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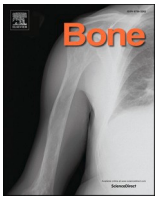

Full Length Article

Small animal DXA instrument comparison and validation

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ABSTRACT

Several new peripheral dual-energy X-ray absorptiometry (DXA) devices designed for assessment of bone and body composition in rodents have been developed. We compared the performance (accuracy and precision) of two of these devices, the InAlyzer and the iNSiGHT, to those of an established device, the PIXImus. We measured total body bone mineral content (BMC), bone mineral density (BMD), and body composition (lean and fat mass) on the three DXA devices in 18 male C57Bl/6 J mice (6 each of ages 8, 14, and 24 weeks, weighing 22 to 33 g). DXA body composition measures were compared to whole-body nuclear magnetic resonance (NMR) outcomes. BMC of the femur was also compared to ex vivo micro-computed tomography (microCT). Total body BMD from the InAlyzer and iNSiGHT devices was strongly correlated to that from PIXImus (R^2 = 0.83 and 0.82, respectively), but was ~25 % higher than PIXImus. Total body BMC measures by InAlyzer were strongly associated with those from PIXImus (R^2 = 0.86), whereas those from iNSiGHT were only weakly correlated (R^2 = 0.29). Femur BMC from InAlyzer was strongly correlated with microCT outcomes, whereas iNSiGHT was only weakly correlated. InAlyzer and iNSiGHT fat mass measures were very strongly correlated with PIXImus and NMR outcomes (R^2 = 0.91 to 0.97), with slightly weaker associations for lean mass (R^2 = 0.81 to 0.76). Short-term precision of InAlyzer and iNSiGHT measurements were excellent, and akin to those from the PIXImus for both body composition and bone measures, ranging between 0.39 and 3.2 %. With faster scan times, closed X-ray source and excellent precision, the new devices are both satisfactory replacements for the now discontinued PIXImus system. However, given the accuracy of the bone and body composition measures, the InAlyzer may be preferable for studies where musculoskeletal changes are the main interest.

1. Introduction

Obesity and osteoporosis are highly prevalent conditions for which rodent models are frequently used to study pathophysiology and examine possible treatments. Peripheral dual-energy X-ray absorptiometry (DXA) has been used to measure bone mass and body composition in vivo in rodents for several decades. Unlike carcass analysis and bone ashing, DXA is a noninvasive technique that allows for longitudinal assessments of body composition and bone mineral density (BMD) with limited radiation. In particular, the PIXImus peripheral DXA system (GE-Lunar, Madison, WI), has been used extensively for whole-body in vivo BMD measures of mice [1–4]. However, as the PIXImus has been discontinued and newer devices have become available, there is a need to evaluate the performance of these newer instruments with respect to accuracy and precision of BMD and body composition measurements.

Thus, we aimed to evaluate two new devices, the InAlyzer (Medikors, Seoul, South Korea) and the iNSiGHT (Osteosys, Seoul, South Korea), by comparing them to the existing PIXImus system. While the PIXImus system has been widely used for several decades, the InAlyzer and iNSiGHT systems may provide faster and safer means of evaluating body composition and BMD in mice. Unlike the PIXImus, the InAlyzer and iNSiGHT systems are closed cabinet systems with lead shielding inside the devices, effectively blocking radiation exposure to the operator during the scan. Moreover, the InAlyzer and iNSiGHT measurements are acquired in <2 min, whereas the PIXImus measurements take 5 min per mouse. Given these advantages, we aimed to determine the accuracy, precision and comparability of body composition and BMD measurements from InAlyzer, iNSiGHT, and PIXImus devices to determine the suitability of these new devices for research studies in mice.

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2. Methods and materials

2.1. Overview of study design

To compare the precision and accuracy of the DXA devices, we obtained mice of three different ages to have a broad distribution of body composition. We assessed the precision of body composition (fat mass, lean mass) and bone (bone mineral density, BMD; bone mineral content, BMC) measurements from the three DXA systems by performing three repeat measurements on each mouse and repositioning between measurements. We evaluated the accuracy of body composition measurements by comparing the DXA measurements to those obtained by whole-body nuclear magnetic resonance (NMR) imaging, which we considered as an alternative for in vivo for body composition measurements. We assessed the accuracy of whole-body bone mass measurements made by the InAlyzer and iNSiGHT DXA systems by comparing measurements to those from the PIXImus. Finally, we compared the accuracy of regional in vivo assessment of bone mass measurements of the femur made by the three DXA systems to ex vivo microCT.

2.2. Animals

We obtained C57BL/6 J male mice aged 8, 14, and 24 weeks ($n = 6$ per group, The Jackson Laboratory, Bar Harbor, ME). Mice were sacrificed by CO_2 inhalation. All animal procedures were approved by and performed in accordance with the guidelines of the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee (IACUC). Mice were immediately weighed after euthanasia by electronic scale.

