Original Article

Age-related changes in lumbar bone mineral density measured using quantitative computed tomography in healthy female cynomolgus monkeys

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Background: Cynomolgus monkeys are widely used in studies related to osteoporosis, and there is no evidence of age-related changes in volumetric bone mineral density (vBMD) measured using quantitative computed tomography (QCT) in nonhuman primates. This study aimed to describe changes in the characteristics of lumbar vBMD with age, to analyze the relationship between lumbar vBMD and body composition, and to investigate the precision of QCT measurements in healthy female cynomolgus monkeys.

Methods: Age-related changes in lumbar vBMD were described using cubic regression models, and the accumulated bone loss rates (ABLR) of the lumbar spine were calculated. Spearman rank correlation and ridge regression analysis were used to investigate the relationship of the average lumbar vBMD and body components. Thirty animals were selected to analyze the short-term *in vivo* precision of the QCT measurements. The precision was expressed as the root-mean-square coefficient of variation (RMS-CV%) or root-mean-square standard deviation (RMS-SD).

Results: A total of 72 healthy female cynomolgus monkeys, aged 1–25 years, were included in this study. The average lumbar vBMD of female cynomolgus monkeys increased with age until the age of 10 years, around which it reached peak bone mass, with a relatively marked decline after the age of 13 years. The ABLRs of female cynomolgus monkeys in the premenopausal (13–19 years) and postmenopausal age groups (20–25 years) were –4.9% and –21.2%, respectively. Ridge regression analysis showed that age and subcutaneous adipose tissue (SAT) contributed positively to the average lumbar vBMD in animals aged ≤10 years, whereas in animals aged >10 years, age contributed negatively to lumbar vBMD. The RMS-CV% (RMS-SD) of the lumbar vBMD measured using QCT ranged from 0.47% to 1.60% (1.91–6.31 mg/cm^3).

Conclusions: Age-related changes in lumbar vBMD measured using QCT in healthy female monkeys showed similar trends to those in humans. Age and SAT may affect the lumbar vBMD in female cynomolgus monkeys. QCT revealed good precision in measuring the lumbar vBMD in female cynomolgus monkeys.

Keywords: Cynomolgus monkeys; quantitative computed tomography (QCT); volumetric bone mineral density (vBMD); precision

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Introduction

Osteoporosis is a systemic disorder characterized by a reduction in bone mass and deterioration of the bone tissue microarchitecture, leading to increased bone fragility and a higher risk of fracture (1). A meta-analysis showed that the global prevalence of osteoporosis based on World Health Organization criteria was 19.7%. The prevalence varied among countries, ranging from 4.1% in Netherlands to 52.0% in Turkey. In terms of gender, the prevalence of osteoporosis was 10.6% in males and 24.8% in females, while it was higher in postmenopausal women at about 27.4% (2). A multicenter study based on a Chinese population showed that the prevalence of osteoporosis in people >50 years of age was approximately 29.0% in women and 13.5% in men (3). An increasing number of people suffer from osteoporosis, and the occurrence of osteoporotic fractures poses a heavy economic burden on the healthcare system (4). Several species of animals are available for osteoporosis research, among which nonhuman primates such as cynomolgus monkeys are highly valuable. Female cynomolgus monkeys are similar to women with regard to the menstrual cycle, estrogen and progesterone secretion patterns, and reduced bone mass associated with natural menopause, making them an ideal model for studying osteoporosis (5). Ovariectomized cynomolgus monkeys are commonly used in studies related to osteoporosis, including investigations into the therapeutic effects of specific drugs on osteoporosis (6,7), which contributes greatly to the treatment and prevention of osteoporosis in humans.

Several methods are available for clinically diagnosing osteoporosis, including dual-energy X-ray absorptiometry (DXA), quantitative computed tomography (QCT), peripheral QCT (pQCT), quantitative ultrasound, and magnetic resonance imaging (8,9). In nonhuman primates, methods used for bone mineral density (BMD) measurements include DXA, pQCT, and micro-CT (6,7), among which DXA is the most commonly used. DXA can be used to measure the areal BMD (aBMD) of the whole body, lumbar spine, and radius using a region of interest (ROI) that includes both the trabecular and cortical bone (10). Whole-body aBMD of cynomolgus monkeys measured by DXA mainly reflects changes in the cortical bone and cannot reflect changes in the cancellous bone (11), and so this measure is not recommended for monitoring therapeutic efficacy. The International Society for Clinical Densitometry (ISCD) recommends measuring the BMD of the lumbar spine to monitor efficacy. The trabecular bone in the spine is approximately eight times more metabolically active than the cortical bone, and changes in lumbar trabecular BMD measured by QCT are greater than changes in aBMD measured by DXA; therefore, QCT is more sensitive for monitoring age- and treatment-related changes in the BMD (12). Several studies have shown that QCT has a higher detection rate than DXA in diagnosing osteoporosis because QCT measures the true volumetric bone mineral density (vBMD), which is not size dependent and unaffected by calcifications, osteophytes, and spinal deformity (13-16). During the natural growth of nonhuman primates, spinal osteoarthritis becomes more common in older monkeys and it may be more accurate to measure their lumbar vBMD using QCT (17).

