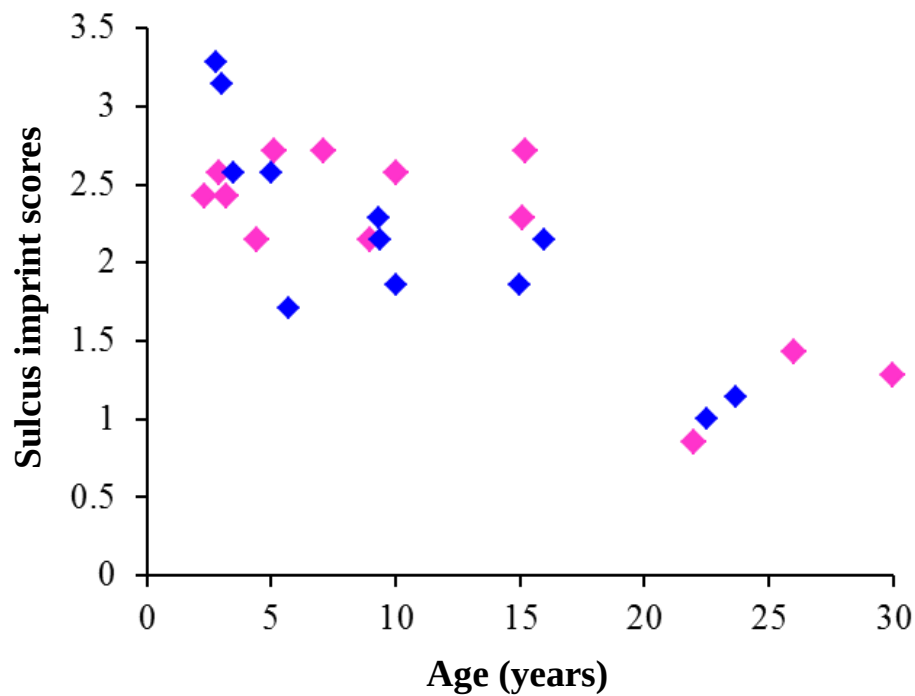


Figure 3.7 Scatter plots showing age-related changes of sulci on the surface of the endocast in all crania. Male, blue diamond; female, pink diamond.

Table 3.2 Age-related changes in the imprints of sulci on the surface of the endocasts in Japanese macaques

Age group (years)	Mean	SD	Range	Age difference
2–4	2.74 (n = 6)	0.38	2.43–3.29	d ^{**}
4–6	2.29 (n = 4)	0.45	1.71–2.71	g [*]
7–10	2.31 (n = 6)	0.35	1.86–2.71	k ^{**}
15–16	2.25 (n = 4)	0.36	1.86–2.71	l [*]
>20	1.14 (n = 5)	0.23	1.14–1.43	d ^{**} , g [*] , k ^{**} , l [*]

^{a-l} Statistically significant differences between age groups (Kruskal-Wallis) at the * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ level for ^a 2–4 vs. 4–6 years, ^b 2–4 vs. 7–10 years, ^c 2–4 vs. 15–16 years, ^d 2–4 vs. >20 years, ^e 4–6 vs. 7–10 years, ^f 4–6 vs. 15–16 years, ^g 4–6 vs >20 years, ^h 7–10 vs. 15–16 years, ^k 7–10 vs. >20 years, ^l 15–16 vs. >20 years.

Table 3.3 Age-related changes in the endocranial volumes of Japanese macaques

Age group (years)	Mean (mm ³)	SD	Range (mm ³)	Age difference
2–4	94,500 (n = 6)	5,600	87,300–100,200	a [*] , b [*] , c [*] , d ^{***}
4–6	103,800 (n = 4)	3,800	99,900–107,800	a [*] , g [*]
7–10	104,400 (n = 6)	4,000	99,400–110,600	b [*] , k [*]
15–16	105,100 (n = 4)	6,800	96,400–113,100	c [*] , l [*]
>20	114,400 (n = 5)	2,800	110,400–118,200	d ^{***} , g [*] , k [*] , l [*]

^{a-l} Statistically significant differences between age groups (ANOVA) at the * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ level for ^a 2–4 vs. 4–6 years, ^b 2–4 vs. 7–10 years, ^c 2–4 vs. 15–16 years, ^d 2–4 vs. >20 years, ^e 4–6 vs. 7–10 years, ^f 4–6 vs. 15–16 years, ^g 4–6 vs >20 years, ^h 7–10 vs. 15–16 years, ^k 7–10 vs. >20 years, ^l 15–16 vs. >20 years.

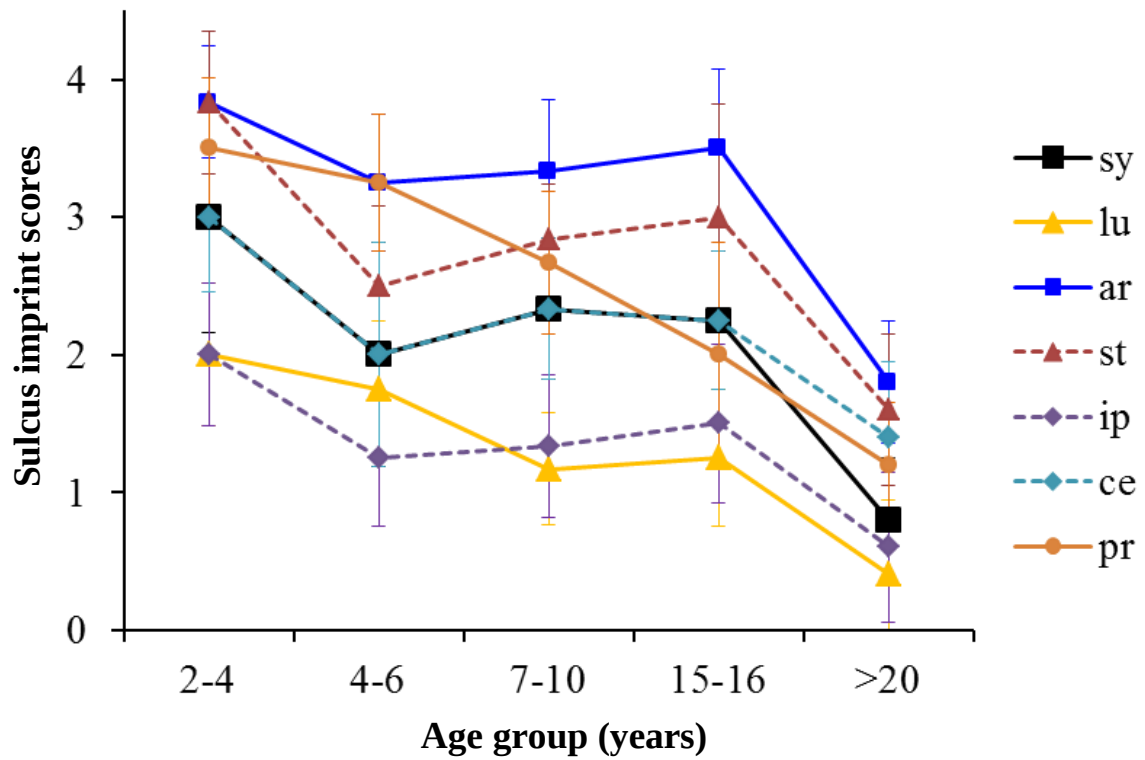


Figure 3.8 Age-related changes in the imprints of sulci on the surface of the endocranial cast; sulci abbreviated as in Figure 3.2.