2.3. Body composition and bone mass measurements by dual-energy X-ray absorptiometry

2.3.1. PIXImus

Mice were scanned using the PIXImus II peripheral DXA bone densitometer (GE-Lunar, Madison, WI) with a pixel size of 180 × 180 μm (1.6-line pairs/mm). A quality control phantom was scanned prior to the mouse measurements, as the instrument software does not allow samples to be scanned unless the measurements of the quality control phantom (scanned within prior 24 h) are within ±2 % of the expected value. High and low energy scans were acquired using 80 kV and 35 kV, respectively (Table 1) from a stationary cone beam X-ray source. Each mouse was placed on a specimen tray, spread out in a prostrate position with limbs extended away from the body and the tail curled around the animal's left side without intersecting any long bones. After each scan, the mouse was lifted off the specimen tray and replaced on the tray as previously described. This process was repeated three times per mouse. We excluded the head from the whole-body region of interest, and the software (version 1.46.007, GE Medical Systems Ultrasound and BMD, Bedford, United Kingdom) calculated total body (less head) bone mineral content (BMC, g), bone mineral density (BMD, g/cm^2), fat mass (g), lean mass (g), and fat mass percent (%) from the total body image. Total animal mass was estimated by adding total body BMC, lean, and fat mass.

Table 1
Technical specifications and scan times of the NMR, PIXImus, InAlyzer, and iNSiGHT systems.

	Image Area (cm)	Low Energy	High Energy	Scan Duration	Beam Type
PIXImus II	8 cm × 6.5	35 kV	80 kV	~5 min	Cone beam
InAlyzer	21 × 31.5	55 kV	80 kV	~28 s	Fan beam
iNSiGHT	16.5 × 25.5	60 kV	80 kV	~25 s	Cone beam

2.3.2. InAlyzer

We also scanned mice using the InAlyzer peripheral DXA system (Medikors Inc., Jungwon-gu, Korea). A quality control phantom was scanned daily prior to each measurement session. Each mouse was placed in the center of the InAlyzer scanning area in the prone position with arms and legs extended out to the side with the tail straight out behind the body. The animal's head was positioned into a nose cone taped to the instrument's scanning area. For each measurement, two scans were taken: first, an image of the nose cone without the animal, then a scan of the animal in the nose cone. The first scan was used as a mask and subtracted from the second to remove the nose cone from the final image. Each mouse was scanned three times with repositioning in between scans. Scans were acquired using the 84 s "Optimum mode," which uses a high energy parameter of 80 kV/1.0 mA and a low energy parameter of 55 kV/1.25 mA via a moving fan beam (Table 1). Scans were acquired with a pixel size of 103×106 μm, with a pixel pitch of 48 μm (108 μm in Analysis). Outcomes obtained using the InAlyzer software version 3.2.3 included animal mass (g), bone mass (BMC, g), bone mineral density (BMD, g/cm^2), lean mass (g), fat mass (g), lean percent (%), and fat percent (%). The InAlyzer offers additional viewing modes to visualize the body composition and bone density outcomes (Fig. 1).

2.3.3. iNSiGHT system

Mice were scanned using the iNSiGHT system which employs a combination of a low energy (60 kV, 0.80 mA) and a high energy (80kv, 0.80 mA) scan (Osteosys, Seoul, Korea) (Table 1). The machine was calibrated using a standard phantom block before scanning each measuring session. Each mouse was positioned and scanned as described for the InAlyzer scan. Outcomes calculated by the iNSiGHT software included total weight (g), BMC (g), BMD (g/cm^2), lean mass (g), fat mass (g), fat proportion (%), bone area (cm^2) and tissue area (cm^2). The iNSiGHT also produced images of whole-body, bone, and body fat visualization for each scan (Fig. 1).

2.4. Body composition by Nuclear Magnetic Resonance Imaging

We used whole-body nuclear magnetic resonance (NMR) imaging (3-in-1 EchoMRI Composition Analyzer, Echo Medical Systems, Houston, Texas) to measure lean mass (g) and fat mass (g) and compare the values to the DXA instruments. Each mouse was individually scanned once in the EchoMRI, using the manufacturers' recommended settings, as previously published [5].

2.5. Region of interest identification evaluation

We assessed the accuracy of bone measures (bone mineral content, BMC) measurements at the femur from the three DXA systems by comparing them to those obtained by whole bone microCT, which we considered the gold standard for bone mass measurements. For each instrument, the whole right femur was included in the region of interest.

2.6. Bone mineral content by MicroCT

We evaluated the excised right femur using a high-resolution desktop micro-computed tomographic (μCT/microCT) imaging system (μCT40, Scanco Medical AG, Brüttisellen, Switzerland) to measure bone mineral content (BMC, g). Femurs were cleaned of all non-osseous tissue. Scans were acquired using a 10 μm^3 isotropic voxel size, 70 kVp and 114 mA peak X-ray tube potential and intensity, 200 ms integration time, and were subjected to Gaussian filtration, in accordance with the ASBMR guidelines for the use of μCT in rodents [6]. A threshold of 672 mg HA/cm^3 was used for evaluation of the whole femur.

