Osteoporosis and Sarcopenia 4 (2018) 79–85

Contents lists available at ScienceDirect

Osteoporosis and Sarcopenia

journal homepage: http://www.elsevier.com/locate/afos

Review article

Bone mineral density assessment for research purpose using dual energy X-ray absorptiometry

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ARTICLE INFO

Article history:
Received 1 August 2018
Received in revised form
7 September 2018
Accepted 17 September 2018
Available online 27 September 2018

Keywords:
Dual energy X-ray absorptiometry
Bone mineral density
Research purpose
Human study
Small animal

ABSTRACT

Dual energy X-ray absorptiometry (DXA) has become the most common method for measuring bone mineral density (BMD) of small animals in metabolic bone disease research, and errors should be minimized in all procedures involved in research studies in order to increase the accuracy of the study results. DXA is simpler and rapid compared to micro-computed tomography for quantitative analysis of change in trabecular bone of test subject. In human research, measuring BMD is widely used; post-operative evaluation on orthopedic surgery, evaluation of osteoporosis medication in menopause and many other areas of study. For the study, the inspector should be trained by the equipment manufacturer regarding the utilization and analysis of the equipment and regular phantom testing should be conducted to ensure the stability of the equipment, and precision tests should be conducted to analyze the positioning and data analysis. They should also be familiar with the clinical trials and conduct studies based on the approval of the Institutional Review Board. In the absolute BMD measurement of the human body, it is necessary to apply and compare the position and condition, rotation degree, region of interest, and area of the scan in the follow-up test. In the case of small animals, animal selection, measurement and equipment should be modeled to match the research. Therefore, we would like to provide information for researchers to minimize the errors, effective data management and accurate data presentation. This article reviews the process of DXA measurement for research purpose including plan for DXA examination, BMD measurement in a human body study and small animal studies.

1. Introduction

Dual energy X-ray absorptiometry (DXA) is widely used method in research of bone disease in human and small animals. It is essential to minimize errors in every procedure during the scanning examination for more accurate result for the research.

One who performs the exam should be trained for utilizing the device and methods and techniques for acquiring and analyzing bone mineral density (BMD) scans. Also, they have to complete regular phantom test and precision test for positioning and data analysis.

Furthermore, it is necessary to follow general guidelines for clinical trials and instructions based on the research approval from Institutional Review Board (IRB).

For measuring human absolute BMD, proper scan position, degree of rotation, region of interest (ROI) and area should be maintained identically for serial follow-up exams.

In addition, studies using small animals have to be planned accordingly for research purpose by selecting proper animal, measurement method and equipment. Also in small animal research using DXA, quality control for animal condition and the equipment and processing thoroughly with acknowledging the check points in every step of the exam to minimize the errors; which can actually lead to confirm minor biological change statistically in the research.

Therefore, equipment quality control and precision test is basic principle for the research examinations.

DXA has become the most common method for measuring BMD

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Peer review under responsibility of The Korean Society of Osteoporosis.

https://doi.org/10.1016/j.afos.2018.09.003

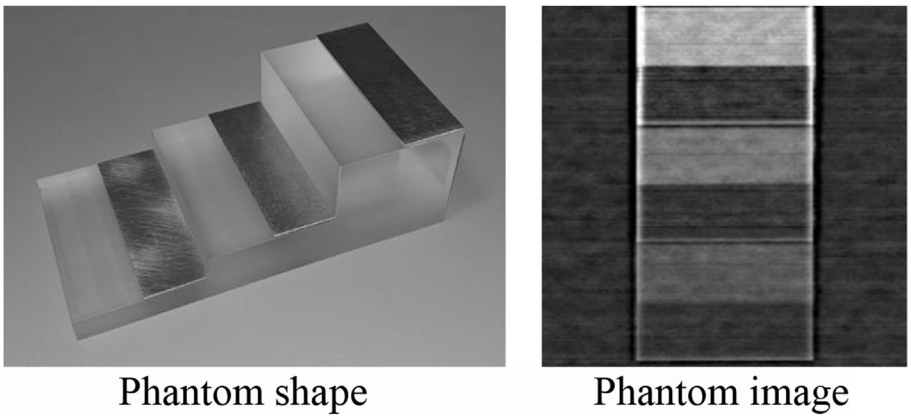


Fig. 1. Dual energy X-ray absorptiometry phantom for Hologic's animal scan only.