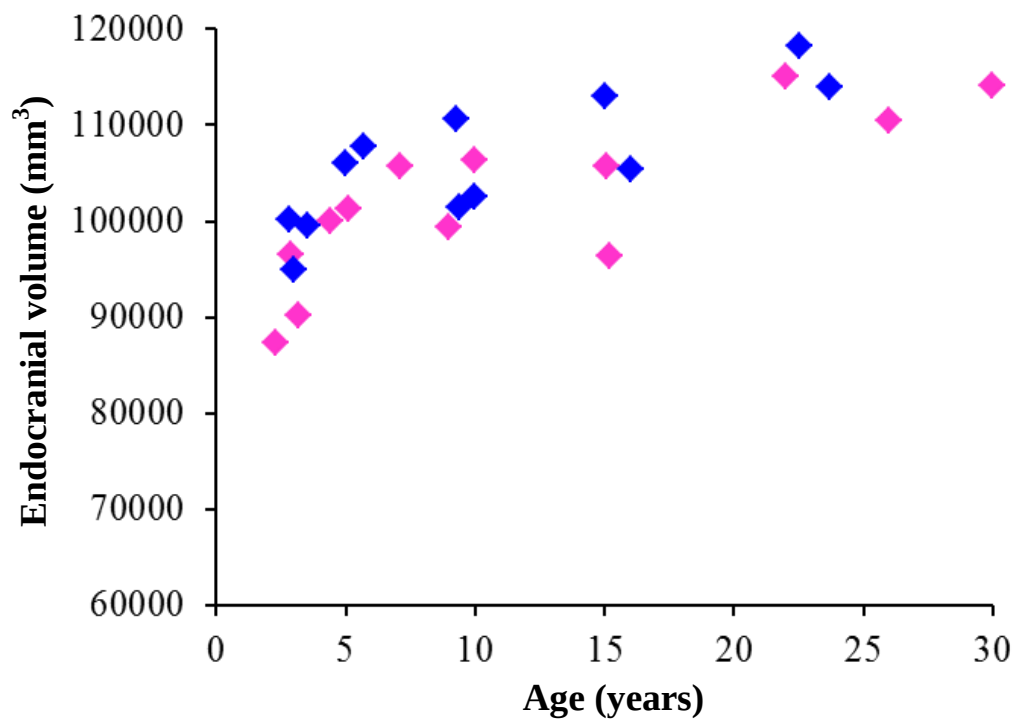
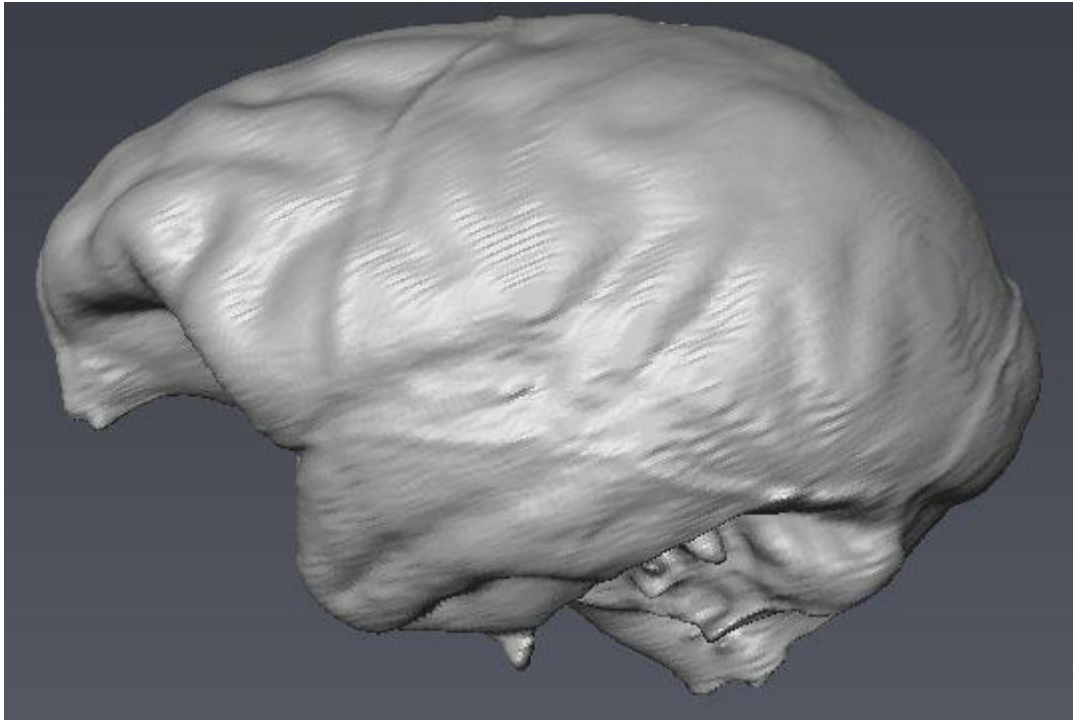


Figure 3.9 Endocranial volume in relation to age. Male, blue diamond; female, pink diamond.