2.7. Statistical analysis

We assessed the precision of the PIXImus, InAlyzer, and iNSiGHT

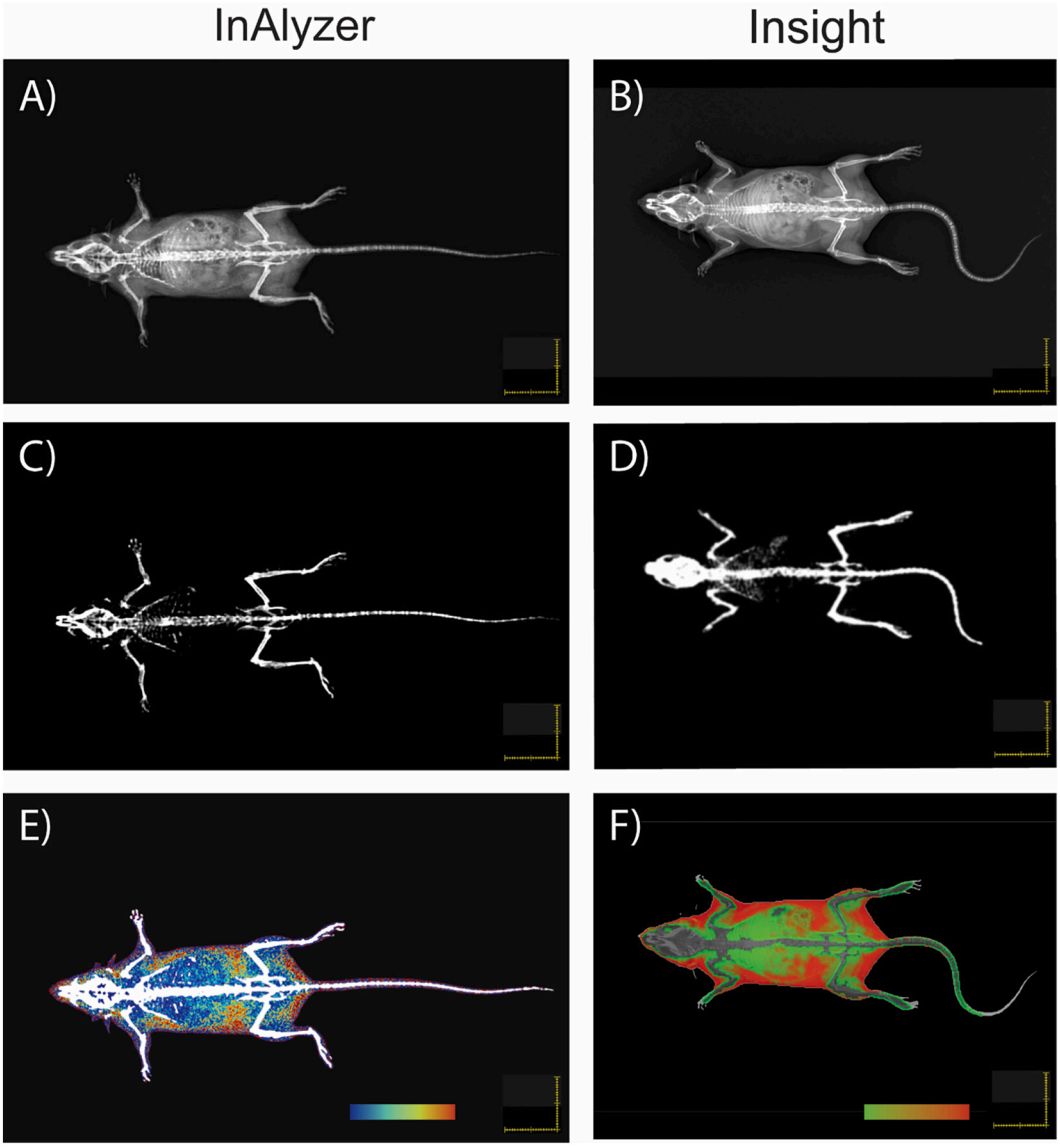

Fig. 1. Representative scan and viewing modalities of the InAlyzer software include A) total body composition, C) skeleton, and E) body fat and representative scan and viewing modalities of the iNSiGHT software include B) total body, D) skeleton, and F) body fat. Color scale bar from Green to red represents lower/higher attenuating tissue (lean mass) to red (fat mass) for the iNSiGHT. Similarly, for the InAnalyzer color scale bar from blue to (lean mass) to red (fat mass). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

systems by computing the coefficient of variance (CV) for repeat measures of each outcome measure. We used paired *t*-tests to assess differences in CV by instrument. The accuracy of body composition measurements was evaluated by comparing lean mass and fat mass measurements from the DXA systems to those from the EchoMRI. Standard descriptive statistics (mean and standard deviation) were calculated for each instrument's body composition and bone mass measures. We calculated the percent error of the PIXImus', InAlyzer's, and iNSiGHT's body composition outcome measures compared to the NMR results. We used repeated measures ANOVA, followed by multiple pairwise paired *t*-tests to determine if measures between instruments were significantly different. We adjusted the *p*-values using the Bonferroni multiple testing correction method. We also assessed accuracy via Bland-Altman plots comparing NMR body composition measures to PIXImus, InAlyzer, and iNSiGHT results. Additionally, the comparability of the PIXImus, InAlyzer, and iNSiGHT measurements was assessed by Pearson's correlation coefficient (r) and linear regression models for each measure. Finally, the BMC of the DXA femur ROI compared to ex vivo microCT was compared by Pearson's correlation coefficient (r) and linear regression models.