Table 1
Things to check and prepare before a research dual energy X-ray absorptiometry test.

Training related to image acquisition and analysis
Evaluate precision of equipment
Regular Measurements with Phantom
Evaluation of the precision of the Technologist
Calculate the least significant change value
Assess the reliability of a device

of small animals in metabolic bone disease research [1–5]. DXA is simpler and rapid compared to micro-computed tomography (micro-CT) for quantitative analysis of change in trabecular bone of test subject. Also, DXA requires less radiation exposure and more cost effective [6–8]. In human research, measuring BMD is widely used; postoperative evaluation on orthopedic surgery, evaluation of osteoporosis medication in menopause or reduced hormone levels after gynecologic surgery, effectiveness of medication, diet supplement or exercise, body fat composition change after diet control and many other areas of study.

In clinical research of small animals or human studies, DXA is used for monitoring BMD changes after taking certain drug or diet supplement or postoperative changes. For using statistical data from several species of animals, it is able to monitor BMD changes throughout one's life cycle with lesser cost and less invasive by no need to harm the animals for data collection.

To monitor changes in BMD accurately, it is necessary to complete quality control on the safety, accuracy and precision of the equipment and perform accuracy measure of test method and data analysis ability of examiner.

These studies should verify the precision and accuracy of the diagnostic method before starting the study [3,9–11]. To monitor the change in BMD with minimal error, it is essential to prove the quality control to ensure the stability of the instrument and the ability of the inspector to analyze the test methods and results consistently.

2. DXA BMD measurement

2.1. Plan for research examination

For starting the research project, researcher should ensure that the technician who performs the exam to be trained about equipment operation and software analysis. Also, researcher should review quality control for the equipment, measure precision of the technician and check safely of the equipment to minimize measurement errors. In addition, designated researcher for clinical research using DXA should complete education from Centers for Disease Control and Prevention according to each institute's IRB regulation [12]. This subject corresponds to the content of the South Korea and may differ in other countries.

2.1.1. Importance of precision in research examination using DXA

The most important indicator in determining the objectivity whether the difference in BMD over time in individual subjects using DXA is precision. Thus, many factors, such as equipment and factors related to the examiner and test subject influence the accuracy in measuring bone density [13].

Clinically, DXA bone density measurements require caution in object location and image analysis. Inadequately performed DXA analysis can lead to errors in diagnosis and treatment [14]. And in small animals, it can be more influenced by positioning error than in humans [15]. Therefore, it is particularly important in small

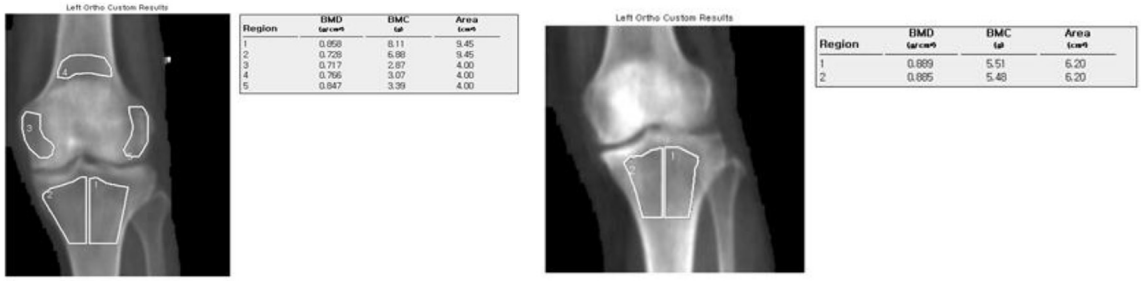
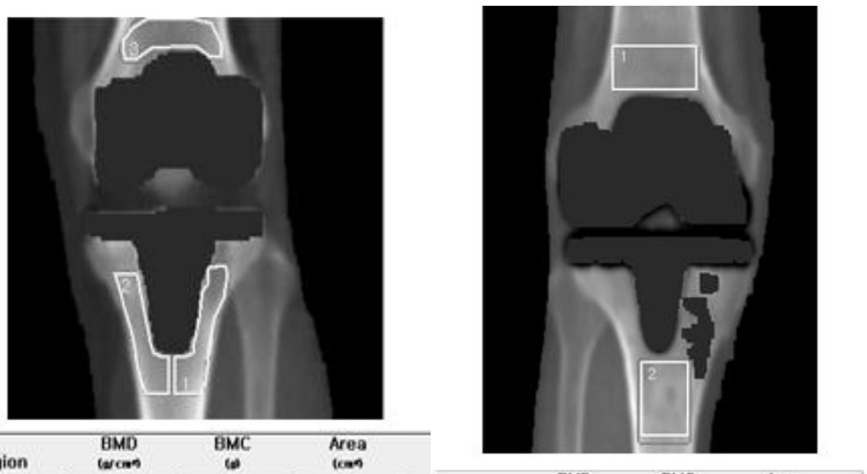