a



b

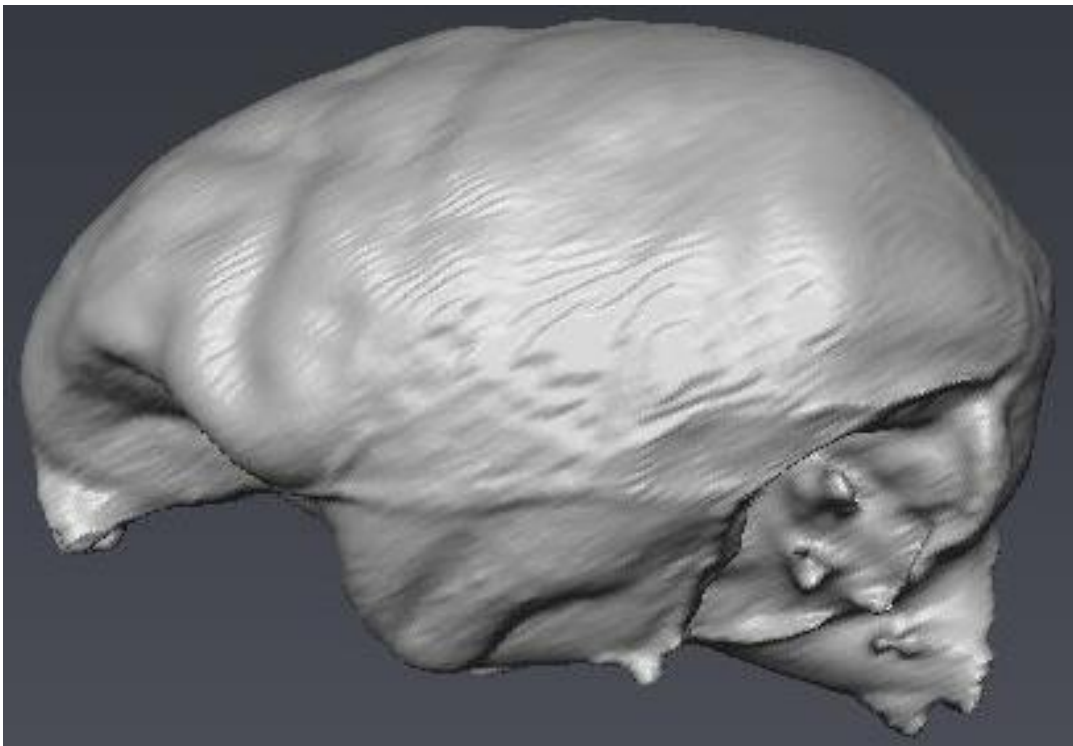


Figure 3.10 The surface of the endocranium. (a) the surface of the endocranium showing marked imprints of the sulci in juvenile (2.9 years); (b) the imprints of the sulci on the surface of the endocranium showing poorly definite in old adult (23.7 years).

Discussion

Endocasts of Japanese macaques, which were generated using CT data, show marked imprint patterns of the major cerebral sulci (Figure 3.2). This corresponds with previous suggestions that primate species with smaller brains produce clearer endocasts than related species with larger brains (Le Gros Clark et al., 1936; Radinsky, 1972, 1974; Holloway, 1974; Falk, 2014; Kobayashi et al., 2014; Bruner, 2015). Impressions of the cerebral sulci have been represented on the surface of endocasts of long-tailed macaques (*Macaca fascicularis*; Kobayashi et al., 2014) and many species of New World and Old World monkeys (Radinsky 1974; Falk, 1978). In contrast, great apes, humans, and fossil hominids endocasts show poorly defined sulcal patterns (Le Gros Clark et al., 1936; Radinsky, 1972; Holloway, 1974; Kobayashi et al., 2014). Falk (1978) attributed the difference in the definiteness of sulcal patterns to difference in the thickness of protective tissue and cerebrospinal fluid, though further studies are needed to support this claim.

Results of the present study show that the definiteness of sulcal imprints differs between sulci. Arcuate, superior temporal, and principal sulci are well defined, whereas lunate and intraparietal sulci are poorly represented. Holloway (1974) suggested that the poorer representation of brain features in great apes and human endocasts is owed to the presence of thicker layers of protective tissue between the brain and skull such as the pia mater, arachnoid tissue, dura mater, and cerebrospinal fluid. It is also suggested that the endocast sulcal patterns would be produced by the pressure of brain (to support brain) on the inner-surface of cranium. Therefore, the differences between sulcal imprints in our study may be related to local differences in the thickness of protective tissue between the brain and internal surface of the skull and/or the pressure of brain on the inner surface of cranium.

Future studies will examine the thickness of such protective layers and pressure applied to various regions of the skull.

Developmental and age-related change in individual sulcal imprints has not been previously studied. It has been shown that sulcal imprints in nonhuman primates such as macaques, langurs, and baboons are better defined in juveniles than in adults (Falk, 1978, 2014). The main finding of the present study is that sulcal imprints show significant age-related changes in Japanese macaques from juvenile to elderly. Sulcal imprints showed a slight decrease in definiteness from the juvenile period (2–4 years) to adolescence (4–6 years), and then remained unchanged until mid-adulthood (15–16 years). The definiteness of the sulcal imprints significantly decreased from mid-adulthood to old age (>20 years).

The definiteness age-related changes differed between sulci. The definiteness of sulcal imprints of five sulci out of the 7 studied decreased slightly from juvenile to mid-adulthood and then dramatically decreased in old age. On the other hand, the principal and lunate sulci showed gradual decrease in definiteness from juvenile to old age.

The decrease in the definiteness of the sulcal imprints between the younger age groups (<20 years) and old age group (>20 years) may be associated both with brain shrinkage (Falk, 1978) and expansion of endocranium (chapter 2), both of which are likely to reduce contact of the brain (pressure) with the inner surface of skull (Falk, 1978). Various studies have shown that both the weight and volume of the brain decrease with age in humans (Mettler, 1956; David and Wright, 1977; Miller and Corsellis, 1977; Matsumae et al., 1996; Dekaban and Sadowsky, 1978; Svennerholm et al., 1997), especially from 45–50 years onwards (Mettler, 1956; Dekaban and Sadowsky 1978). For example, brain volume decreased by 6.7% in men and 7.9% in women between 30–39 years and 80–89 years of age (Mettler, 1956), or brain weight declined by 7.3% in men and 7.4% in women between 20–30 years and 70–80 years of age (Dekaban and Sadowsky, 1978). Brain shrinkage with aging has

also been found in nonhuman primates, including Japanese macaques, rhesus macaques (*Macaca mulatta*), and mouse lemurs (*Microcebus murinus*) (Kumakura, 1994; Shamy et al., 2006; Picq et al., 2012). The brain weight of rhesus macaques decreased by 8.1% from young adulthood (9–12 years) to late adulthood (24–29 years) (Shamy et al., 2006), while that of Japanese macaques decreased by 9.3% from 20 years to 30 years of age (Kumakura, 1994). The brain shrinkage of Japanese macaques is thought to start around 20 years of age (Kumakura, 1994). The present result suggests that the definiteness of sulcal imprints may start decreasing around 20 years of age in Japanese macaques.

The endocranial volume in Japanese macaques shows increases with age from mid-adulthood to the older. Endocranial volume undergoes a 9.8% increase between the juvenile and adolescent periods, remains unchanged from the adolescent period to mid-adulthood (15–16 years), and then undergoes an 8.8% increase in old age (>20 years). In humans the endocranium expands with age, and has been shown to increase by approximately 3–5% in women between 35 and 55 years of age (Israel, 1973a). It has therefore been suggested that the endocranial cavity in humans may continue to grow throughout adult life (Israel, 1973a; Lazenby, 1990).

Both brain shrinkage and increasing endocranial volume reduce pressure between the brain and internal bone table of the skull, resulting in a less definite impression of the sulci in older Japanese macaques. The sulcal part of endocranium (bone) is absorbed, making the endocranium less rugged. It is also possible that not only mechanical loading but also the whole-body aging and estrogen (testosterone) depletion render the inner surface absorption (bone loss) along with the thinning of the cortex of long bones (postcranium).

General Discussion and Conclusion

General discussion

Physiognomic change in the craniofacial skeleton with age is great in both humans and non-human primates. The morphology of the craniofacial skeleton is considered to be a dynamic process involving changes in size, shape, and structure with advancing age. In humans, although age-related changes in the skull have been studied (e.g., Albert et al., 2007), there is still a controversy with respect to age-related changes in the outer surface morphology and cortical thickness of the skull in adults.

With sexual maturation, cranial growth in humans has been considered to cease or be insignificant (Tallgren, 1974). However, it has been recognized that cranial growth continues at a slow rate throughout adulthood (Hrdlička, 1936; Israel, 1977; Ruff, 1980; Forsberg et al., 1991). Some studies (Israel, 1973a; Adeloje et al., 1975) suggested a small increase in the thickness of the skull cortex during adult life. However, others found no correlation between age and cranial thickness during adult life (Tallgren, 1974; Lynnerup, 2001). It has been suggested that the endocranial cavity tends to enlarge during adult life in humans (Israel, 1973a).