Studies on the lumbar BMD of cynomolgus monkeys using pQCT have been reported, mainly measuring the *in vitro* vertebral BMD of monkeys in a single age group (18-20). Currently, pQCT is not routinely used in clinical practice, and the QCT lumbar vBMD measurement is widely used in clinical and scientific research. Previous study has shown that the aBMD and bone mineral content measured by DXA in female cynomolgus monkeys experience age-related changes (21). However, no studies have used QCT-based techniques to investigate age-related changes in the BMD in nonhuman primates. In addition, QCT is used to measure body components, which include abdominal adipose tissue and paravertebral muscles (22,23). Lumbar vBMD measured by QCT may be affected by body components in humans, which include visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), and lean mass (LM) (23-27). At present, there are no reports on the measurement of abdominal adipose tissue and LM using QCT in nonhuman primates. In addition, the relationship between lumbar vBMD and body composition in nonhuman primates is unclear. To ensure the accuracy of QCT measurements, the ISCD recommends establishing a new *in vivo* precision level for spine QCT operations (12). Several studies have reported the precision of pQCT for measuring BMD in nonhuman primates, but they measured peripheral

bones, and some of these studies were performed *in vitro* (28-30). This study aimed to establish *in vivo* precision data of QCT to measure lumbar vBMD in female cynomolgus monkeys.

QCT is an accurate method for measuring vBMDs, and cynomolgus monkeys are ideal models for studying osteoporosis. Therefore, this study aimed to explore the value of QCT in healthy female cynomolgus monkeys. Our study was performed on female cynomolgus monkeys to investigate the following: (I) age-related changes in lumbar vBMD and in the relationship between lumbar vBMD and body composition, and (II) short-term *in vivo* precision of QCT measurements. We present the following article in accordance with the ARRIVE reporting checklist (available at https://qims.amegroups.com/article/view/10.21037/qims-22-763/rc).

Methods

Animals

This study included 72 healthy female cynomolgus monkeys. All experimental animals were absent of severe organ diseases, and none of the animals showed evidence of clinical disorders. Animals with signs of disease were excluded. All female cynomolgus monkeys were specific pathogen-free animals in compliance with national laboratory animal standards. Animals were obtained from Guangdong Landau Biotechnology Co., Ltd., Guangzhou, China, which holds international accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care. The animals were housed in groups of 10–15 monkeys, which consisted of one mature male, several mature females, and their progenies. The animal room was mainly illuminated by natural lighting, adjusted with incandescent lighting to achieve 12/12 h light/dark cycles. The animals were fed fruits, a semi-purified diet containing ~1.0% calcium, ~0.7% phosphorus, and drinking water ad libitum. The sitting height was recorded as the trunk length, which was approximately the distance from the crown to the base of the pubic bone. Body mass index (BMI) was calculated as the weight (kg) divided by the square of the trunk length (m^2) (31). Experiments were performed under a project license (No. LDIACUC2018-0004) granted by the Laboratory Animal Ethics Committee of Guangdong Landau Biotechnology Co., Ltd., in compliance with international Association for Assessment and Accreditation of Laboratory Animal Care guidelines for the care and use of animals. Our protocol was not registered. Based on the progression of sexual maturation and development, healthy female cynomolgus monkeys were stratified into different age groups in our study: a juvenile group (≤4 years old), young group (5–10 years old), middle-aged group (11–19 years old), and older group (≥20 years old) (32).

QCT examination

All animals underwent whole-body CT scans using a PET/CT scanner (GE Discovery Elite 690, USA) at the PET CT/MRI center of the First Affiliated Hospital of Jinan University. For quality control purposes, we calibrate the machine with a phantom once a month. Asynchronous QCT was performed according to the following parameters: tube voltage 120 kV; tube current 300 mA; slice thickness 1.25 mm; slice interval 1.0 mm; and field of view 500 mm. Asynchronous QCT calibrates data in Hounsfield units by using phantom data originally obtained from CT scans for measuring vBMD. The installation of the QCT Pro Asynchronous Calibration Module makes it possible to measure vBMD directly from CT images without simultaneously using the calibration phantom during the subject scan (33). All monkeys were starved for 8–12 h and weighed prior to scanning. Before examination, the animals were anesthetized by a specialized veterinarian using ketamine hydrochloride (5–10 mg/kg, intramuscularly) and 3% pentobarbital sodium (0.5–1 mL/kg, intravenously). A few cynomolgus monkeys may vomit during anesthesia. In order to avoid causing asphyxia or death, the vital signs of the animals need to be monitored every 10 minutes to ensure that they can successfully complete the QCT scan. If the animal's vital signs were not stable after anesthesia, the QCT scan was not performed. The animals were placed in a supine position on the scanning bed, with the spine parallel to the long axis of the examination bed. All CT images were sent to the QCT workstation and analyzed using the three-dimensional spine function and tissue composition module in the application (QCT Pro Version 6.1, Mindways Software).

QCT measurements of lumbar vBMD

QCT measurements of the lumbar vBMD were performed on the L2-4 vertebrae. Most cynomolgus monkeys have seven lumbar vertebrae, whereas some have six. In this study, we calculated the vertebral sequences from bottom to top, meaning that the bottom vertebra was defined as L7.

On the axial and sagittal images, the ROIs were placed in the central trabecular bone of the vertebral bodies, parallel to the endplates of the upper and lower margins of the vertebral bodies, avoiding the inclusion of the cortical bone. The area of each ROI was approximately 20–30 mm^2. If any vertebra from among L2-4 vertebrae showed deformation or an uneven increase in bone density, three adjacent vertebrae from among L1-7 vertebrae were selected for vBMD measurements. To reduce the error in manually drawing the ROI, the vBMD of each vertebra was measured three times by the same well-trained investigator, and the average of the three measurements was chosen as the final vBMD of the vertebra. The vBMD of each cynomolgus monkey was the average vBMD of L2-4 or of the three adjacent vertebrae.

