Verification of precision, accuracy, and characteristics of dual-energy X-ray absorptiometry and nuclear magnetic resonance in the analysis of mouse body composition

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Research

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Abstract

Background: As an instrument for measuring body composition in experimental animals, dual-energy X-ray absorptiometry (DXA) is ideal for accuracy, cost, and measurement efficiency. However, there is too little insight into the effectiveness of the various aspects of applying DXA to experimental animals. Therefore, we investigated whether to compare and verify the precision and accuracy of DXA and nuclear magnetic resonance (NMR) animal body composition analyzers. We used 30 ICR mice in the study. First, in order to evaluate the reproducibility of DXA and NMR, we did repeated measurements by repositioning each mouse in anesthesia and euthanasia states. Subsequently, the accuracy of each device was evaluated by comparing the weight measured before the experiment, the weight of the tissue extracted from the mice after the experiment, and the measured DXA and NMR. In addition, when measuring the body composition of animals, we compared the time and the measurable body composition parameters and summarized the advantages and disadvantages of the two devices.

Results: Compared to NMR, DXA had the advantage of a fast measurement of bone composition and rapid image analysis. In addition, DXA showed a higher correlation ($> 95\%$) with FM, body weight, and fBMC baseline than did NMR ($> 85\%$).

Conclusion: In conclusion, DXA was confirmed to have higher precision and measurement accuracy than did NMR. Therefore, DXA is an effective method for evaluating the body composition of experimental animals.

Background

Animal clinical trials are conducted in various ways in order to verify the effects of various physical activities of exercise on health as well as for research on the treatment of diseases and the efficacy of food. In addition, animal clinical trials are a procedure that verifies the safety of new drugs or treatments before humans are exposed to new molecular entities [1, 2].

The most common model for clinical trials uses mice, because they are mammals who share 99% of their genes with humans [3], and because there are outstanding genetic resources and sophisticated genetic engineering techniques available for genome manipulation [4]. In addition, such a model allows one to check the results in a short time because of mice's short life. Of the many indicators used to confirm the condition in animal clinical trials, the most basic ones are body components, such as weight, fat mass, and bone mineral content. Therefore, there are various experimental methods for measuring these body components.

Methods of measuring body composition in human clinical practice include underwater weighing [5] and Bod Pod [6], which are very accurate. However, they require a lot of space, are hassle in measurement, and are expensive. In addition, skinfold thickness measurements [7] and bioelectric impedance analysis [8] have a disadvantage, in that the errors caused by the measuring operators and measurement timing are very large. Therefore, dual-energy X-ray absorptiometry (DXA) is recognized as the "gold standard"

because of its accuracy, cost, and measurement efficiency [9–11]. As with humans, animal body composition measuring equipment should be able to identify factors such as body weight, body fat, and bone mineral content, and also consider the cost and accuracy of measurement. Given these factors, DXA is the most suitable instrument for measuring body composition for animals. Nevertheless, insight on efficiency in various aspects to apply DXA to animals is lacking.

We investigated whether to compare and verify basic performance in terms of precision and accuracy for DXA and nuclear magnetic resonance (NMR) animal body composition analyzers.

Methods

Experiment outline

We divided the mice into three groups based on weight. At the time of initial preparation, each mouse was measured once to determine whether it could grow according to the reference range of each group. Then, it was measured once again on the day of the experiment to find out if had reached the target range. We did the same for all the prepared mice. The entire experiment was done in accordance with the guidelines of the Animal Experimental Ethics Committee of the Agency for Korea National Food Cluster (approval number: KNFC-IACUC-20-001).

Animals

We purchased male ICR mice ($n = 30$) from Orient Bio (Seongnam, Gyeonggi-do, Republic of Korea). We provided all animals with *ad libitum* access to both water and normal diet feed. We kept the mice in a room maintained at $23\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$ with $50\% \pm 20\%$ relative humidity and a 12-h light/12-h dark cycle. After the one-week adaptation period, we divided the mice into a low-weight group ($n = 10$), mid-weight group ($n = 10$), and high-weight group ($n = 10$). The target body weight of the low-weight group was 10–20 g, that of weight of the mid-weight group was 20–30 g, and that of the high-weight group was more than 30 g. We sacrificed all mice in each group when they reached the target weight.

Procedure

When measuring DXA (iNSIGHT VET DXA, Osteosys, Seoul, Korea) or NMR (EchoMRI™-700, EchoMRI, Houston, TX, USA), there are a total of two measuring conditions for animals, which are defined as euthanasia with repositioning (RE), anesthesia with repositioning (RA). In the initial preparation of all animals, the weight is measured once using an electronics scale. In addition, this value is defined as a reference value of weight for accuracy verification by measuring it once more on the day of the experiment.