Aging is a deterioration process that occurs in most animals after reproductive maturity. Age-related changes in bones indicate smaller inter-individual variation than other organs, and therefore, bones are considered to be good representative of physical aging. Age-related changes in postcranial bones have been extensively studied on aspects of morphology, density (osteoporosis), and osteoarthritis; because postcranial bones are of importance for positional behavior and quality of life in the elderly.

Bones are a dynamic tissue undergoing continuous remodeling through the absorption and deposition of bone minerals. With advancing age, the long bones, e.g. rib, femur, tibia,

humerus, and metacarpals, represent a general remodeling, which increases bone cross-sectional diameter through the continuous periosteal apposition and endosteal resorption, however, their cortices become thinner both in humans (Smith and Walker, 1964; Epker and Frost, 1965; Garn et al., 1967; Ericksen, 1976; Pfeiffer, 1980; Ruff and Hayes, 1982; Riggs et al., 2004) and non-human primates (macaques; Bowden et al., 1979; Kimura, 1994; chimpanzees; Morbeck et al., 2002). Factors that influence bone turnover are physical stress, hormones, and aging, which are inter-related with each other. Age-related changes in the long bones are influenced by these factors. Therefore, by the remodeling process, the long bones can be maintained to adapt and to fulfil their function (against bending stress) with aging, though the bones become weak against shear stress.

Physical stress, hormones, and whole body aging are the factors which are considered to play the important roles in the regulation of bone remodeling and therefore, both of the long bones and skull might be under the influences from these factors. Although bone structure in both the long bones and skull is similar, function and mechanical loading between the long bones and skull may differ. The main functions of the long bones are to support body weight and to bear physical stress from body weight as well as contraction of muscles. With advancing age, the long bones show generalized remodeling, which increases bone cross-sectional diameter but decreases cortical thickness.

The question, therefore, arises whether age-related changes of the skull, including 3 aspects, outer surface morphology (morphometry), cortical thickness, and inner table bone surface, are similar to those in postcranial skeletons. The primary functions of the skull are to protect the brain and nasopharyngeal organs and to receive physical stress from masticatory and postural muscles. Also the position of skull is held and moved by nuchal muscles, sternocleidomastoideus, and digastric muscles, and so on, and is also affected by inner pressure from the brain activity. Although age-related changes in the skull have been studied in

humans (e.g., Albert et al., 2007), there is still a controversy with respect to age related changes of the outer surface morphology and cortical thickness of the skull in adult humans. Potential reasons for these contradictory conclusions might arise from sampling bias, small sample size, the confounding effects of pathology, methodologies in data collection, and the statistical methods adopted (Roos, 1998; Lynnerup, 2001; Albert et al., 2007).

Macaques are considered to be a good animal model for human postcranial skeletal changes with advancing age, bone loss and osteoarthritis aggravation as in humans (Bowden et al., 1979; Kimura, 1994; Cerroni et al., 2002; Colman and Binkley, 2002). It is, thusly, supposed that macaques may show age-related changes in the skull clearly. In the present study, I controlled origins of subjects, physical activity (corral cages), and nutrition (monkey chow and potato supplements) during their life span. Taking these conditions together, age-related changes in the skulls of Japanese macaques are discussed in this study.

The whole aspects of age-related changes in the skull of Japanese macaques (*Macaca fuscata*) were studied, comprising outer surface morphology (morphometry), cortical thickness, and inner table bone surface using morphometric measures, computed tomography scans, and virtual endocasts generated from CT scans, respectively. The influential factors, such as physical stress (muscular forces), hormones (estrogen depletion), brain, and aging as a whole, on all aspects of age-related changes in the skull were considered. I discussed sex differences and age-related changes of the skull of macaques in comparison with that of humans.

In chapter 1, I investigated age-related skull morphometric (outer surface) changes of Japanese macaques. Because long bones tend to increase their diameters (cross-sectional diameters), and thus, I examined whether similar age-related changes occur in outer surface morphometrics of the neurocranium of nonhuman primates. The major findings of the present study indicate that the neuro-cranial dimensions increased from young adulthood to mid-

adulthood, and then, remained or decreased; in total there are slight decreases in females as in males. Continuous increase of craniometric measurements in adulthood was also reported in humans (Goldstein, 1936; Garn et al., 1967; Nasjeleti and Kowalski, 1975; Israel, 1973a, 1977; Susanne, 1977; Ruff, 1980; Bartlett et al., 1992), however, the magnitude of age related changes in the dimensions of the skull is much greater in macaques than in humans. The differences between humans and macaques may be associated with the differences of the mechanical stress from mastication on which larger masticatory forces are applied to the skull and face in macaques (Dechow and Carlson, 1990). Furthermore, facial and mandibular dimensions tended to show greater and significant changes with age than neurocranial dimensions in both sexes. Furthermore, as for proportional changes in the face, facial height increased more than bizygomatic breadth in both sexes, making the face relatively longer with age in macaques. Also rather great change was observed in biorbital breadth.

Age at the peak of skull size in many measurements tended to be attained later in females than in males. This sex difference might relate to reproductive burden in Japanese macaques, which is different between the two sexes. Females keep reproducing until the cessation of reproduction in older age (individual variation is wide, but around 18–25 years; Takahata et al., 1995), and therefore, adult females may spend more energy for reproductive costs in pregnancy and the lactation period, which may lead to the slowing of cranial bone remodeling or growth. On the other hand, after the peak, many craniometric measurements are maintained from mid-adulthood to very old age in both sexes. Perhaps sex hormone deficiency might be a potential factor to stop or constrain the increasing process of the skull from mid-adulthood to very old age in macaques.

Sex differences in cranial dimensions in relation to age in macaques are larger than those in humans (Ruff, 1980). Sex differences in macaques may be associated with the stress of mastication and the holding and moving of the head (postural musculature). Larger

masticatory forces are applied to the face in male macaques (Dechow and Carlson, 1990). On the other hand, the increase in cranial and posterior basicranial lengths and the large decrease in intertemporal distance in male macaques may be associated with the development of the nuchal crest or insertion processes (tubercles) on which nuchal muscles attach to keep and move developed (projecting) faces and canines. On the other hand, in the facial cranium, mid-face region showed a greater decrease, especially bizygomatic breadth and zygomatic height.

In general, neurocranium dimensions tended to expand with age slightly in males and to shrink slightly in females, which have also been documented in the cranium of humans (e.g. Israel, 1973a). However, the facial cranium and mandible dimensions increased considerably, which is similar to age-related changes of long bones in both humans and nonhuman primates (Smith and Walker, 1964; Garn et al., 1967; Ericksen, 1976; Ruff and Hayes, 1982; Bowden et al., 1979; Kimura, 1994). On the other hand, thinning of the cortex of long bones with advancing age has been documented for various bones in both humans and nonhuman primates (Smith and Walker, 1964; Garn et al., 1967; Pfeiffer, 1980; Ruff and Hayes, 1982; Bowden et al., 1979; Kimura, 1994; Morbeck et al., 2002). Therefore, craniofacial thickness is supposed to decrease with advancing age in macaques.