Fig. 2. TKRA (total knee replacement arthroplasty) preoperation evaluation example using dual energy X-ray absorptiometry. BMD, bone mineral density; BMC, bone mineral content.



Region	BMD (g/cm²)	BMC (g)	Area (cm²)
1	1.040	3.56	3.42
2	0.988	3.38	3.42
3	0.823	2.81	3.42

Region	BMD (g/cm²)	BMC (g)	Area (cm²)
1	0.779	4.97	6.38
2	1.075	6.87	6.39

Fig. 3. TKRA (total knee replacement arthroplasty) postoperation evaluation example using dual energy X-ray absorptiometry. BMD, bone mineral density; BMC, bone mineral content.

animal studies to obtain high-quality images in a well-placed, consistent position. To improve the accuracy, proper quality control should be achieved by using a dedicated phantom (Fig. 1). For each test, each subject should be able to reproduce the same posture, and it is advisable to record the relevant contents so that they can be applied to the follow-up exam. Finally, the results analysis using images should be an objective analysis (Table 1).

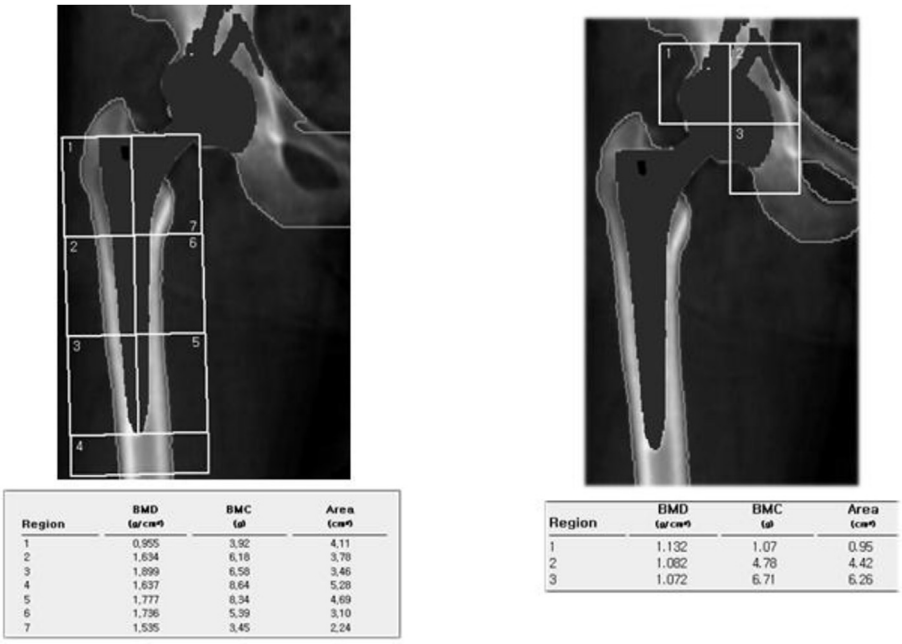
2.1.2. Clinical study and IRB

Clinical studies are conducted to identify the clinical and pharmacological effects of the drug and to investigate the adverse reaction to establish the safety and efficacy of the drug or medical device used in the clinical trial.

IRB is an independent committee established within the research organization to protect rights, safety and welfare of trial participants by examines the clinical trial plan, plan for changes, and forms and information used to obtain written consent from the subject continuously.

The person responsible for the research is who has entire authority and responsibility for conducting clinical trials at the testing laboratory and the researcher is a person who has proper education and training essential for clinical research and has adequate experience in the related field.

The clinical trial sponsor is the manufacturer of the drug or medical device which is responsible for the planning, administration, and financing, and the clinical trial entrustment organization is the individual or institute authorized by the sponsor [16,17].