Data are presented as mean ± standard deviation unless otherwise noted. Statistical analyses were performed with R version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria). All tests were two-tailed, and differences were considered statistically significant at $P < 0.001$.

3. Results

3.1. Animal parameters

To evaluate the DXA instruments, mice at three different ages (8, 14, and 24 weeks, $n = 6$ per group) were used to ensure varied body compositions. Scale weights were taken following sacrifice and ranged from 22.28 g to 33.21 g with a mean weight of 28.18 g.

3.2. Comparison of whole-body bone mass and body composition measurements to PIXImus

Whole body BMC and BMD outcomes from InAlyzer were strongly correlated with corresponding PIXImus measures ($R^2 = 0.86$ and $R^2 = 0.83$, respectively, Fig. 2). InAlyzer's whole-body BMD values were higher than those from PIXImus (+26.2 %, $p < 0.001$), whereas the InAlyzer's whole-body BMC did not differ from that of the PIXImus (Table 2). The iNSiGHT measures of whole-body BMD were also strongly correlated with PIXImus measures ($R^2 = 0.82$, Fig. 2), but whole-body BMC measures were only weakly correlated with PIXImus ($R^2 = 0.29$, Fig. 2). The iNSiGHT overestimated BMD (+24.6 %, $p < 0.001$) and underestimated BMC (−24.2 %, $p < 0.001$) as compared to the PIXImus (Table 2). BMD values did not differ between the InAlyzer and the iNSiGHT ($p = 0.172$). Bland-Altman plots indicated good agreement and minimal notable bias in bone outcomes from InAlyzer and iNSiGHT systems compared to PIXImus (Supplemental Fig. 1).

Body composition measurements (Total Mass, Fat Mass, Lean Mass, Percent Fat Mass, and Percent Fat Mass) from the InAlyzer and iNSiGHT systems were strongly correlated with PIXImus measures ($R^2 = 0.87$ to 0.99, Table 3).

Table 2
Whole body BMC and BMD measures from PIXImus, InAlyzer, and iNSiGHT systems (mean ± SD).

	PIXImus	*InAlyzer*	*iNSiGHT*
BMC (g)	0.46 ± 0.05	0.45 ± 0.06	0.35 ± 0.05*, **
BMD (g/cm^2)	0.050 ± 0.003	0.063 ± 0.004*	0.062 ± 0.004*

* Denotes statistically significant from PIXImus at $P < 0.001$.
** Denotes statistically significant from InAlyzer at $P < 0.001$. Repeated measures ANOVA and post hoc multiple pairwise paired *t*-tests with *p*-values adjusted using the Bonferroni multiple testing correction method.

Table 3
Measurement agreement of body composition and bone mass measures of InAlyxer and iNSiGHT to PIXImus.

	InAlyzer	*iNSiGHT*
Total Mass	$R^2 = 0.99$, $y = 3.2 + 0.9\times$	$R^2 = 0.99$, $y = 2.7 + 0.9\times$
Fat Mass	$R^2 = 0.87$, $y = -2.8 + 0.79\times$	$R^2 = 0.95$, $y = 0.024 + 1.4\times$
Lean Mass	$R^2 = 0.98$, $y = 3.1 + 1.2\times$	$R^2 = 0.93$, $y = 1.5 + 0.91\times$
Percent Fat Mass	$R^2 = 0.96$, $y = -22.0 + 1.1\times$	$R^2 = 0.93$, $y = -0.75 + 1.4\times$
Percent Lean Mass	$R^2 = 0.96$, $y = 13 + 1.1\times$	$R^2 = 0.93$, $y = -40.0 + 1.4\times$
BMC	$R^2 = 0.86$, $y = 0.11 + 0.77\times$	$R^2 = 0.29$, $y = 0.30 + 0.46\times$
BMD	$R^2 = 0.83$, $y = 0.011 + 0.62\times$	$R^2 = 0.82$, $y = 0.013 + 0.6\times$