Age-related changes in the craniofacial thickness were investigated on Japanese macaques using computed tomography scans. The main findings of the present study are that the cranial thickness at many sites in the neurocranium showed significant age-related changes, with an increase from young adulthood to mid-adulthood followed by a decrease to the oldest age group, in total, females decreased from young adulthood while males increased or decreased. The previous study on human skull thickness suggested that there was a slight decrease in thickness over 50–60 years of age in women, but not in men (Lynnerup, 2001); and in Japanese women but not in men (Torimitsu et al., 2014). These studies suggested that

cranial thickness might be greatly affected by lower bone metabolism, which is essentially caused by postmenopausal bone loss in women (Lynnerup, 2001; Torimitsu et al., 2014). In the present study, the cranial thickness at many sites in macaques showed a significant decrease from mid-adulthood to the oldest age group in both sexes. It is especially true in females, revealing greater decreases in thickness than males. This sex difference may be associated postmenopausal estrogen depletion in female macaques on the developed (projecting) face in males. Like craniometric age changes (chapter 1), the peak of the cranial thickness was attained later in females at many sites in the neurocranium than in males.

Facial cranial thickness significantly continuously decreased from young adulthood to old age. The thickness at the two facial sites (MFZ and MLZ) showed an exceptionally distinctive pattern of decreasing from young adulthood to the oldest age group in both sexes. This coincides with the findings in craniometry, that is, zygomatic height, biorbital breadth decrease greatly.

Sex difference was found in age-related changes in cranial thickness. The thickness at sites on the mid-sagittal plane significantly increased in males from young adulthood to mid-adulthood, though they did not show any significant change from mid-adulthood to very old age. This was especially evident in the thickness at the inion, which increased significantly with age in males. In contrast, the thickness at these sites in females did not increase significantly from young adulthood to mid-adulthood, but did show a significant decrease from mid-adulthood to the oldest age group. This sex difference may be associated with the differences in the sizes of the projecting face and canines between males and females, which leads to the development of masticatory and postural muscles and stimulates the increase in the cranial thickness at these sites in males.

The thickness at the temporal line increased significantly from young adulthood to mid-adulthood (19–24 years in both males and females) with a greater increase in males than

in females, and they remained stable from mid-adulthood to the oldest age group in both sexes. The greater increase in the thickness at the temporal line in males may be the result of development or stress on the temporal line on which greater forces from the temporalis muscle are applied. The thickness at the left and right of the bregma, which represents thickness of the cranial plain cranial vault, showed the same age-related trend between the two sexes: a slight increase from young adulthood to mid-adulthood followed by a significant decrease from mid-adulthood to the oldest age group.

From the craniometrics and cortical thickness age-changes, the endocranial volume is supposed to increase with age. As the definiteness of sulcal pattern depends on the brain size and endo-cranial volume, it is supposed that the definiteness of sulcal pattern would decrease with age in macaques. It has been suggested that the endocranial cavity enlarges during adulthood in humans (Israel, 1973a). The internal surface of the skull is influenced by a complex interaction between bone, meninx, and the brain (Moss and Young, 1960; Richtsmeier et al., 2006), and therefore, the changes of the brain volume and/or endocranial cavity may influence on the surface morphology of the endocranium.

Age-related changes in the definiteness of sulcal imprints on the endocranium and in the endocranial volume were investigated (Chapter 3) in Japanese macaques from juvenile to old age, using virtual endocasts generated by computed tomography scans. The main findings of the present study indicated that the endocranial volume in Japanese macaques showed a significant age-related increase, and thus, it is suggested that the endocranial surface in macaques may be resorbed with advancing age, which is similar to that of long bones in both humans and nonhuman primates. The definiteness of the sulcal imprints revealed significant age-related decreases in adults. The definiteness of sulcal imprints showed a slight decrease from juvenile to adolescence, and then, remained unchanged until mid-adulthood. After that, the definiteness of the sulcal imprints greatly decreased to old age. The great decrease starts

at around 20 years of age.

Shrinkage of the brain with age in the elderly has been found in both humans and nonhuman primates (Mettler, 1956; Dekaban and Sadowsky 1978; Kumakura, 1994). Therefore, the decrease in the definiteness of the sulcal imprints from the younger age class to the old age class, especially from mid-adulthood to the oldest, may be associated both with the shrinkage of the brain (Falk, 1978) and increase of endocranial volume with aging. These may create larger gaps between the brain and the internal bone table of the skull, and decrease pressure on the internal bone table, resulting in less of an impression of the sulci in old individuals of Japanese macaques.

Factors on craniofacial skeleton age-related changes

Physical stress from muscle action is considered to play an important role to the adaptation of overall bone morphology. In humans, the cortices of long bones have been demonstrated to be thicker at muscle attachment sites compared to non-attachment sites (Niinimäki et al., 2013). Moreover, there are fewer cases of statistically significant age-related declines in cortical thickness of long bones at attachment sites compared to non-attachment sites, and thus, it has been suggested that cortical thickness could be maintained site-specifically at muscle attachments (Niinimäki et al., 2013). In the present study, cranial thickness of macaques at muscle attachment sites such as the temporal line and inion in the neurocranium also showed the same trend as the cross-section of long bones with advancing age in which the thickness at the temporal line and inion tends to increase with advancing age, and is considered to be associated with the development of bones responding to physical stress from masticatory (temporalis) and postural muscles, respectively. As males have a projecting face and large canines, the posture holding and moving the head results in considerable stress on the skull, and the cortical thickness at the mid-sagittal plane showed an

increase with age in males, though the cortex at plain cranial part, e.g., besides the bregma, showed a slight decrease.

Unlike thickness at the neurocranium, those in the facial cranium showed a great and continuous decrease with advancing age in adults. In humans, the area in the mid-face (especially in the mid-cheek) has been demonstrated to be more prone to bony resorption with aging (Pessa et al., 1999; Pessa, 2000; Shaw and Kahn, 2007; Mendelson and Wong, 2012). It is suggested that a lack of stress may be a factor resulting in bone loss in this area (Mendelson and Wong, 2012). Cross-sectional areas and densities of masseter muscles decrease significantly with aging in humans (Newton et al., 1993; McComas, 1998). Although both the masseter and temporalis muscles tend to decrease their activity with advancing age in humans, masseter muscles have smaller activation than in temporalis muscles in all age periods (Cecílio et al., 2010). Combining atrophy and loss of density, smaller activations in masseter muscles contribute to less stress on the mid-face. Zygomatic height (position of masseter muscle inserts) also significantly decreased from mid-adulthood to old age in both males and females (Chapter 1). Therefore, the thickness and zygomatic height decreasing in the facial area of macaques with age may be associated with lower stress from muscles, especially from the masseter muscles. This decrease in dimension in the mid-facial region relates with physiognomic change both in macaques and humans.

The thickness of the neurocranial cortex and facial cranium, especially those from mid-adulthood to the old age, that is, 15 years to >20 years of age, may be related with estrogen/testosterone depletion with menopause and andropause in females and males, respectively, and/or whole body aging, such as the assimilation efficiency of calcium and vitamins of the intestine.