Region	BMD (g/cm²)	BMC (g)	Area (cm²)
1	0.955	3.92	4.11
2	1.634	6.18	3.78
3	1.899	6.58	3.46
4	1.637	8.64	5.28
5	1.777	8.34	4.69
6	1.736	5.39	3.10
7	1.535	3.45	2.24

Region	BMD (g/cm²)	BMC (g)	Area (cm²)
1	1.132	1.07	0.95
2	1.082	4.78	4.42
3	1.072	6.71	6.26

Fig. 4. THRA (total hip replacement arthroplasty) postoperation evaluation example using dual energy X-ray absorptiometry. BMD, bone mineral density; BMC, bone mineral content.

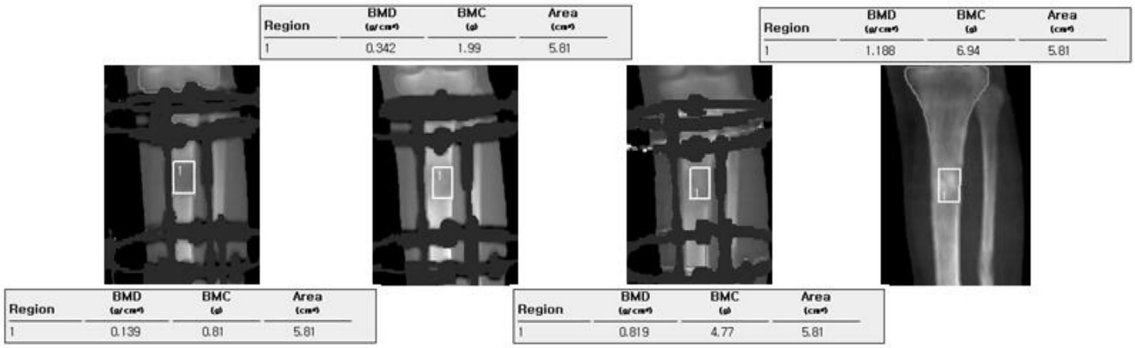


Fig. 5. Example of dual energy X-ray absorptiometry bone mineral density (BMD) evaluation over time in the same area. BMC, bone mineral content.

Table 2
Considerations for absolute bone mineral density measurements.

Are the images before and after surgery scanned in the same position and condition?
Is the measurement part rotating the same? Is the region of interest the same and the same area? Is the inclusion of soft tissue the same?

2.2. BMD measurement in a human body study

2.2.1. Absolute BMD

DXA is used to measure the BMD of a specific location in the body skeleton. The soft tissue is excluded and the ROI of bone alone is used for the evaluation (Figs. 2–5). Therefore, consideration should be given to the measurement of Absolute BMD in Table 2.

In addition, it is necessary to repeat measurements on the same equipment and to compare and observe changes over a certain period of time according to the Standard Operating Procedure.

2.2.2. BMD measurement of central bone and peripheral bone

In case of Cohort studies, scans are achieved once per subject, but follow-up tests are required for long-term studies that require drug administration for a certain period of time or follow-up after exercise. During the follow-up scan, images should be acquired under the same conditions in the same position, and the same inspector is advised to be inspected on the same equipment. The analyzed output of the results should be comparable with the same area in the same ROI.

2.3. BMD measurement of small animal studies

2.3.1. Selection of animals used in the research

In 2017, the number of animals used in the laboratory in Korea increased by nearly 70% compared to 2012, with an estimated 3 million animals by Animal and Plant Quarantine Agency. Rats account for 70%–80% of them. Rats are widely used for experimental purposes since they are vertebrate and biologically similar to humans. In addition, they are easy to keep and breeding because they require only small space. In addition, the life span is about 2 years, therefore the generation increases rapidly, which makes the experiment easy.

The experimental animals have differenct characteristics (Table 3). Due to the relatively small body size of the mice, it is difficult to perform the same examination with the previous DXA scan after long time, and the error of the bone edge is uncertain due to the relatively low resolution of DXA [18].

The study modeling of small animals is based on setting the similar conditions for small animals' breeding environment (photoperiod, temperature and humidity of the breeding room, food supply), and select the age and weight at the time of research examination. In addition, the test equipment and the inspector should be the same, and they shall be divided into a control group and a group to change the experiment condition.

Method of anesthesia and number of tests should be same for each group. After completion of the experiment, the schedule should be determined based on whether or not to sacrifice small animals and measurement of bone volume are to be performed after the study.

Table 3
Characteristics and research areas of experimental animals.