QCT measurements of body components

Abdominal adipose tissue was measured at the L4-5 intervertebral disc level. The QCT Pro software was used to automatically color the adipose tissue based on the CT value. Anatomical positions in cynomolgus monkeys differ from those in humans. The selected slice may include the limbs, which required manual adjustment of the closed spline to remove non-abdominal tissues, after which the data were recorded as the total adipose tissue (TAT). The closed spline was adjusted to the external edge of the abdominal muscles to distinguish VAT from SAT, and the data within the closed spline were recorded as the VAT. SAT values were obtained by subtracting the VAT from the TAT.

The fascial boundaries of the LM of the paravertebral muscles (including the bilateral psoas major and erector spinae muscles) were manually traced and segmented from the image at the L3 mid-plane level. Voxels with density values in the muscle tissue range were defined as the LM (23). Some animals had low abdominal adipose tissue levels, and the contours of the paravertebral muscles could not be clearly displayed and were therefore outlined based on the CT images.

Precision of QCT measurements

The ISCD recommends a degree of freedom of 30 for precision studies, so the scanning methods include scanning 4 times each of 10 individuals, or 3 times each of 15 individuals, or twice each of 30 individuals (34,35). We selected the last scanning method in order to reduce the radiation exposure of female cynomolgus monkeys. Older female cynomolgus monkeys in the group aged ≥20 years old may experience osteoporosis and are not suitable for precision study (36). Due to the differences in lumbar vBMD in female cynomolgus monkeys of different ages, we used stratified random sampling method to randomly select 10 animals among those aged ≤10 years and 20 animals among those aged 11–19 years, adding up to a total of 30 animals for precision study. Two animals in the middle-aged group were excluded from the precision study due to an uneven increase in bone density of any lumbar vertebra on QCT images. Therefore, we randomly selected two more animals among those aged 11–19 years. Finally, there were 30 female cynomolgus monkeys included in the short-term *in vivo* precision study, consisting of seven from the juvenile group (1–4 years old), three from the young group (5–10 years old), and 20 from the middle-aged group (11–19 years old). All intact animals were scanned twice under the same conditions within 10 min with repositioning before the second scan.

A well-trained investigator measured the vBMD of the seven vertebrae in L1-7. The vBMD of each vertebra was recorded separately, and the average vBMD of the combination of two and three adjacent lumbar vertebrae was calculated. TAT, VAT, and SAT levels were measured at each intervertebral disc level from L1-2 to L6-7 in each cynomolgus monkey. The LM of the paravertebral muscles was measured in the mid-plane of each vertebra from L2 to L6. All the QCT measurements above were performed by the same investigator on the same day. In order to control the potential measurement bias from non-blinding, the investigator performed the QCT measurements strictly following the pre-defined criteria, as mentioned in the *QCT measurements of lumbar vBMD* and *QCT measurements of body components* above. The QCT images of the two scans were measured and recorded separately. The investigator measured the QCT images of the 1st scan first and recorded the results, while measuring the images of the 2nd scan, the investigator was not allowed to read the results of the first recording.

The root-mean-square standard deviation (RMS-SD) and root-mean-square coefficient of variation (RMS-CV%) are generally used to express short-term precision:

$$\text{RMS-SD} = \sqrt{\frac{\sum_{i=1}^{m}\left(SD^2\right)}{m}} \quad [1]$$

$$\text{RMS-CV\%} = \sqrt{\frac{\sum_{i=1}^{m}\left(\text{CV\%}^2\right)}{m}} \quad [2]$$

In Eqs. [1] and [2], m is the number of animals, SD is the standard deviation, and CV% is the percent coefficient of variation.

Once the precision of the measurement at a site is determined, the least significant change (LSC) representing the real biological change at that site can be calculated. Generally, an 80% confidence interval is adequate for clinical purposes, even though a 95% confidence interval is ideal. The formula for the LSC is as follows:

$$\mathrm{LSC} = Z^{'}(\mathrm{Pr})\sqrt{\frac{1}{n_1}+\frac{1}{n_2}} \quad [3]$$

where Z' is the statistical confidence of the desired level obtained from tables or mathematical statistical texts, Pr is the precision value (RMS-SD or RMS-CV%), n_1 is the number of measurements at baseline, and n_2 is the number of measurements at follow-up. In general, one measurement is performed for both baseline and follow-up (34).

Statistical analysis

All data were analyzed using the SPSS software (version 26.0) and R software (version 4.2.1). For normally distributed variables, values are presented as the mean ± standard deviation (SD), and for non-normally distributed variables, values are presented as the median and interquartile range. For comparisons between groups, one-way ANOVA was used for normally distributed variables, and the Kruskal–Wallis H test was used for non-normally distributed variables. By evaluating and comparing different models such as linear, logarithmic, quadratic, cubic, compound, power, growth, and exponential, we found that the cubic regression model was the best for describing age-related changes in vBMD and body components. The mean value of peak vBMD and accumulated bone loss rates (ABLR) were calculated. ABLR was computed using the following formula: (mean vBMD – mean peak vBMD)/mean peak vBMD × 100%. The correlations between vBMD and body components were analyzed using Spearman rank correlation. We found strong correlations between some of the variables and performed collinearity diagnostics, which revealed the existence of multicollinearity. Finally, we used ridge regression instead of multiple linear regression in order to make the regression coefficients more stable. Ridge regression analysis was applied to evaluate the degree of contribution of each factor to vBMD using vBMD as the dependent variable, whereas age, LM, VAT, and SAT were independent variables, adjusted for BMI. Differences were considered statistically significant at P<0.05. The precision of the vBMD and body components was expressed using RMS-SD or RMS-CV%, and the LSCs with 80% and 95% confidence intervals were calculated, respectively, as shown in the above formula.