General conclusion and future study

Three aspects of age-related changes in the skulls of macaques, outer surface morphometry, cortical thickness, and inner table bone surface, are similar with those of postcranial skeletons. In general, outer surface morphometrics showed an age-related pattern of increasing from young adulthood to mid-adulthood followed by stability in males and a slight decrease in females in very old age. Cranial cortex thickness increased from young- to mid-adulthood followed by a great decrease to very old age. Muscular forces (physical stress), including masticatory and postural muscles may play an important role to regulate age-related changes of outer surface skull morphometrics and cranial thickness, especially in male macaques e.g. the increases of cranial and posterior basic cranial lengths, the decrease of intertemporal distance (coming close to the mid-line; outer surface morphometrics); the increases in cranial thicknesses at sites on mid-sagittal plane, and temporal lines. The cranial thickness at many sites in macaques showed a significant decrease from mid-adulthood to the oldest age group in both sexes, and females revealed greater decrease in thickness than males. Cortical thickness and zygomatic height, which decrease greatly in the facial area of macaques with age, are associated with lower stress from muscles, especially from the masseter muscles.

The sex difference in craniometry and cranial cortical thickness may be associated with postmenopausal estrogen depletion in female macaques. Age at the peak of skull size in many measurements tended to be attained later in females than in males. This sex difference might relate to female-biased reproductive burden in Japanese macaques. However, it is also possible that males start aging earlier than females.

The definiteness of the sulcal imprints (relief forms) in inner table bone surface (endocranium) rapidly decreased in old age. This decrease may be associated both with the shrinkage of the brain (Falk, 1978; Kumakura, 1994) and increasing endocranial volume with

aging, because of the thinning of the cranial cortex, which creates larger gaps between the brain and the internal bone table of the skull to decrease pressure on the latter.

It is suggested that factors such as sex hormones, physical activity (stress), and whole body aging may influence on age-related changes of the skull in macaques, though experiments of these factors were not included, and thus, further study examining relations of sex hormones, social status in males, reproductive physiology in females, and physical activity on the skull are better to understand age-related changes of skull morphology. Although secular trends or environmental factors may not have had any effect on cranial dimensions in this study, cross-sectional studies still have some limitations e.g. inter-variation of individuals, selective survival, sex effects, and so on, and thus longitudinal studies are necessary. Since osteophytosis is considered to affect positional behavior and quality of life in the elderly, further studies examining the relationship between osteophytosis (mandibular joints) and age-related changes of the skull are needed.

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Appendices

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Appendix 1.1 Age and scores of PC1, PC2, and PC3 of craniometric variables in each individual obtained from the principal component analysis using both sexes

Age	Sex	PC1	PC2	PC3
7.0	Male	-1.366	0.956	-0.158
7.3	Male	-0.768	0.306	0.793
7.4	Male	1.795	2.531	-1.183
7.6	Male	3.807	1.424	1.287
8.0	Male	0.200	-0.042	0.742
8.1	Male	4.709	2.926	0.176
8.5	Male	1.839	1.307	0.311
8.6	Male	-1.025	-1.268	0.224
8.6	Male	-4.666	0.914	2.257
9.0	Male	0.158	0.894	-0.496
9.1	Male	3.377	2.580	0.687
9.3	Male	0.659	1.468	-0.183
9.3	Male	3.700	-0.121	0.868
9.4	Male	1.304	-1.881	-0.543
9.7	Male	3.014	-0.296	0.701
9.8	Male	0.544	-0.154	0.173
9.8	Male	2.269	-0.177	-2.327
10.1	Male	0.750	-0.979	1.006
10.3	Male	2.514	-0.863	0.192
10.3	Male	1.466	0.045	0.794
10.4	Male	5.330	-0.528	-0.040
10.5	Male	-0.009	-0.364	-0.688
10.6	Male	1.367	0.083	-1.736
10.6	Male	3.099	-1.342	-0.475
10.6	Male	-0.002	-2.576	-0.648
10.9	Male	5.471	-1.003	0.514
11.4	Male	3.520	0.568	-1.149
11.4	Male	4.754	-1.282	2.189
11.7	Male	3.377	-1.597	1.784
12.2	Male	1.843	1.176	1.746
12.3	Male	5.027	0.088	0.411
12.3	Male	2.552	-1.194	-0.818
13.3	Male	4.339	1.384	-1.004
13.5	Male	2.643	0.718	2.202
13.6	Male	3.217	0.785	0.856
13.9	Male	1.970	0.035	-0.639
14.5	Male	0.712	-2.739	1.207
14.5	Male	3.692	-2.280	1.344
14.6	Male	6.897	0.007	-1.263
14.6	Male	3.572	0.784	0.579
14.9	Male	2.057	-1.584	2.203
15.0	Male	5.720	-0.396	1.015
16.0	Male	6.142	-0.306	1.050
16.0	Male	4.456	-2.693	-0.928
16.1	Male	5.409	2.736	-0.994
16.2	Male	6.240	-0.677	0.161
16.5	Male	5.482	1.077	-0.565

Appendix 1.1 (Continued)

Age	Sex	PC1	PC2	PC3
16.5	Male	4.033	0.345	0.263
16.7	Male	2.875	-1.019	-0.914
17.0	Male	2.558	0.083	0.683
18.1	Male	2.701	1.560	1.015
18.8	Male	1.961	-1.186	-1.894
19.0	Male	5.559	0.677	-0.609
19.5	Male	3.263	-0.821	0.585
19.7	Male	1.514	-1.730	-0.928
20.0	Male	4.332	-0.127	-0.044
20.3	Male	5.480	-0.749	0.241
20.6	Male	2.738	-4.012	0.226
20.7	Male	3.260	-1.703	1.241
22.5	Male	1.145	-2.526	-1.104
23.3	Male	4.429	1.272	-0.253
24.1	Male	4.614	-1.127	-0.275
24.7	Male	2.665	-2.722	-0.831
24.7	Male	1.787	-1.269	-1.663
25.2	Male	2.549	-1.472	-0.920
26.9	Male	3.994	-0.081	-1.339
7.0	Female	-3.007	0.632	0.621
7.1	Female	-3.591	0.526	0.678
7.8	Female	-2.620	0.191	1.197
7.9	Female	-5.243	0.710	-1.670
8.3	Female	-2.761	0.279	-1.814
8.3	Female	-3.248	0.744	-0.593
8.4	Female	-4.530	1.375	0.053
8.5	Female	-2.540	0.930	0.220
8.7	Female	-1.568	1.298	0.430
8.8	Female	-3.060	0.895	1.073
9.1	Female	-6.645	-1.494	-0.294
9.2	Female	-3.788	-0.098	-0.268
9.3	Female	-2.562	-1.193	1.350
9.7	Female	-2.370	0.159	1.127
9.9	Female	-2.173	1.397	-1.296
10.0	Female	-8.153	-0.706	1.512
10.0	Female	-6.734	-1.563	-0.221
10.2	Female	-5.268	-0.906	0.006
10.3	Female	-2.484	0.165	2.278
10.5	Female	-4.562	0.268	1.144
10.7	Female	-2.864	1.370	0.937
11.0	Female	-3.323	0.000	-0.317
11.1	Female	0.047	1.156	-0.447
11.1	Female	-2.967	-0.464	0.320
11.2	Female	-0.268	0.230	0.018
11.2	Female	-2.505	1.188	0.468
11.3	Female	-2.924	0.312	1.932