Dual energy X-ray absorptiometry (DXA) measurement

On the day of the experiment, we measured all mice seven times each with DXA after anesthesia. We repositioned animals at the initial three measurements, and posture at the 3rd measurement was maintained for the next four measurements. On the day of the experiment, after anesthesia, we measured

all mice seven times with DXA. After relocating the animals in the initial three measurements, the posture was maintained during subsequent measurements. The time required for one measurement of DXA equipment was measured with a stopwatch. The time required was defined as the time until the operation was completed after pressing the measurement start button of the equipment software. We used the DXA equipment to collect fat mass (FM), lean mass (LM), body weight, bone density (BMD), bone mineral content (BMC), and fat percentage of the entire body range. In addition, we collected left femur BMC (lfBMC) and femur BMC (fBMC) by designating the femur region as a local region of interest (ROI) value. Finally, we collected images provided by DXA.

Nuclear magnetic resonance measurement

On the day of the experiment, we measured all mice three times each by NMR after anesthesia. We repositioned the animals at every measurement. On the day of the experiment, we measured all mice three times each by NMR after euthanasia. We repositioned the animals at every measurement. We measured the time required for one measurement with the NMR equipment with a stopwatch. The time required was defined as the time until the operation was completed after pressing the measurement start button of the equipment software. We collected FM, LM, and body water over the entire body range using NMR.

Autopsy

After we completed the measurement with all equipment, the mouse was autopsied, and after we extracted the main tissue, the mouse was weighed by using an electronic scales. We combined the weights of kidney adipose tissue, epididymal adipose tissue, intestine adipose tissue, and subcutaneous fat, and defined it as a reference value of FM for accuracy verification. The sum of the measurements of left femur and right femur was defined as femur mass and as the reference value of fBMC for accuracy verification. We selected one mouse from each group (low-weight, moderate-weight, and high-weight groups) and measured the time required for autopsy, which was defined as the time from the moment of taking the action necessary to autopsy the euthanasia animal to the completion of weighing for all items.

Statistical analysis

To evaluate the precision, we calculated the coefficient of variation (CV) of each item in the repeated measurement data. The mean $\pm$ standard deviation (S.D.) of the CV values for each measurement is summarized according to the mouse condition. In order to evaluate the accuracy, we summarized average residual values for each measurement r^2 value of the fitting linear model together with reference values. In addition, we did independent two-sample t -tests with equipment measurement and reference values to find the statistical significance of the mean values. Statistical analysis of the fitting linear model and independent two sample t -test used R software (version 3.6.1).

Results

Precision

We derived the CV of body composition measurement results according to the measuring conditions of all mice (Table 1). For the analysis of body composition elements, we derived the average value by classifying the repositioning condition (RE, RA) for each equipment for precision analysis. For both RE (3.88 ± 2.53) vs. RA (17.00 ± 12.91), FM had lower CV than NMR (5.05 ± 3.58 vs. 14.96 ± 10.46). Also, in both RE (0.43 ± 0.25) and RA (2.75 ± 1.70) in LM, DXA had lower CV than NMR. We measured body weight, BMC, and IfBMC in DXA but not in NMR. In DXA, the CV values of body weight, BMC, and IfBMC were lower in RE than in RA, which was consistent with the direction of FM and LM (FM > LM). We measured CV of body water only in NMR, but it was lower in RA than in RE as in FM and LM. In particular, the CV value in the RE (26.66 ± 43.77) condition of body water was the highest regardless of all measurement tools, measurement conditions, or measurement items.

Table 1

Results of dual energy X-ray absorptiometry (DXA) and nuclear magnetic resonance (NMR) according to repeated measures

		DXA (total, $n = 30$)	NMR (total, $n = 30$)
FM	RE	3.88 ± 2.53	17.00 ± 12.91
	RA	5.05 ± 3.58	14.96 ± 10.46
LM	RE	0.38 ± 0.18	3.91 ± 2.39
	RA	0.43 ± 0.25	2.75 ± 1.70
Body weight	RE	0.27 ± 0.15	NA
	RA	0.41 ± 0.34	NA
Body water	RE	NA	26.66 ± 43.77
	RA	NA	13.65 ± 29.73
BMC	RE	2.36 ± 1.44	NA
	RA	2.90 ± 1.85	NA
IfBMC	RE	6.38 ± 3.73	NA
	RA	7.70 ± 5.95	NA
Unit: gram. All data are presented as the mean $\pm$ standard deviation (S.D.). RE = euthanasia with repositioning, RA = anesthesia with repositioning, FM = fat mass, LM = lean mass, BMC = bone mineral content, IfBMC = left femur bone mineral content.			