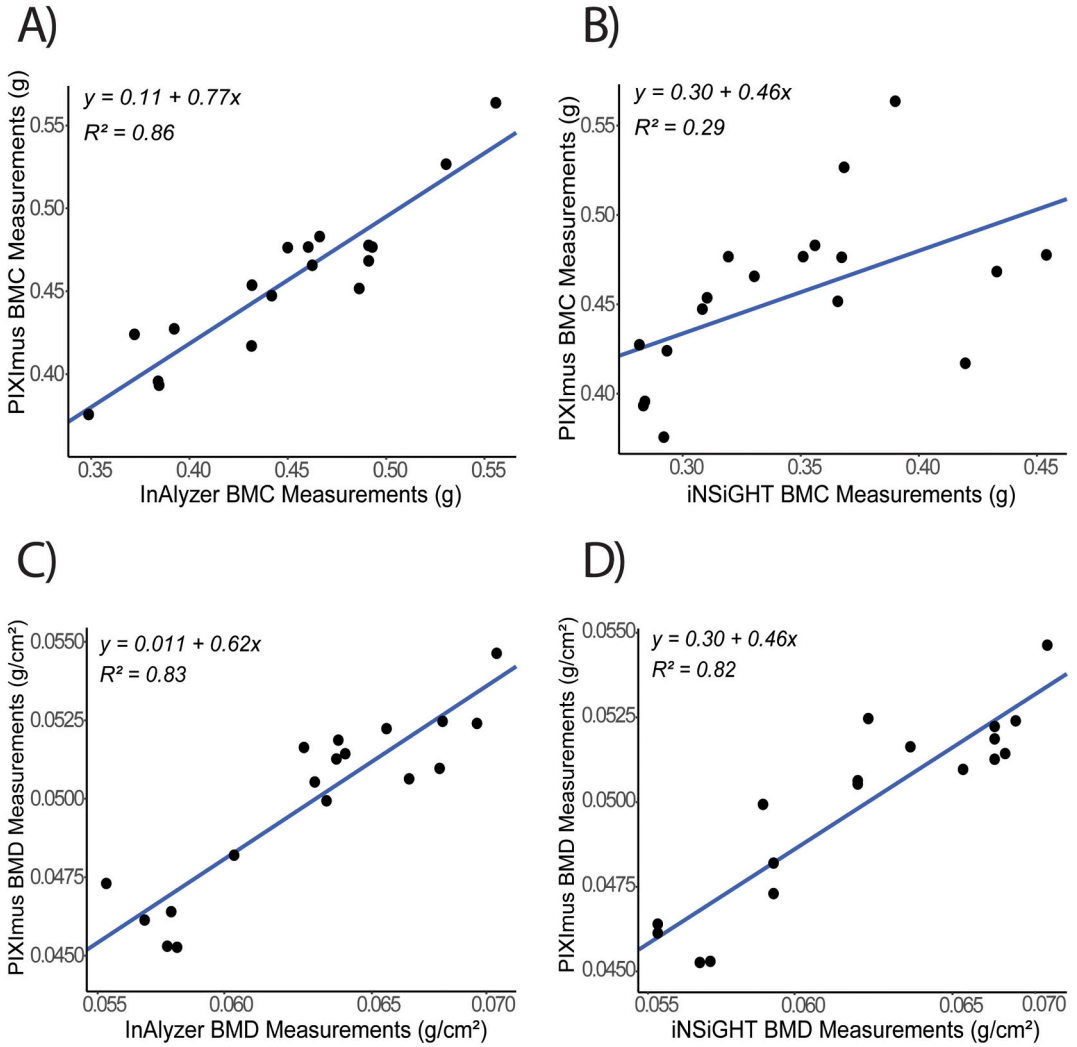

Fig. 2. Correlations of whole-body bone mineral density measures of DXA instruments. Regression plots of PIXImus measures of BMC and A) InAlyzer Whole Body BMC outcome measures ($R^2 = 0.86$) and B) iNSiGHT Whole Body BMC outcome measures ($R^2 = 0.29$). Regression plots of PIXImus measures of BMD and C) InAlyzer Whole Body BMD outcome measures ($R^2 = 0.83$) and D) iNSiGHT Whole Body BMD outcome measures ($R^2 = 0.82$).

3.3. Short-term precision

All three instruments had excellent precision, with coefficients of variation for repeat measurements ranging from 0.28 % to 3.16 % (Table 4). Compared to PIXImus, the InAlyzer had better precision for total mass (0.43 % vs. 1.12 %, $p < 0.001$), but worse precision for lean mass (0.97 % vs. 0.39 %, $p < 0.001$). The iNSiGHT also had better precision for BMC than the PIXImus (1.38 % vs. 2.42 %, $p < 0.001$). Precision for all other outcomes was similar among the three devices.

3.4. Comparison of DXA body composition measurements to NMR

All three DXA devices provided comparable measures of body mass compared to the actual scale weight of the animals (Table 5). The PIXImus over-estimated lean mass (+14.1 %, p < 0.001), but reported similar fat mass values compared to the EchoMRI measurements. The InAlyzer under-estimated lean mass (−16.4 %, p < 0.001) and over-estimated fat mass (151.0 %, p < 0.001) compared to the EchoMRI system. The iNSiGHT overestimated lean mass (+17.4 %, p < 0.001), but had similar fat mass measures as the EchoMRI.

PIXImus, InAlyzer, and iNSiGHT outcome measures were strongly correlated with scale weight and NMR-based body composition values (Table 6). Specifically, total mass measurements were strongly correlated with body mass assessed via scale ($R^2 \geq 0.99$ for all, Table 6). Fat and percent fat mass from all DXA systems were strongly associated with the corresponding NMR measurements ($R^2 = 0.91$–0.98, Fig. 3). Bland-Altman plots indicated good agreement and no notable bias in DXA-based and EchoMRI-based fat measurements (Supplemental Fig. 2). Lean mass from all three DXA systems was strongly correlated with lean mass from EchoMRI ($R^2 = 0.76$ to 0.81), whereas DXA-based percent lean mass measurements were only moderately correlated with those from EchoMRI ($R^2 = 0.36$ to 0.44, Table 6).