Results

Characteristics of lumbar vBMD and body composition

A total of 72 healthy female cynomolgus monkeys, with an age range of 1–25 years and a weight range of 2.0–9.1 kg, were included in this study and divided into four groups according to age (*Table 1*). There were statistically significant differences in the average lumbar vBMD between the juvenile and young groups, young and older groups, and middle-aged and older groups (P<0.05). The differences in BMI, LM, TAT, VAT, and SAT were statistically significant (P<0.05) between the juvenile and young groups, juvenile and middle-aged groups, and juvenile and older groups. In addition, the LM of the older group was significantly different from that of the young group (P<0.05), and the SAT of the older group was significantly different from that of the middle-age group (P<0.05).

Age-related changes in the vBMD and body composition

Figures 1,2 demonstrate the changes in the average lumbar vBMD and body components associated with age in healthy female cynomolgus monkeys. The average vBMD of the lumbar spine tended to increase with age until the age of 10 years, reaching a peak bone mass at approximately 10 years, with bone mass plateauing between 8 and 12 years of age. After the age of 13 years, the average lumbar vBMD showed a decreasing trend with age (*Figure 1*). From the scatter plot, a few female cynomolgus monkeys had relatively high vBMD after reaching peak bone mass, which may be due to the individual differences, so we mainly described the result of the fitted curve of 72 monkeys above.

The LM of the paravertebral muscles increased with age until the age of 10 years, decreased mildly with age from 11 to 15 years, and decreased more rapidly with age after 15 years (*Figure 2A*). With regard to abdominal adipose tissue, TAT, VAT, and SAT levels showed a rapid increase before the age of 10 years, stabilized around the ages of 11–15 years, and showed a decreasing trend after the age of 15 years (*Figure 2B-2D*).

Table 1 Descriptive characteristics of female cynomolgus monkeys

Variables	Juvenile (≤4 years)	Young (5–10 years)	Middle-aged (11–19 years)	Older (≥20 years)
Number of animals	16	19	24	13
Age (years)	1.5 (1.0–4.0)	9.0 (7.0–10.0)	14.0 (13.0–15.0)	22.0 (21.0–24.0)
BMI (kg/m^2)	22.14±6.75	34.41±9.08[a]	33.17±6.23[a]	30.18±8.31[a]
Average vBMD (mg/cm^3)	382.17±31.02	433.08±51.44[a]	402.93±47.53	337.14±61.94[bc]
LM (g)	1.21±0.26	1.72±0.26[a]	1.65±0.28[a]	1.50±0.23[ab]
TAT (g)	0.85 (0.80–1.35)	9.50 (4.10–11.70)[a]	10.10 (5.98–11.90)[a]	8.50 (4.95–9.90)[a]
VAT (g)	0.80 (0.60–1.05)	6.60 (3.20–8.50)[a]	7.05 (4.55–8.10)[a]	6.80 (4.00–7.90)[a]
SAT (g)	0.20 (0.10–0.30)	2.20 (1.50–3.20)[a]	3.10 (1.45–3.50)[a]	1.50 (0.65–2.15)[ac]

Values are presented as the mean ± standard deviation (SD) for normally distributed variables and as the median and interquartile range (IQR) for non-normally distributed variables. [a], P<0.05 versus juvenile group; [b], P<0.05 versus young group; [c], P<0.05 versus middle-aged group. BMI, body mass index; vBMD, volumetric bone mineral density; LM, lean mass; TAT, total adipose tissue; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

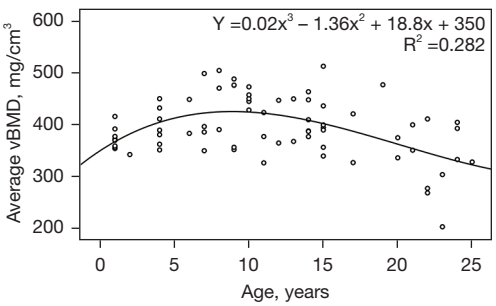

Figure 1 Changes in the average vBMD of the lumbar spine with age in healthy female cynomolgus monkeys. vBMD, volumetric bone mineral density; R^2, coefficient of determination.

ABLR in female cynomolgus monkeys

As shown in *Figure 1*, the bone mass of female cynomolgus monkeys was at a high level between approximately 8 and 12 years of age, and the mean value of their lumbar vBMD was calculated. After reaching the peak bone mass, the lumbar vBMD of female cynomolgus monkeys tended to decrease with age. Female cynomolgus monkeys were divided into premenopausal (13–19 years old) and postmenopausal groups (20–25 years old) according to the age of menopause, and their ABLRs were calculated separately, as shown in *Table 2*.

Spearman correlation analysis of the average lumbar vBMD and body components

According to the age at peak bone mass, the female cynomolgus monkeys were divided into a pre-peak bone mass group and a post-peak bone mass group with the age of 10 years as the cut-off. In the group aged ≤10 years, the average lumbar vBMD was positively correlated with age, BMI, LM, VAT, and SAT. In the group aged >10 years, the average lumbar vBMD was positively correlated with BMI and SAT, while it was negatively correlated with age (*Table 3*).

Ridge regression analysis of average lumbar vBMD and body components

Table 4 shows the results of the ridge regression analysis with the average lumbar vBMD as the dependent variable and age, LM, VAT, and SAT as independent variables, adjusted for BMI. In the group aged ≤10 years, age and SAT had a positive contribution to the average lumbar vBMD, and in the group aged >10 years, only age showed an independent negative influence on vBMD. The LM and VAT were not significantly correlated with the average lumbar vBMD in the ridge regression analysis in either group.