In order to confirm the error of measurement according to the body weight, the weight was divided in the three grades; the measured CV is shown in Table 2. For DXA, we confirmed that the CV value in all items decreased as the body weight increased, regardless of the measurement conditions. However, for NMR, the CV value of the low-weight group was high in both RE and RA. In addition, NMR showed that in all

items except for FM under RA, the CV values were high in the order of low-weight, high-weight, and mid-weight. For LM, unlike FM, there was no tendency to increase or decrease in group average and CV.

Table 2
Results of DXA and NMR according to repeated measurements classified by body weight

		RE		RA	
		DXA	NMR	DXA	NMR
Low-weight (n = 10)	FM	5.63 ± 2.90	27.03 ± 14.69	6.92 ± 4.72	19.69 ± 13.08
	LM	0.36 ± 0.21	6.11 ± 2.17	0.44 ± 0.32	2.75 ± 1.93
	BMC	3.51 ± 1.56	NA	3.51 ± 1.56	NA
Mid-weight (n = 10)	FM	3.47 ± 2.01	6.11 ± 2.17	5.48 ± 2.58	15.28 ± 8.69
	LM	0.41 ± 0.17	2.51 ± 1.49	0.42 ± 0.25	2.75 ± 1.93
	BMC	1.70 ± 1.08	NA	2.47 ± 2.11	NA
High-weight (n = 10)	FM	2.54 ± 1.61	8.29 ± 4.29	3.17 ± 2.58	8.29 ± 4.29
	LM	0.38 ± 0.15	2.91 ± 1.49	0.44 ± 0.21	2.66 ± 1.70
	BMC	1.86 ± 0.89	NA	2.47 ± 2.11	NA
Unit: gram. All data are presented as the mean ± standard deviation (S.D.). RE = euthanasia with repositioning, RA = anesthesia with repositioning, FM = fat mass, LM = lean mass, BMC = bone mineral content, BMC = bone mineral content.					

Accuracy

We compared the measurements of each body component according to the measuring conditions with the reference values (Table 3, Fig. 1). For indirect comparison of the measurement accuracy of DXA and NMR, we compared them against a reference value measured by an electronic scale and expressed the values as mean signed difference (MSD) and r^2 values [MSD (r^2)]. For FM, there was no significant difference in either RE or RA of DXA compared to the reference value. However, compared to the reference value, NMR had a significantly difference in both RE and RA ($p < 0.05$). NMR could not measure body weight and fBMC, because of the relatively long measurement time compared to DXA. Although comparison with NMR was not possible, DXA showed a high correlation ($r^2 > 0.95$) with body weight.

Table 3
Correlation between measurement results and reference values of DXA or NMR

		DXA (total, <i>n</i> = 30)	NMR (total, <i>n</i> = 30)	Ref. (electronic scale)
FM	RE	0.61 (0.959)	2.08 (0.851) *	1.63 ± 1.51
	RA	0.49 (0.965)	1.63 (0.916) *	
Body weight	RE	-0.80 (0.996)	NA	29.36 ± 10.16
	RA	-0.60 (0.996)	NA	
lfBMC	RE	-0.108 (0.954)	NA	0.133 ± 0.060
	RA	-0.108 (0.961)	NA	
Unit: gram. All data are presented as the mean ± standard deviation (S.D.). Ref = reference value, RE = euthanasia with repositioning, RA = anesthesia with repositioning, FM = fat mass, LM = lean mass, BMC = bone mineral content, BMC = bone mineral content. * <i>p</i> < .05, vs. Ref.				

Discussion

DXA is a method of deriving body composition results using the difference between high-energy X-ray images and low-energy X-ray images [12]. NMR is a technology that generates high frequencies, measures the difference in signals from each tissue, and then reconstructs them by means of a computer to image them.

Compared to X-ray or computed tomography (CT), NMR has no radiation exposure. Its image contrast and resolution has excellent advantages for soft-tissue and brain examination [13], but it has a disadvantage in that it takes longer to measure than does DXA. In addition, the caustic ratio is poor for measuring only body composition. Therefore, if the measurement precision and accuracy of body components are higher than those of NMR, using DXA is much more effective.

In order to reduce the errors in measuring the experimental animals, what is most important is the degree of fixation at the time of measurement. It is natural that accuracy is higher in euthanasia than in anesthesia, because under anesthesia, there is a high possibility of errors caused by differences in fine movements that depend on the degree of breathing and level of anesthesia.