3.5. Femur bone mineral content

Finally, we evaluated the DXA instruments' measures of femur BMC compared to ex vivo microCT of the same bone. The InAlyzer's BMC measure was strongly correlated with the microCT-based BMC measure ($R^2 = 0.81$), whereas the iNSight and PIXImus values of BMC were less strongly correlated with microCT measures ($R^2 = 0.42$, and $R^2 = 0.68$, respectively) (Fig. 4).

4. Discussion

This study evaluated the performance of two relatively new peripheral bone densitometry machines, the InAlyzer and the iNSiGHT systems, for in vivo body composition and bone outcomes in mice. We compared these systems' measures to those of the now discontinued PIXImus DXA system, whole-body NMR EchoMRI, and ex vivo microCT outcomes. Measurements from both of the new DXA systems had excellent precision that was similar to the PIXImus. Given that these instruments will likely be used for longitudinal studies, precision is of great importance and both the InAlyzer and iNSiGHT will be suitable replacements for the PIXImus for these applications.

Table 4
Short-term precision (% CV) of body composition and bone mass measures from PIXImus, InAlyzer, and iNSiGHT systems.

Measure	*PIXImus*	*InAlyzer*	*iNSiGHT*
Total Mass	1.12 ± 0.71	0.43 ± 0.24*	1.37 ± 0.48
BMC	2.42 ± 1.0	2.02 ± 1.12	1.38 ± 0.65*
BMD	1.04 ± 0.47	0.80 ± 0.48	1.08 ± 0.91
Fat Mass	2.35 ± 1.81	2.24 ± 1.63	3.15 ± 3.83
Lean Mass	1.10 ± 0.73	0.75 ± 0.38	0.73 ± 0.97
Percent Fat	1.76 ± 0.68	1.93 ± 1.37	3.16 ± 3.81
Percent Lean	0.39 ± 0.27	0.97 ± 0.61*	0.51 ± 0.96

* Denotes statistically significant from PIXImus at $P < 0.001$.

Table 5
Body composition measures from EchoMRI, PIXImus, InAlyzer, and iNSiGHT systems (mean ± SD).

	Scale weight	*EchoMRI*	*PIXImus*	*InAlyzer*	*INSiGHT*
Body mass (g)	29.3 ± 4.2	NA	26.7 ± 3.5	25.9 ± 3.9	26.6 ± 3.9
Fat Mass (g)	NA	3.9 ± 1.9	4.2 ± 1.8	8.9 ± 2.1*	3.1 ± 1.3
Lean Mass (g)	NA	19.7 ± 2.3	22.5 ± 3*	16.5 ± 2.6*	23.2 ± 3.2*
Percent Fat (%)	NA	13.1 ± 5.1	15.6 ± 5.2	34.4 ± 4.7*	11.5 ± 3.6
Percent Lean (%)	NA	67.8 ± 5.8	84.4 ± 5.2*	63.9 ± 4.6	87.4 ± 3.6*

* Denotes statistically significant from EchoMRI at P < 0.001, Repeated measures ANOVA and post hoc multiple pairwise paired *t*-tests with *p*-values adjusted using the Bonferroni multiple testing correction method.

Table 6
Regression outcomes of body composition measures from PIXImus, InAlyzer, and iNSiGHT systems as compared to EchoMRI.

	PIXImus	*InAlyzer*	*iNSiGHT*
Total Mass vs. Scale Weight	$R^2 = 0.99$, $y = -2.3 + 1.2\times$	$R^2 = 1$, $y = 1.3 + 1.1\times$	$R^2 = 1$, $y = 0.64 + 1.1\times$
Fat Mass vs. EchoMRI	$R^2 = 0.98$, $y = -0.64 + 1.1\times$	$R^2 = 0.91$, $y = -3.9 + 0.88\times$	$R^2 = 0.97$, $y = 0.71 + 1.5\times$
Lean Mass vs. EchoMRI	$R^2 = 0.77$, $y = 4.6 + 0.67\times$	$R^2 = 0.81$, $y = 6.2 + 0.82\times$	$R^2 = 0.76$, $y = 5.1 + 0.63\times$
Percent Fat vs. EchoMRI	$R^2 = 0.96$, $y = -1.8 + 0.95\times$	$R^2 = 0.94$, $y = -23 + 1.1\times$	$R^2 = 0.95$, $y = -3.0 + 1.4\times$
Percent Lean vs. EchoMRI	$R^2 = 0.36$, $y = 11 + 0.67\times$	$R^2 = 0.40$, $y = 16 + 0.81\times$	$R^2 = 0.44$, $y = -28 + 1.1\times$

Due to its good approximation of bone mass compared to ash weight [7,8], non-invasive scanning, and ease of use, the PIXImus has been widely used to assess bone in rodent studies of development [9], aging [10], and metabolic bone diseases [7]. Yet, the PIXImus has been discontinued and thus we evaluated two newer DXA systems to determine their agreement with the PIXImus's bone and body composition measures. The short-term precision of the PIXImus measures of whole body BMD and BMC were excellent (1.0 and 2.4 %, respectively) and comparable to previous reports [11–13]. Precision of whole-body BMD and BMC from InAlyzer were equivalent to the PIXImus, whereas the precision of whole-body BMD from the iNSiGHT system was significantly worse than that of either the PIXImus or the InAlyzer, in agreement with findings from Baek et al. [14]. For all three systems in our study, the precision of whole-body BMD was better than BMC.