Short-term in vivo precision of QCT measurements of lumbar vBMD

The RMS-CV% and RMS-SD of lumbar vBMD for repeated QCT measurements of a single vertebra and of the combination of 2 and 3 adjacent vertebrae ranged from 0.47% to 1.60% and 1.91 to 6.31 mg/cm^3, respectively. In addition, the LSCs with 80% and 95% confidence intervals for the above measurement sites are provided in *Table 5*.

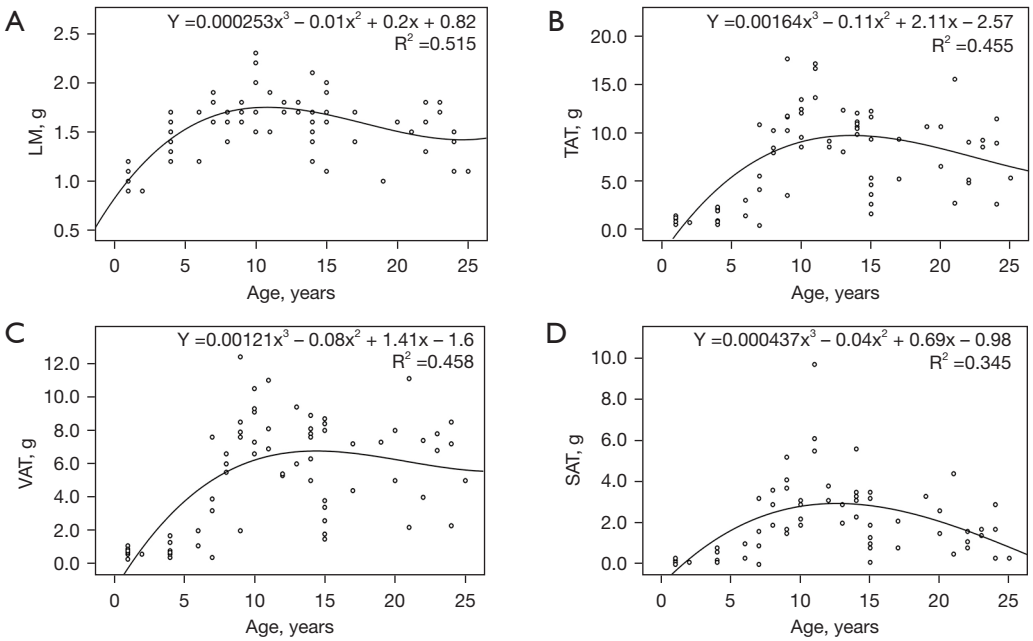

Figure 2 Changes in the LM of paravertebral muscles (A), TAT (B), VAT (C), and SAT (D) with age in healthy female cynomolgus monkeys. LM, lean mass; TAT, total adipose tissue; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; R^2, coefficient of determination.

Table 2 ABLR in female cynomolgus monkeys

Age (years)	Number of animals	vBMD (mg/cm^3)	ABLR (%)
8–12	18	428.05±53.75	–
13–19	19	406.95±47.89	–4.9
20–25	13	337.14±61.94	–21.2

vBMD, volumetric bone mineral density, presented as mean ± standard deviation; ABLR, accumulated bone loss rate; ABLR = (mean vBMD – mean peak vBMD)/mean peak vBMD × 100%.

Table 3 Correlations between average vBMD, age, BMI, LM of paravertebral muscles, VAT, and SAT in female cynomolgus monkeys

Variables	Pre-peak bone mass group (≤10 years; n=35)					Post-peak bone mass group (>10 years; n=37)				
	vBMD	VAT	SAT	LM	BMI	vBMD	VAT	SAT	LM	BMI
VAT	0.508**					0.317				
SAT	0.617**	0.929**				0.362*	0.657**			
LM	0.519**	0.674**	0.589**			–0.053	0.193	0.248		
BMI	0.412*	0.821**	0.800**	0.590**		0.377*	0.691**	0.562**	0.231	
Age	0.534**	0.826**	0.781**	0.813**	0.756**	–0.345*	–0.218	–0.611**	–0.405*	–0.379*

*P<0.05; **P<0.01. vBMD, volumetric bone mineral density; BMI, body mass index; LM, lean mass; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

Table 4 Ridge regression analysis of age, LM of paraspinal lumbar muscles, VAT, and SAT with average vBMD adjusted for BMI in female cynomolgus monkeys

Variables	Average vBMD (≤10 years; n=35)			Average vBMD (>10 years; n=37)		
	Standardized β	t	P value	Standardized β	t	P value
Age	0.229	2.205	0.028	–0.218	2.869	0.004
LM	0.209	1.902	0.057	–0.093	1.165	0.244
VAT	–0.142	1.479	0.139	0.055	0.768	0.442
SAT	0.251	2.537	0.011	0.056	0.760	0.447

Ridge parameter was 0.312 for the group aged ≤10 years and 0.841 for the group age >10 years, which were chosen automatically by the R software. vBMD, volumetric bone mineral density; BMI, body mass index; LM, lean mass; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

Table 5 Short-term *in vivo* precision results of QCT measurements of lumbar vBMD in a single vertebra and in a combination of 2 and 3 adjacent vertebrae in 30 female cynomolgus monkeys