The precision of FM and LM in DXA was higher than that of NMR (Table 1). The rest of the items could not be compared between DXA and NMR, because DXA classifies body components by the contrast of the X-ray image (Fig. 2); so body water is included in the LM and classified. Also, because NMR is analyzed using the hydrogen spin, bone-related contents are not measured (see manufacturer's description). Although it was impossible to compare it with DXA, the standard deviation of body water was very large because of the characteristic of the NMR. Overall, it seems that neither DXA nor NMR seems to be suitable for measuring body water. Therefore, in order to measure body water, we judge that

it would be more accurately measured by other methods, such as electrical bioimpedance measurement [14].

FM had an error of less than about 1 g compared to the reference value and showed a correlation of more than 95%. The error of NMR was + 2.08 g, which was larger than that of DXA. Since NMR showed a correlation of up to about 92% with the reference value, we concluded that DXA has higher estimation accuracy for the reference value than does NMR. When compared to the actual mouse body weight, DXA measured about 2.04% – 2.96% lower. However, we found that the body weight difference between individuals was measured with an accuracy of 99% or more ($r^2 > 0.996$) under all measurement conditions. The reason why the overall weight was measured was not confirmed in this experiment. However, since it is easy to obtain and there is no problem in using it with DXA, we recommend weighing the body using an electronic scale.

The r^2 value of fBMC measured in DXA showed a correlation of more than 95% with the reference value. Therefore, we judge that the difference between the bone components of the mouse individuals is relatively good. In DXA, the fBMC value was estimated to be under about 82% of the reference value. The reason for the large difference is that the reference value and fBMC measured by DXA are not actually the same component. DXA's fBMC measures the mineral component existing in the ash state by drying and completely burning the femur bones, because this study defined the reference value as the weight of the femur bone after extraction. Therefore, the value of fBMC measured by DXA may be more accurate than is the reference value. There are several studies supporting this. In a study of wet weight, dry weight, and ash weight in tibia of 3-week-old rats, the dry weight and ash weight were 53% and 26% of the wet weight, respectively. Also, the ash weight was 47% of the dry weight [15]. In another study, the dried and ash weights were 67% and 32% of the fresh weight of the right tibia and femur of hens [16]. Another study found that the ash weight of the femur in mice was 58% of the dry weight [17]. DXA has the advantage of being able to simultaneously do both bone-related measurements and quantitative analysis of soft tissues, such as fat and lean. In addition, since X-rays used in DXA react with all substances and are attenuated, weight can be accurately calculated, and image analysis information on the shape or fat distribution of bones and organs is also provided through measurement images. However, since the X-ray image provided by DXA is a two-dimensional cross-sectional image, the amount or accuracy of information may be deteriorated compared to the three-dimensional structural information that can be observed by autopsying animals. Also, the DXA method has a disadvantage in that it is impossible in principle to obtain components other than bone, fat, and lean, because the components are classified using the differences in the object transmission characteristics of the two X-rays. However, studies have been done to obtain other components, such as moisture and protein using a statistical estimation method using DXA [18, 19].

NMR has a longer measurement time than DXA, but has the advantage of being able to measure without anesthesia even when the animal is alive. In addition, NMR equipment can measure water in addition to fat and lean. However, since there are body tissues that cannot be measured, because they do not respond well in the NMR method, such as bone, NMR contains a fundamental error in weight estimation.

In addition, there is a disadvantage in that visual information cannot be acquired, because an image of the measured animal is not provided separately.

Conclusion

DXA had higher precision and accuracy of measurement than did NMR. These results showed the same tendency when the experimental animals were anesthetized, euthanized, and classified according to body weight.

Abbreviations

DXA

Dual energy X-ray absorptiometry

NMR

Nuclear magnetic resonance

RE

Euthanasia with repositioning

RA

Anesthesia with repositioning

FM

Fat mass

LM

Lean mass

BMC

Bone mineral content

fBMC

Femur BMC

lfBMC

Left femur BMC

ROI

Region of interest

Declarations

Ethics approval and consent to participate:

The entire experiment was done in accordance with the guidelines of the Animal Experimental Ethics Committee of the Agency for Korea National Food Cluster (approval number: KNFC-IACUC-20-001).

Consent for publication:

Not applicable

Availability of data and material:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests

Funding:

Not applicable

Authors' contributions:

KWB, JSK and SJK dissected the experimental mice. KWB operated animal body composition analyzer, and was a major contributor in writing the manuscript. JSP and YCH analyzed all the data in this study. JIY planned and led this study. All authors read and approved the final manuscript.

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Not applicable

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Figures

Figure 1

Scatter plot of correlations between measurement results and reference values of dual-energy X-ray absorptiometry or nuclear magnetic resonance. RE = euthanasia with repositioning, RA = anesthesia with repositioning, FM = fat mass, fBMC = femur bone mineral content.

Figure 2

Measurement images provided by dual-energy X-ray absorptiometry used in this study.