Experimental animal	Characteristic	Research areas
Mouse	Short cycle of various systems Lifetime (Fast Generation Replacement) Excellent food skills, good breeding	Oncology, pharmacology, genetics, endocrine study
Rat	Similar to humans in pharmacology, metabolism, biochemistry, and nutrition	Oncology, toxicology experiment
Hamster	Special sensitivity to nocturnal and mild viruses (studies of rabies, Japanese encephalitis, etc.) Observe *in vivo* reproduction and spread of cancer cells	Oncology, development and aging studies, virus studies
Guinea pig	A study on the sensitivity to antibiotics (penicillin) (no synthesis in vitamin C) Measurement of oxygen consumption	Pharmacology, immunology, pathology, metabolic research
Rabbit	For the purpose of injecting and collecting blood vessels using large ears and thick vessels	Pharmacology, immunology, blood and pathology, metabolic research, endocrine study
Pig	Similar to humans in formal and physiological characteristics	Circulation system, digestive tract research metabolism, nutritional studies, skin studies
Dog	Gentle, easy to raise, highly prolific and easy to handle	Surgery, circulation, neurophysiology, digestive studies, endocrine studies, respiratory, dental and urinary studies

Adapted from Ministry of Food and Drug Safety (Korea).

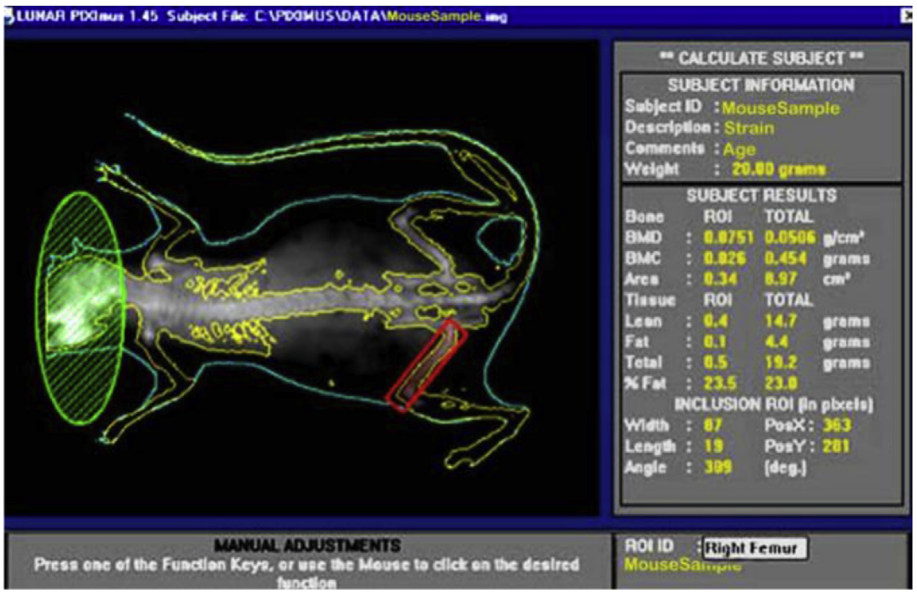
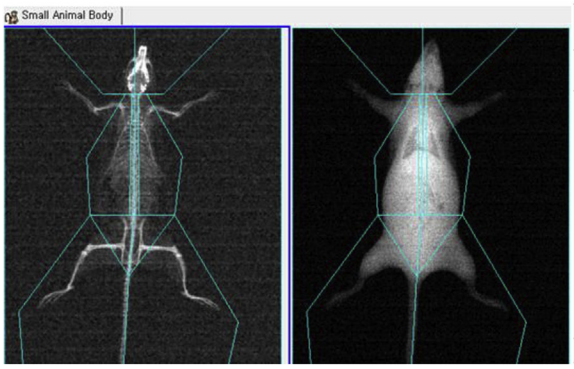


Fig. 6. Mouse scanned with PIXImus, a small animal only equipment.