Lumbar vertebrae	Average vBMD (mg/cm^3)	RMS-CV% (%)	RMS-SD (mg/cm^3)	LSC^{80}		LSC^{95}	
				RMS-CV% (%)	RMS-SD (mg/cm^3)	RMS-CV% (%)	RMS-SD (mg/cm^3)
L1	418.38±41.18	0.75	3.11	1.35	5.64	2.06	8.63
L2	411.30±45.17	0.72	2.97	1.31	5.37	2.00	8.22
L3	402.40±44.88	1.09	4.24	1.97	7.68	3.01	11.75
L4	393.71±44.40	1.08	4.21	1.95	7.61	2.99	11.65
L5	390.41±48.82	1.60	6.31	2.89	11.42	4.25	17.48
L6	391.12±49.48	1.51	5.91	2.73	10.69	4.18	16.36
L7	395.70±48.48	0.94	3.64	1.71	6.59	2.62	10.08
L1-2	414.84±42.72	0.50	2.08	0.90	3.76	1.38	5.75
L2-3	406.85±44.52	0.61	2.39	1.10	4.33	1.68	6.62
L3-4	398.06±44.22	0.80	3.09	1.45	5.59	2.21	8.56
L4-5	392.06±46.08	0.93	3.72	1.68	6.73	2.58	10.29
L5-6	390.77±48.86	1.15	4.53	2.09	8.20	3.19	12.55
L6-7	393.41±48.04	0.94	3.77	1.70	6.82	2.60	10.44
L1-3	410.69±43.03	0.47	1.91	0.84	3.45	1.29	5.28
L2-4	402.47±44.11	0.58	2.26	1.05	4.09	1.60	6.26
L3-5	395.50±45.34	0.82	3.23	1.48	5.84	2.27	8.92
L4-6	391.74±46.85	0.84	3.32	1.52	6.01	2.32	9.19
L5-7	392.41±47.72	0.85	3.38	1.53	6.11	2.34	9.36

QCT, quantitative computed tomography; vBMD, volumetric bone mineral density, presented as mean ± standard deviation; RMS-CV%, root-mean-square of coefficient of variation; RMS-SD, root-mean-square of standard deviation; LSC^{80}, least significant changes at 80% confidential intervals; LSC^{95}, least significant changes at 95% confidential intervals.

Short-term in vivo precision of QCT measurements of body components

Table 6 shows the RMS-CV% (RMS-SD) for the repeated QCT measurements of LM of the paravertebral muscles, TAT, VAT, and SAT at different levels, as well as the corresponding LSCs at 80% and 95% confidence intervals.

Discussion

Female nonhuman primates experience menarche at approximately 2.5–3 years of age, with regular menstrual cycles at around 4 years of age, and experience natural menopause at approximately 20 years of age (32). Accordingly, we divided them into juvenile, young, middle-aged and older groups. The time of epiphyseal closure of different skeletons varies in cynomolgus monkeys, with their long bones closing at about 5–6.5 years of age (37), while the epiphyses of the spine close at least 15 years of age (38). There has still existed confusion as to which skeletal epiphyseal closure should be used as a marker of bone maturity. X-ray radiography was commonly used to determine the closure of the epiphyses (37), while we only performed QCT in this study. There were differences in the determination of epiphyseal closure by different devices, leading to differences in the determination of skeletal maturity age. Therefore, we grouped monkeys according to their reproductive age rather than their skeletal maturity age. Our study showed that the young group had the highest average vBMD of the lumbar spine, followed by the middle-aged and juvenile groups, and the older group had the lowest average vBMD. There were some differences in lumbar vBMD between the different age groups in our study, consistent with the process of growth and physiological changes in healthy female cynomolgus monkeys. Our study showed that female cynomolgus monkeys in the postmenopausal group had higher ABLR than the premenopausal group, suggesting that postmenopausal animals lose bone mass more rapidly. Colman *et al.* [1999] also showed that the aBMD of the lumbar spine measured using DXA was higher in adult premenopausal female rhesus monkeys than in growing and postmenopausal animals (17).

In the ridge regression analysis, our study revealed that age contributed positively to the average lumbar vBMD in the group aged ≤10 years, while it contributed negatively to vBMD in the group aged >10 years. Our study showed that the age at peak bone mass in healthy female cynomolgus monkeys was approximately 10 years. Studies based on DXA have reported an age at peak bone mass of 9 years in female cynomolgus monkeys (21,39), and Champ *et al.* [1996] reported an age at peak bone mass of 11 years in female rhesus monkeys (10). The results from our study were similar to those reported in the above studies. Hence, we recommend using cynomolgus monkeys aged no less than 10 years for osteoporosis-related studies based on QCT. The average lumbar vBMD of female cynomolgus monkeys decreased significantly after 13 years of age in our study. As reported by Jayo [1994], skeletally mature monkeys showed a decreasing trend in bone mass with age after reaching peak bone mass (21), which was consistent with the results of the present study. However, after reaching peak bone mass, some studies based on DXA have shown that the bone mass of the lumbar spine in nonhuman primates remains relatively stable with age (10,39), which may be due to the increased susceptibility of older monkeys to osteoarthritis, leading to overestimation of the bone mass of the lumbar spine (17). Since DXA measures aBMD, it may not always reflect the true bone strength of animals or patients with osteochondrosis or osteoarthritis. The vBMD of the trabecular bone measured by QCT reflects more accurately bone mass and is less likely to be affected by osteoarthritis (12). In our study, lumbar vBMD showed a decreasing trend with age after reaching peak bone mass, which was consistent with the findings in female humans (23). However, nonhuman primates are distinguished from humans by their diverse positional modes, including tripedal walking, quadrupedal walking and so on (40). Different postural or locomotor habits may affect the skeleton development of monkeys and thus indirectly affect lumbar vBMD. The impact of these factors should be taken into account when using monkeys as animal models. Currently, there is a lack of QCT-based diagnostic criteria for osteoporosis in female cynomolgus monkeys. Our study calculated the mean peak vBMD and ABLRs in female cynomolgus monkeys, and the data provided in this study may be used to determine whether the osteoporosis model in cynomolgus monkey was successfully established and to provide reference information for screening suitable animal models.