Appendix 1.1 (Continued)

Age	Sex	PC1	PC2	PC3
11.5	Female	-1.398	-2.020	-0.564
11.6	Female	-3.957	0.671	1.220
11.8	Female	-2.071	1.251	-1.495
12.0	Female	-4.035	-0.865	0.776
12.3	Female	-1.092	0.380	-0.326
13.3	Female	-1.122	1.287	-0.996
13.6	Female	-2.002	1.381	1.025
14.0	Female	-3.174	-0.009	-0.862
14.7	Female	-6.481	-0.709	0.368
14.8	Female	-5.019	-0.851	-0.165
14.8	Female	-1.893	1.529	-0.761
15.1	Female	-4.173	0.887	-0.565
15.1	Female	-2.150	1.636	-0.216
15.2	Female	-3.620	-0.721	-0.145
15.5	Female	-6.386	-2.248	-1.531
15.8	Female	-4.983	-1.130	-0.889
16.1	Female	-1.821	1.422	-0.159
16.2	Female	-0.348	0.618	-0.860
16.5	Female	-4.278	0.060	-0.631
16.6	Female	-0.109	1.001	-0.584
16.6	Female	-0.221	2.387	-0.778
16.9	Female	-4.411	0.522	2.032
17.0	Female	-5.960	-1.687	-0.875
17.7	Female	-2.302	-1.139	0.890
17.9	Female	0.106	1.492	-0.522
18.2	Female	0.150	0.296	-1.170
18.2	Female	0.518	0.628	-0.019
18.7	Female	-4.420	-1.136	-0.239
18.9	Female	0.477	0.979	0.176
18.9	Female	-0.422	-0.944	0.054
19.7	Female	1.346	1.118	0.129
20.2	Female	-0.707	-0.346	-0.116
21.3	Female	-2.140	0.707	0.384
22.2	Female	-0.861	1.898	-0.728
22.6	Female	-2.509	0.876	0.612
23.3	Female	0.911	0.946	0.566
23.7	Female	0.558	1.596	-1.536
25.3	Female	-1.262	-0.012	-2.083
25.6	Female	-1.395	-0.057	0.029
25.8	Female	-4.867	-0.128	-0.570
26.5	Female	-1.742	-1.123	-0.129
29.3	Female	0.161	1.807	-0.755
30.7	Female	-3.798	-1.499	-1.549

Appendix 2.1 Age and scores of PC1, PC2, and PC3 of variables of cranial thickness in each individual obtained from the principal component analysis using both sexes

Age	Sex	PC1	PC2	PC3
7.0	Male	-0.104	1.311	0.461
7.1	Male	-1.020	1.206	1.403
7.3	Male	-3.862	0.318	0.713
7.4	Male	-0.574	2.238	4.173
7.6	Male	3.798	1.996	0.336
8.1	Male	3.737	2.153	1.349
8.5	Male	-0.475	1.810	1.555
8.6	Male	-3.354	0.365	1.336
8.6	Male	0.057	0.525	0.877
9.0	Male	-2.020	-0.597	0.794
9.1	Male	1.554	0.117	-0.111
9.3	Male	-1.921	-0.127	-0.422
9.3	Male	0.153	0.867	1.702
9.4	Male	-0.632	1.034	1.339
9.7	Male	1.745	1.340	-0.075
9.8	Male	0.215	0.976	-0.522
9.8	Male	-0.408	-0.393	1.748
10.1	Male	-1.480	1.570	1.084
10.3	Male	1.015	1.522	-0.143
10.4	Male	1.590	-0.029	0.724
10.5	Male	-2.417	1.681	0.278
10.6	Male	-2.078	-1.552	1.186
10.6	Male	3.206	-2.395	1.658
10.6	Male	-2.980	-1.284	1.903
10.9	Male	2.781	-0.593	1.322
11.4	Male	-1.643	1.671	1.970
11.4	Male	4.079	-2.774	0.720
11.6	Male	2.579	1.154	1.529
12.2	Male	2.755	0.053	-0.723
12.3	Male	2.831	1.476	1.958
12.3	Male	-2.460	-1.089	-0.154
13.3	Male	4.940	0.877	-1.003
13.5	Male	1.654	1.016	-0.454
13.6	Male	-0.177	1.299	-0.337
13.9	Male	0.101	0.682	-0.524
14.5	Male	0.923	-1.686	2.946
14.5	Male	4.106	0.742	0.735
14.6	Male	2.562	-0.316	1.737
14.6	Male	0.116	-0.927	0.407
14.9	Male	1.173	-0.485	-0.655
15.0	Male	1.151	0.851	0.984
16.0	Male	1.342	-0.034	0.486
16.0	Male	5.815	-4.252	0.816
16.1	Male	2.010	1.962	-0.502
16.2	Male	7.443	-0.671	-0.114
16.5	Male	3.921	1.130	0.515

Appendix 2.1 (Continued)

Age	Sex	PC1	PC2	PC3
16.5	Male	-1.411	-0.378	-0.066
16.7	Male	3.754	0.000	0.036
17.0	Male	0.285	-0.080	-0.347
18.1	Male	2.253	1.463	-1.288
18.8	Male	1.938	-1.056	0.513
19.0	Male	1.751	2.073	2.192
19.5	Male	1.099	0.383	-0.192
19.7	Male	-1.295	-2.070	-0.118
20.0	Male	7.037	-0.237	0.156
20.3	Male	2.355	-1.119	0.139
20.6	Male	6.550	-4.020	0.167
20.7	Male	2.504	-1.896	-0.542
22.5	Male	1.931	-4.257	1.127
23.3	Male	-1.200	-2.734	-0.759
23.7	Male	3.574	-0.240	-0.989
24.1	Male	-1.005	-4.538	0.767
24.7	Male	-1.045	-1.030	-0.200
24.7	Male	1.912	-4.790	-0.003
25.2	Male	-0.982	-3.768	0.557
25.8	Male	-2.768	-0.704	0.185
26.9	Male	2.560	-0.861	-0.033
7.0	Female	-2.262	0.288	-0.060
7.1	Female	-1.465	0.850	0.436
7.8	Female	-1.301	0.885	0.702
7.9	Female	-1.366	-0.121	0.159
8.3	Female	-1.701	1.176	0.584
8.3	Female	1.234	1.176	-1.202
8.4	Female	0.175	0.864	-0.781
8.5	Female	-1.204	0.275	-0.396
8.7	Female	-1.793	-0.532	-0.053
8.8	Female	0.612	-0.280	-0.328
9.1	Female	-3.830	0.853	1.518
9.2	Female	-1.768	0.215	-1.230
9.3	Female	-1.198	-0.810	-0.329
9.7	Female	-0.161	1.261	0.801
9.9	Female	-2.060	0.526	0.346
10.0	Female	-3.475	0.109	-0.220
10.0	Female	-2.621	-0.448	-0.172
10.2	Female	-3.205	1.474	0.660
10.3	Female	-0.412	0.982	-0.624
10.5	Female	-1.895	0.906	1.249
10.7	Female	-0.691	-1.396	0.574
11.0	Female	-3.100	-0.227	0.137
11.1	Female	0.893	0.758	0.071
11.1	Female	-2.381	0.603	-0.258
11.2	Female	0.782	2.303	-0.584
11.2	Female	-1.878	-0.800	-0.863
11.3	Female	0.849	1.396	-0.228