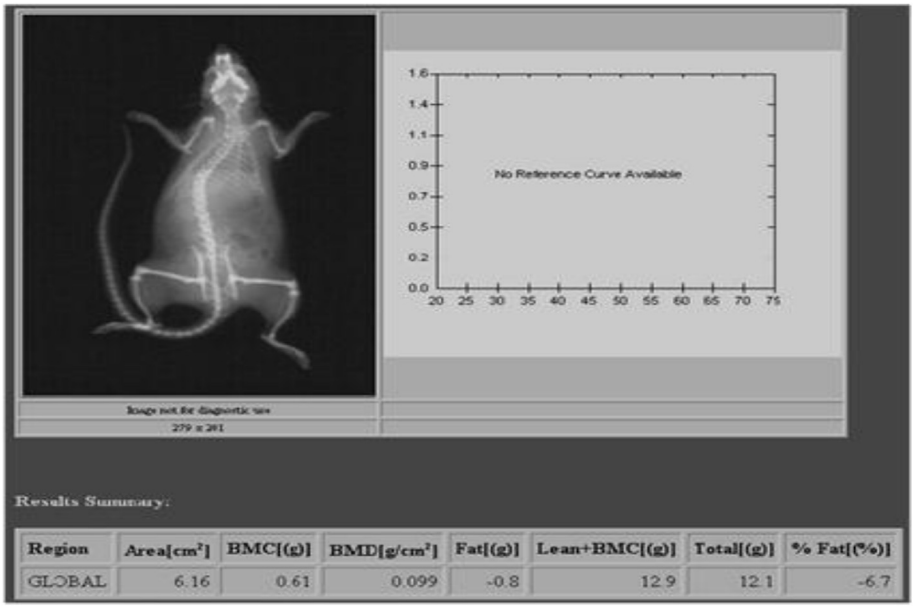


Densitometry | Composition | Information

Densitometry

Region	BMD (g/cm²)	BMC (g)	Area (g/cm²)
Head	0,285	2,4	8
Left Arm	0,112	0,3	3
Left Leg	0,141	1,0	7
Left Trunk	0,151	2,4	16
Left Total	0,175	5,6	32
Right Arm	0,116	0,3	3
Right Leg	0,140	1,3	9
Right Trunk	0,151	2,3	15
Right Total	0,150	4,4	29
Arms	0,114	0,6	5
Legs	0,141	2,3	16
Trunk	0,151	4,7	31
Ribs	0,110	1,5	14
Pelvis	0,172	1,6	10
Spine	0,196	1,6	8
Total	0,163	10,0	61

Fig. 7. iDXA small animal scan and standard analysis image. BMD, bone mineral density; BMC, bone mineral content.



Region	Area[cm²]	BMC[(g)]	BMD[g/cm²]	Fat[(g)]	Lean+BMC[(g)]	Total[(g)]	% Fat[(%)]
GLOBAL	6.16	0.61	0.099	-0.8	12.9	12.1	-6.7

Fig. 8. Hologic QDR (Marlborough, MA, USA) animal scan analysis image.

Table 4
Types and characteristics of small animal bone mineral density measurement system.

Manufacturer	MEDIKORS	Norland	GE Lunar
Equipment name	InAlyzer	pDEXA sabre	PIXImus
Scan method	Fan beam	Multiple detection Rectilinear scan	Cone beam
Field size	140 mm × 210 mm	150 mm × 120 mm	80 mm × 65 mm
Small animal size	10–50 g	Less than 27 kg	10–50 g
Scan and processing time	5 min	1 sec–40 min	5 min
Precision	Less than 1% (CV)	Less than 2%	1%–2%
Resolution	108 μm × 108 μm	0.1 mm × 0.1 mm	0.18 mm × 0.18 mm
Equipment image			

CV, coefficient of variation.

Table 5
Check lists for dual energy X-ray absorptiometry bone mineral density measurement in small animals.

Check lists before examination	
Research animals	Check for cleanliness, food intake, sleep (anesthesia) status
Examination equipment	Verification of quality control (Phantom scan)
Radio-technologist	Confirm precision test for equipment use and result analysis
Check lists during examination	
Research animals	Check movement and sleep (anesthesia) status
Examination equipment	• Check for foreign body in the inspection area • Check for normal operation in Examination equipment
Radio-technologist	• Enter information for small animals, Examination position of small animal (Image verification if there is previous examination) • Preview scan (check ROI setting status), scan mode, scan area, length and width of scan area
Check lists after examination	
Research animals	Identification of small animal species and sleep (anesthesia) state
Examination equipment	Check for error in examination equipment
Radio-technologist	Image verification (movement, foreign body, flexion of vertebra, posture of limb) and calculation of result

Analysis of the images follows the manual of each equipment.

Table 6
Examples of mice positions in dual energy X-ray absorptiometry (DXA) measurements for research purpose.