In our study, the TAT, VAT, and SAT of female cynomolgus monkeys increased with age before the age of 10 years, while after the age of 15 years, they showed a decreasing trend. Ng *et al.* [2013] reported that in women, total body fat measured by DXA, VAT and STA measured by QCT increased with age until approximately 70 years, after which it decreased (41), showing a similar trend to

Table 6 Short-term *in vivo* precision results of QCT measurements of LM and abdominal adipose tissue at different anatomic slices in 30 female cynomolgus monkeys

Anatomic slices	RMS-CV% (%)	RMS-SD (g)	LSC^{80}		LSC^{95}	
			RMS-CV% (%)	RMS-SD (g)	RMS-CV% (%)	RMS-SD (g)
L2–LM	5.40	0.08	9.77	0.15	14.95	0.23
L3–LM	2.47	0.04	4.47	0.07	6.84	0.11
L4–LM	2.78	0.05	5.03	0.09	7.70	0.13
L5–LM	3.90	0.07	7.07	0.12	10.81	0.18
L6–LM	4.21	0.07	7.62	0.13	11.66	0.20
L1/2						
TAT	6.31	0.17	11.43	0.31	17.49	0.47
VAT	7.18	0.18	13.00	0.32	19.89	0.50
SAT	2.94	0.05	5.32	0.09	14.73	0.14
L2/3						
TAT	5.22	0.09	9.81	0.16	14.46	0.25
VAT	6.75	0.17	12.22	0.31	18.70	0.47
SAT	16.78	0.13	30.37	0.24	46.48	0.37
L3/4						
TAT	3.81	0.09	6.90	0.16	10.57	0.25
VAT	6.11	0.14	11.07	0.26	16.93	0.40
SAT	10.54	0.09	19.08	0.16	29.20	0.24
L4/5						
TAT	3.18	0.13	5.76	0.23	8.82	0.36
VAT	3.86	0.14	6.99	0.26	10.69	0.40
SAT	8.71	0.10	15.76	0.19	24.12	0.29
L5/6						
TAT	3.44	0.10	6.23	0.18	9.53	0.28
VAT	3.45	0.11	6.24	0.20	9.55	0.30
SAT	6.58	0.07	11.92	0.12	18.24	0.19
L6/7						
TAT	4.98	0.10	9.02	0.18	13.81	0.28
VAT	7.67	0.09	13.88	0.16	21.24	0.25
SAT	8.22	0.07	14.89	0.13	22.78	0.20

QCT, quantitative computed tomography; LM, lean mass; TAT, total adipose tissue; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; RMS-CV%, root-mean-square of coefficient of variation; RMS-SD, root-mean-square of standard deviation; LSC^{80}, least significant changes at 80% confidential intervals; LSC^{95}, least significant changes at 95% confidential intervals.

our study in female cynomolgus monkeys. LM increased in female cynomolgus monkeys with age until the age of 10 years and decreased rapidly with age after the age of 15 years. Colman *et al.* [2005] showed that whole-body LM measured by DXA reached a peak at approximately 14–15 years of age, after which it declined significantly with age in female rhesus monkeys (42). Although there were some differences between the whole-body LM measured by DXA and LM of paravertebral muscles measured by QCT, they exhibited similar characteristics regarding the changes associated with age. Our study describes age-related characteristics of the LM of paravertebral muscles, which may provide valuable information for sarcopenia-related studies.

The relationship between lumbar vBMD and abdominal adipose tissue remains controversial. Our study revealed that SAT had a positive effect on the average lumbar vBMD in the group aged ≤10 years, adjusted for BMI. Zhang *et al.* [2019] also showed a positive contribution of SAT to lumbar vBMD in women (23). Leptin is produced by SAT and affects bone metabolism and promotes bone formation (43). SAT may exert mechanical stress on the bone and therefore has a positive effect on BMD (26). However, some studies have shown no correlation between the SAT and the average lumbar vBMD in women (24-26). The reasons for this discrepancy may be due to differences in the sample size or the different groupings of the studies. Our study demonstrated that VAT was positively associated with the average lumbar vBMD in the Spearman correlation analysis, whereas it had no effect on vBMD in the ridge regression analysis in the group aged ≤10 years adjusted for BMI. This may be due to factors such as the BMI, which may confound the effect of VAT on the vBMD. Similarly, previous human studies showed that VAT was not correlated with lumbar vBMD (23,25). However, studies have shown that VAT has a negative effect on lumbar vBMD in women, and that increased VAT may be detrimental to the maintenance of bone mass (24,26). This may be due to the secretion of inflammatory mediators and adipokines by VAT, which affects the health of bones (44). These results suggest that the effects of VAT on vBMD may be complicated, and some differences may exist between humans and nonhuman primates. The correlation between adipose tissue and lumbar vBMD in nonhuman primates reported in this study needs to be further confirmed.