Appendix 2.1 (Continued)

Age	Sex	PC1	PC2	PC3
11.5	Female	0.383	−0.224	−0.618
11.6	Female	−2.555	−0.030	−0.221
11.8	Female	−1.491	0.201	0.042
12.0	Female	0.562	0.304	−1.031
12.3	Female	−1.951	−0.338	−0.785
13.3	Female	−1.236	−0.096	0.029
13.6	Female	0.357	0.107	0.234
14.0	Female	−1.648	−1.510	−0.644
14.6	Female	4.006	1.351	−1.297
14.7	Female	−3.445	−0.170	−0.890
14.8	Female	−0.976	−0.015	0.095
14.8	Female	−3.047	−0.266	−0.833
15.1	Female	−0.720	1.273	0.135
15.1	Female	−0.374	0.038	−0.614
15.2	Female	−3.409	0.050	−0.267
15.5	Female	−2.018	−1.224	−1.485
15.8	Female	−2.667	−0.638	−0.701
16.1	Female	1.758	−0.081	−1.891
16.2	Female	−0.497	0.271	0.146
16.5	Female	−4.596	0.687	0.537
16.6	Female	0.168	−0.226	−1.371
16.6	Female	1.660	0.562	−1.185
16.9	Female	0.038	0.420	−1.257
17.0	Female	−3.248	−0.073	−1.385
17.7	Female	0.011	1.690	−0.866
17.9	Female	−0.839	0.141	−0.574
18.2	Female	−0.367	0.570	−1.401
18.2	Female	−0.737	−0.834	0.129
18.7	Female	−2.376	−0.491	−1.056
18.9	Female	2.925	1.855	−0.946
18.9	Female	−1.284	0.231	0.082
19.7	Female	6.145	0.697	−2.685
20.2	Female	−0.984	0.215	−1.796
21.3	Female	−0.210	0.070	−0.726
22.2	Female	3.609	1.690	−2.735
22.6	Female	−1.475	−0.913	−1.470
23.3	Female	0.678	0.969	−0.125
23.7	Female	1.592	2.210	−0.633
25.3	Female	0.688	1.287	−0.043
25.6	Female	−2.436	−0.781	−1.777
25.8	Female	−1.367	−0.913	−1.588
26.5	Female	−2.448	−2.440	1.008
29.0	Female	−4.870	−1.011	−1.686
29.3	Female	−2.594	−0.458	0.297
29.6	Female	−2.017	−0.674	−1.196
30.7	Female	−3.018	−0.653	−1.174

Appendix 2.2 Pearson's correlation between all variables of the cranial thickness and the cortical thickness at medial and lateral sides in the midlength of the femur in males and females

Variable		Male		Female	
		Medial thickness	Lateral thickness	Medial thickness	Lateral thickness
MBN	Correlation	0.316**	0.302*	0.271*	0.226
	Sig. (2-tailed)	0.009	0.013	0.021	0.055
AB	Correlation	0.223	0.237	0.291*	0.335**
	Sig. (2-tailed)	0.069	0.053	0.013	0.004
B	Correlation	0.223	0.277*	0.242*	0.382**
	Sig. (2-tailed)	0.070	0.023	0.039	0.001
LB	Correlation	0.264*	0.456***	0.245*	0.253*
	Sig. (2-tailed)	0.031	0.000	0.037	0.031
RB	Correlation	0.287*	0.411**	0.293*	0.300*
	Sig. (2-tailed)	0.018	0.001	0.012	0.010
PB	Correlation	0.223	0.297*	0.282*	0.394**
	Sig. (2-tailed)	0.069	0.015	0.016	0.001
MBI	Correlation	0.036	0.148	0.124	0.164
	Sig. (2-tailed)	0.771	0.232	0.297	0.165
LTL	Correlation	0.104	0.184	0.132	0.117
	Sig. (2-tailed)	0.403	0.136	0.264	0.324
RTL	Correlation	0.096	0.131	0.144	0.151
	Sig. (2-tailed)	0.439	0.289	0.224	0.203
I	Correlation	0.110	0.013	0.297*	0.194
	Sig. (2-tailed)	0.376	0.914	0.011	0.100
MFZ	Correlation	0.277*	0.448***	0.163	0.228
	Sig. (2-tailed)	0.024	0.000	0.167	0.053
MLZ	Correlation	0.145	0.235	0.118	0.261*
	Sig. (2-tailed)	0.240	0.056	0.321	0.026
PC1	Correlation	0.260*	0.346**	0.308	0.353**
	Sig. (2-tailed)	0.034	0.004	0.008**	0.002

Appendix 3.1 Average imprint scores of all specimens (SD) in sulcus on the surface of the endocasts in Japanese macaques

Sulcus	Mean	SD	Range
Principal sulcus	2.48	0.96	1–4
Arcuate sulcus	3.16	0.85	1–4
Sylvian sulcus	2.00	0.87	0–4
Superior temporal sulcus	2.76	0.88	1–4
Central sulcus	2.12	0.67	1–3
Intraparietal sulcus	1.28	0.61	0–2
Lunate sulcus	1.28	0.61	0–2

Appendix 3.2 Age-related changes in the imprints of sulci on the surface of the endocast

Sulcus		Age group (years)				
		2–4	4–6	7–10	15–16	>20
Principal sulcus	Mean	3.50	3.25	2.67	2.00	1.20
	SD	0.52	0.50	0.52	0.82	0.45
	Range	3–4	3–4	2–3	1–3	1–2
Arcuate sulcus	Mean	3.83	3.25	3.33	3.50	1.80
	SD	0.41	0.50	0.52	0.58	0.45
	Range	3–4	3–4	3–4	3–4	1–2
Sylvian sulcus	Mean	3.00	2.00	2.33	2.25	0.80
	SD	0.84	0.82	0.52	0.50	0.45
	Range	2–4	1–3	2–3	2–3	0–1
Superior temporal sulcus	Mean	3.83	2.50	2.83	3.00	1.60
	SD	0.52	0.58	0.41	0.82	0.55
	Range	3–4	2–3	2–3	2–4	1–2
Central sulcus	Mean	3.00	2.00	2.33	2.25	1.40
	SD	0.55	0.82	0.52	0.50	0.55
	Range	2–3	1–3	2–3	2–3	1–2
Intraparietal sulcus	Mean	2.00	1.25	1.33	1.50	0.60
	SD	0.52	0.50	0.52	0.58	0.55
	Range	1–2	1–2	1–2	1–2	0–1
Lunate sulcus	Mean	2.00	1.75	1.17	1.25	0.40
	SD	0.52	0.50	0.41	0.50	0.55
	Range	1–2	1–2	1–2	1–2	0–1