The position of the mouse in DXA measurements	
Whole body	Confirm scan range with increasing length and weight
Head	Measurement by site: no reposition
Spine	Keep your tail and head as close as possible to a straight line.
Fore legs	Head direction/not overlap or rotate
Back legs	Head direction/not overlap or rotate
Tail	Included in the scan range/not overlap

2.3.2. BMD measurement of small animals

There are invasive and noninvasive methods in BMD measurements of small animals. In the invasive method, there is bone content measurement (ash content weight). Bone ash is a white particles obtained by burning animal bones without fat or colloid. Measurements of bone ash volume are done by measuring the weight and volume of the femur of the small animal, drying it at low temperature and then convert into ash in high temperature. After the process, we quantify the remaining inorganic substances.

Compared to the noninvasive method, the accuracy is comparatively high, but the process takes 5–7 days [19]. The noninvasive method is to measure the BMD, bone mass (bone mineral content, BMC), and muscle mass (lean), and fat by DXA scan using DXA, micro-CT equipment on live animals. BMD and BMC can be analyzed on specific ROI, and dual energy can be used to reduce the measurement error due to under measurement of soft tissue. Studies using DXA are also useful for verifying the effects of food or medicines on bone disease such as osteoporosis by analyzing BMD changes. Other benefits include cost effectiveness and less time consuming.

2.3.3. Selection of measuring equipment for small animal study

In general, DXA, which is the most suitable method for BMD measurement, is mainly used in small animal study [20]. Since X-ray is used for the scan, resolution is good and it take less time with negligible radiation exposure (<3 mR) [21]. The device for measuring the spine and femur, which is the central bone, is Central device, and the device for measuring the peripheral bone is Peripheral device. Also, the animal BMD measuring device is called Small animal device and it is widely used in animal trials.

Micro-CT, which scans small animals in high resolution is easy to observe microstructure of living tissues because various functions of living organisms are closely related to their structures in medical and biological studies.

In addition, it is possible to visualize the progress of the disease and the treatment in noninvasive method during the life span of small animal without sacrificing the experimental animal. As bone structure can be quantified in three dimensions, it is convenient and accurate therefore it can analyze bone in 2 dimensions and

three dimension [22].

It is known that BMD, a conventional bone strength measure and micro-CT index are relevant. The relationship between histomorphometrical parameters and micro-CT index has also been suggested [23,24]. Therefore, micro-CT can be used in many life sciences researches by accurate diagnosis of disease status such as gene expression and protein toxicity test and new drug development.

2.3.4. BMD measurement equipment used in small animal studies

In general, BMD equipment used in the study is typically dedicated to the characteristics of small animals (Fig. 6). However, if there is a restriction on the use of dedicated equipment, additional software for animal scanning can be used in the center bone DXA equipment (Figs. 7 and 8). However, DXA instruments for small animals have different specification by each company, changes in BMD trend can be confirmed but it is inappropriate to make comparison (Table 4). Therefore, it is appropriate to use the same equipment when follow-up inspection is required.

2.3.5. Checklist for DXA BMD of small animals

In the case of DXA BMD measurement using small animals, it is necessary to check study animal, test equipment and examiner's checklist before, during, and after the test to minimize errors (Table 5).

2.3.6. Positioning of small animals in DXA BMD

The most common error in DXA measurements of small animals is derived from the positioning of small animals [3,9–11]. Therefore, standardization of posture during BMD measurement is required. For example, mouse is a small animal preferred for osteoporosis research because of its genetic, developmental, and biochemical similarity to humans and its response to hormones related to calcium metabolism is similar to that in humans [25–28] and the position of the head, spine, foot and tail should be established so that they can be examined consistently (Table 6). Measurements of length and weight are essential before measuring changes in body and partial bone density, since long-term measurements can vary significantly by size and weight than short-term measurements.

Previous studies have reported that there is no significant difference in the precision of the positional change, except for the skull [24].

3. Conclusion

The DXA BMD exam for the study is usually applied on human or small animals. It is important to minimize the errors caused by the condition of the equipment or the inspection and analysis of the inspectors, to detect incidence of small biological changes. Therefore, quality control of equipment and inspectors is essential. In long-term studies, the human body should be tested under the same conditions (same area). In case of small animals, follow-up tests should be performed considering development status, foreign body and test position for possible analyses of statistically significant results.

However, since bone density measurement using DXA provides results for areas other than volume, it should be considered that there is a difference from the actual BMD to apply to the research study.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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