A study has shown that LM contributes to BMD and that an increase in LM is beneficial for maintaining bone mass (27). Muscles may produce osteocyte viability factors that exert a protective effect on osteocytes. The mechanical view implies that a decrease in muscle function leads to a reduction in skeletal load and consequently to a reduction in bone mass (45). Wagner *et al.* [2018] also showed that men with low LM may have accelerated bone deterioration, whereas those with higher LM and muscle strength had less deterioration of the bone microarchitecture (46). In female cynomolgus monkeys, our study showed no contribution of LM of the paravertebral muscles to the average lumbar vBMD in ridge regression analysis. This might be due to the fact that the postural or locomotor habits of primates are different from those of humans, and therefore the effect of LM on vBMD in primates may not be consistent with that of humans. Very few studies have reported similar issues, and more research is needed to elucidate these issues further.

This is the first study to report the short-term *in vivo* precision of QCT measurements of the lumbar vBMD and body composition in female cynomolgus monkeys. Short-term precision reflects the reproducibility of the measurement technique and allows for the calculation of the LSC, which can be used for efficacy assessment and estimation of the follow-up interval. Precision study is statistically required to have a degree of freedom of no less than 30 (34), and the ISCD recommends that new QCT techniques establish *in vivo* precision levels (12). Previous studies using QCT-based techniques to analyze the precision of BMD in nonhuman primates did not meet the above requirements (28-30), whereas our study met the relevant requirements and the results were reliable. For the short-term *in vivo* precision of our study, the RMS-CV% of single lumbar vBMD measured by QCT ranged from 0.72% to 1.60%, among which the precision of the L2 vertebra was the best. The RMS-CV% of the vBMD for the two-and three-lumbar combinations ranged from 0.50% to 1.15% and 0.47% to 0.85%, respectively. The precision of the average vBMD of the three adjacent lumbar combinations was relatively better than that of the two adjacent lumbar combinations, with the best combination of precision being for the L1-3 vertebrae. We selected the L2-4 vertebrae for vBMD measurements, as these sites also showed good precision. Wang *et al.* [2017] used asynchronous QCT in the European Spine Phantom to determine the RMS-CV%, which ranged from 0.2% to 0.7% between two scans, and the RMS-CV%, which ranged from 2.2% to 2.6% between observers (47). The precision of QCT in measuring human spinal trabecular BMD has been reported to be 1.3–2.4% (12). Another study reported that the precision

error of QCT measurements of spinal trabecular bone was 2–4% (48). Bligh *et al.* [2009] showed that the precision of helical multidetector-row QCT ranged from 1.4% to 3.6% (49). Regarding the precision of lumbar vBMD in female cynomolgus monkeys, the RMS-CV% of all measurements in our study was <2%, which is generally good and roughly consistent with that reported above. Therefore, QCT can be used for measuring lumbar vBMD in monkeys and accurately reflects their bone mass. In studies based on pQCT in nonhuman primates, Hotchkiss [1999] reported a precision <3% for the measurement of lumbar BMD in cynomolgus monkeys (28).

The present study showed that the RMS-CV% of the LM of paravertebral muscles measured by QCT in cynomolgus monkeys ranged between 2.47% and 5.40%, with the best precision in the L3 mid-plane, which was also the level analyzed in this study. The precision of LM measured in nonhuman primates using QCT has not been reported, and the precision results of our study may provide reference information for future related studies. This study showed that the RMS-CV% was <6.4% for TAT and <7.7% for VAT, and greater variability in the precision for SAT, ranging from 2.94% to 16.78%. The precision at the L4-5 intervertebral disc level was comparatively superior among the measurement levels; therefore, we selected this level to measure abdominal adipose tissue. Baum *et al.* [2012] showed that QCT had a reproducibility error of 0.12% for measuring SAT levels and 3.74% for measuring VAT levels in humans (50). This discrepancy may arise due to differences in measurement software and the fact that the above study measured the volume of abdominal adipose tissue, whereas our study measured the mass of abdominal adipose tissue. Contrarily, the small size of cynomolgus monkeys, the low levels of abdominal adipose tissue in some animals, and the very few animals in which SAT could not be measured may also account for the large variation in precision. In addition, since SAT levels in cynomolgus monkeys are significantly lower than those of TAT and VAT, it is more significantly influenced by respiratory movements, and the error of measurement is greater, resulting in a greater error of precision.

Our study had the following limitations. First, although the present study included female cynomolgus monkeys of different ages ranging from 1 to 25 years, the number of animals was unevenly distributed at each year of age, and the results may have been more conclusive if the number of animals was ≥5 per year of age. Second, due to differences in measurement techniques, our study could only analyze the correlation between lumbar vBMD and body components at the individual level based on QCT, and we could not confirm whether there was a correlation between lumbar vBMD and whole-body fat mass or between lumbar vBMD and whole-body LM. Third, long-term precision is crucial for efficacy monitoring and follow-up; however, due to limited experimental conditions, long-term precision studies were not performed in this study, and relevant data could not be obtained.

Conclusions

In conclusion, age-related changes in lumbar vBMD in healthy female cynomolgus monkeys based on QCT were similar to those in humans. Our study also described the age-related characteristics of body components in healthy female cynomolgus monkeys. Age and SAT levels are factors that may influence the lumbar vBMD in female cynomolgus monkeys. QCT showed good *in vivo* precision for measuring the vBMD of the lumbar spine in female cynomolgus monkeys.

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Ethical Statement: The authors are accountable for all aspects

of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Experiments were performed under a project license (No. LDIACUC2018-0004) granted by the Laboratory Animal Ethics Committee of Guangdong Landau Biotechnology Co., Ltd., in compliance with international Association for Assessment and Accreditation of Laboratory Animal Care guidelines for the care and use of animals.

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