The short-term precision of the InAlyzer and iNSiGHT body composition measurements were also excellent (<3 %), and comparable to those from the PIXImus in this study and in prior studies [13,8]. Likewise, our findings for the precision of iNSiGHT measurements of fat mass and lean mass were consistent with previously reported findings [14]. Similar to the previous studies, we found that the measures of lean mass are more precise than those of fat mass for all three of the DXA instruments in this study.

We also evaluated the absolute values of the bone and body composition measurements from the InAlyzer and iNSiGHT to those of PIXImus, microCT, and NMR. However, a comparison of the values of these measures must be interpreted with care. While PIXImus is commonly used to evaluate rodent bone density and body composition, it is not a true “gold standard”. Additionally, both NMR and microCT are entirely different imaging modalities than DXA, making direct comparisons between values challenging to interpret. Yet, as these other instruments are frequently used to evaluate rodent body composition and bone density, it is important to understand how the InAlyzer and iNSiGHT measures compare.

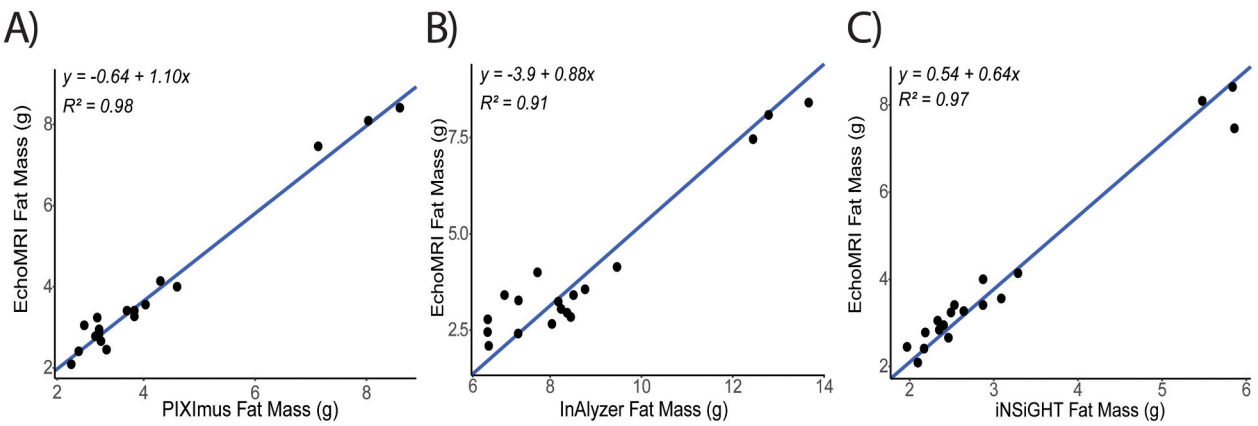

Fig. 3. Correlations of fat mass measures of DXA instruments and NMR. A) Regression of EchoMRI and PIXImus Fat Mass outcome measures ($R^2 = 0.98$). B) Regression of EchoMRI and InAlyzer Fat Mass outcome measures ($R^2 = 0.91$). C) Regression of EchoMRI and iNSiGHT Fat Mass outcome measures ($R^2 = 0.97$).

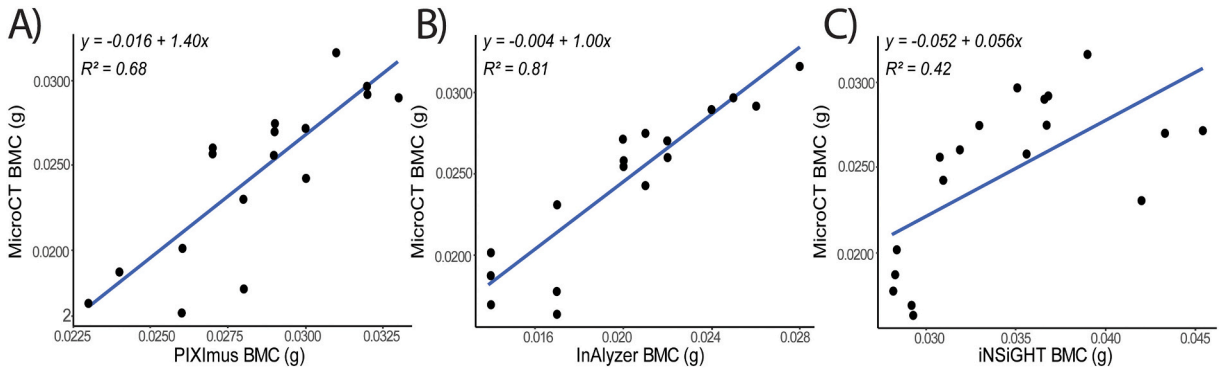

Fig. 4. A) Regression of MicroCT and PIXImus BMC ($R^2 = 0.68$), B) Regression of MicroCT and InAlyzer BMC ($R^2 = 0.81$) and C) Regression of MicroCT and iNSiGHT BMC outcome measures of the femur ($R^2 = 0.42$).

Bone outcomes from the InAlyzer were highly correlated with those of the PIXImus. The InAlyzer measures of whole-body BMC did not differ from the PIXImus, though the InAlyzer overestimated whole-body BMD by ~25 %. This finding suggests that the two devices identify the 'area' of the bone region differently, with InAlyzer selecting a boundary closer to the bone edge and thereby smaller. By contrast, while whole-body BMD from the iNSiGHT was strongly correlated with that from PIXImus, whole body BMC was only weakly correlated with PIXImus measures and underestimated BMC by −24 %. Identification of the bone edge appeared to be somewhat worse with the iNSiGHT system. For example, in Fig. 1F, the iNSiGHT does not correctly identify pixels of lower density bone (parts of the pelvis and sternum), but rather assigns them as lean mass. By contrast, the InAlyzer has a much more accurate reflection of bone area, visualizing not only the entire pelvis and most of the rib cage, but also identifying the entire tail of the animal. Overall, the InAlyzer's tighter bone edge detection may lead to greater accuracy in measures of BMD than either the PIXImus or iNSiGHT.

To further evaluate the ability of these two systems to measure bone mass, we compared BMC measures of the whole femur to ex vivo microCT measures. Femur BMC from the InAlyzer had the highest correlation with the microCT measure ($R^2 = 0.81$). By contrast, the iNSiGHT was only weakly correlated with the microCT measures of the femur BMC ($R^2 = 0.42$). Like the in vivo measures of bone density, these findings demonstrate the greater bone edge detection capabilities of the InAlyzer than the other systems. Collectively, these results suggest that the InAlyzer provides the most reliable bone outcomes, with excellent measurement precision.

Body composition, measurements from the InAlyzer and iNSiGHT were strongly associated with those from the PIXImus. We further compared the fat and lean mass measures of all three DXA devices to those of EchoMRI and found moderate to strong correlations (R^2 values of 0.76–0.98). Yet, despite the correlations with the EchoMRI measures, there were notable differences in the absolute values of fat mass and lean mass when comparing the DXA-based outcomes to those from whole-body NRM. Previous studies have shown that DXA overestimates fat mass by nearly 100 % and underestimates lean mass when compared to gravimetric and chemical extraction techniques [8]. We found that the InAlyzer overestimated fat mass by a similar margin (~150 %), while the iNSiGHT and PIXImus overestimated lean mass by roughly 15 % when compared to whole body NMR measurements.

These discrepancies in soft tissue mass measures may be due to the inherent differences in measurement methodologies. NMR measures fat mass and lean mass, where lean mass is calculated without solid structures such as bone [15]. By contrast, DXA measures fat mass, lean mass and bone mineral content, yet every pixel of the DXA image is initially only resolved into two components: bone mineral and soft tissue [15]. The proportions of fat mass and lean mass are then predicted from the DXA measurements of soft tissue, based upon the different mass attenuations at low and high energy [16]. Another contributor to discrepancies in the body composition measurements may be differences in the material that each manufacturer used to create a standard for the attenuation of fat and lean mass. For example, the InAlyzer system used stearic acid mixed with water for their standard for fat attenuation, which may contribute to the overestimate of fat mass.

There are considerable advantages of the newer InAlyzer and iNSiGHT systems compared to the older PIXImus system. Unlike the open X-ray source system used by the PIXImus, both the InAlyzer and iNSiGHT systems employ a closed cabinet with lead shielding, which

eliminates operator exposure to radiation. Further, the scan time of both the InAlyzer and iNSiGHT systems is less than half that of the PIXImus, enabling a shorter duration of anesthetic exposure for the mice and a more efficient workflow. The InAlyzer system has the potential for even greater efficiency, as it is capable of scanning up to four mice at a time. However, as we only scanned one mouse at a time, future studies are needed to confirm the accuracy and precision of the InAlyzer scan mode for multiple mice. Future studies may also consider including different strains of mice or transgenic models with greater differences in bone mass and body composition.

Altogether, given the safety and efficiency advantages of the InAlyzer and the iNSiGHT for both the user and the animals, and their comparable precision to the PIXImus, both systems appear suitable for longitudinal studies of bone mass and body composition in mice. However, apart from fat mass, the InAlyzer had better correlation to the PIXImus measures of lean mass, BMC, BMD, percentage of fat mass, and percentage of lean mass than the iNSiGHT system. Further, the body composition algorithm in the new InAlyzer2 (not available when the current study was conducted) has been revised to eliminate the overestimate in fat mass (personal communication), though a future study is needed to determine the instrument's correlation with PIXImus values. Considering the accuracy differences between the instruments, as well as more accurate bone edge detection, the InAlyzer may be slightly preferable for studies with an interest in musculoskeletal (bone and lean mass) outcomes.

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CRediT authorship contribution statement

Jennifer C. Coulombe: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **David E. Maridas:** Writing – review & editing, Visualization, Data curation. **Jarred L. Chow:** Writing – review & editing, Visualization, Data curation. **Mary L. Bouxsein:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Data availability

Data will be made available on request.

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