Multi-strain and species comparison of next-generation dual energy x-ray absorptiometry (DXA) machines against benchmark techniques (PIXImus, QNMR, Chemical Carcass Analysis) for BMD and body composition

Husam Bensreti[1], Dima Alhamad[1], Kanglun Yu[1], Maribeth Johnson[1], Meghan E. McGee-Lawrence[1]
[1]Medical College of Georgia, Augusta University, Augusta, GA mmcgeelawrence@augusta.edu **Disclosures:** MM-L: ORS Board of Directors [9]

INTRODUCTION: Dual-energy X-ray absorptiometry (DXA) technology has been used to quantify areal bone mineral density (BMD) and body composition (fat vs. lean tissue) across a wide variety of species including humans and rodents. The first widely used instrumentation for these simultaneous analyses in rodents was the PIXImus densitometer, although this equipment has not been commercially manufactured in several decades. Newer small animal DXA machines have entered the commercial market from a variety of different companies, yet only recently [1] have any of these newer instruments been compared against benchmark technology. In addition, to date, no published studies have compared body composition measurements from next-generation DXA machines against the gold standard of chemical carcass analysis. To address the need for rigorous validation of small animal DXA imaging across the field, in this study, three next-generation DXA systems from a single company (Kubtec Scientific) were compared to current benchmark approaches for quantification of BMD (PIXImus) and body composition (quantitative nuclear magnetic resonance, qNMR, and chemical carcass analysis). A multi-strain and multi-species (mice, rats) approach was utilized to address variability associated with inbred research animals [2].

METHODS: All experiments followed NIH guidelines and were approved by the Institutional Animal Care and Use Committee at Augusta University. Male and female C57BL/6 and CD-1 mice (12-14 weeks old; n=6 per sex and strain) and male and female Sprague-Dawley and Wistar rats (12-14 weeks old; n=6 per sex and strain) were obtained from a commercial supplier (Charles River). Animals were anesthetized via isoflurane anesthesia (2-3%) and subjected to sequential scanning on each of four DXA systems: a PIXImus (GE Lunar), and three next-generation systems from a single supplier (Kubtec Scientific) including a PARAMETER 3D cabinet (P3D; detector size 5" x 6", 50 kV source), PARAMETER 2D cabinet (P2D; detector size 5" x 6", 50 kV source), and XPERT 80 (X80; detector size 10" x 12", 90 kV source). Rats were only imaged on the XPERT 80 next-generation DXA system due to detector size limitations, although site-specific scans were collected on the PIXImus for BMD comparisons. Following DXA scans, each animal was imaged while conscious on a Bruker Minispec qNMR. Animals were sacrificed via carbon dioxide followed by cervical dislocation. Carcasses were sent to the Small Animal Phenotyping core facility at the University of Alabama Birmingham for chemical carcass analysis. Briefly, carcasses were dried (to quantify water content) after which fat was extracted prior to incinerating carcasses at 600 degrees Celsius for ashing. The fractional carcass masses of fat mass and fat-free dry mass (i.e., lean mass) were quantified from the resulting datasets. Agreement was assessed for BMD of the whole body, right femur, and lumbar vertebrae (L3-L5) regions of interest (ROI) produced by next-generation DXA machines as compared to similar ROIs analyzed on the PIXImus, as well as to test for agreement between body composition data (lean mass, fat mass) produced by each of the four DXA machines utilized in this study as compared to qNMR or chemical carcass analysis. For statistical analyses, Pearson correlation (r) was used to assess the strength of the linear agreement relationship between methods, paired t tests (MD) were used to quantify the difference (over- or underestimation) between machines against the benchmark (i.e. "bias"), and Bland-Altman plots [3, 4] were used to plot the "bias" (y-axis) against the mean of the two methods (x-axis) with the relationship assessed by regression (β). Sexes and strains were pooled for each species in these analyses to increase sample sizes for higher statistical power.

RESULTS: For mice, measurements of whole-body BMD generally showed modest linear agreement between next-generation DXA machines and PIXImus (r_{P3D}=0.85, mean difference (MD) ± SD = -0.003±0.006, r_{P2D}= 0.58, MD = 0.002±0.008, r_{X80} = 0.80, MD = 0.011±0.039), with the XPERT 80 under-estimating whole-body BMD in lower BMD animals and over-estimating BMD in higher BMD animals (β = 1.22±0.09). Lumbar vertebrae BMD in mouse models showed modest agreement between the PARAMETER cabinets (r_{P3D}=0.74, MD = -0.007±0.010, r_{P2D}=0.87, MD= -0.006±0.001) and PIXImus but was under-estimated by the XPERT 80 system (r_{X80}=0.92, MD= -0.045±0.006), whereas femur BMD showed modest agreement between the XPERT 80 and PIXImus (r_{X80}=0.70, MD=-0.007±0.015) but was over-estimated by the PARAMETER cabinets (r_{P3D}=0.91, MD=0.023±0.008; r_{P2D}=0.01, MD=0.021±0.009). Detector size of the PIXImus precluded whole-body scans of rats, but site-specific measurements of lumbar vertebrae and right femur BMD for rats showed modest to poor agreement between the XPERT 80 and PIXImus (femur r_{X80}=0.34, MD=0.008±0.022; lumbar r_{X80}=0.48, MD=0.011±0.036), with the XPERT 80 over-estimating BMD more substantially in higher-BMD animals (β=1.03±0.20).

For body composition analyses of mice, when qNMR was considered the benchmark modality, all DXA machines including the PIXImus and the three next-generation systems showed modest linear agreement but over-estimated fat mass as compared to qNMR ($r_{PIXImus}$=0.88, MD = 2.14±0.90; r_{P3D}=0.82, MD=1.42±0.77; r_{P2D}=0.73, MD=1.66±0.87; r_{X80}=0.65, MD=0.94±0.95), where the over-estimation was more substantial for higher-fat animals for the PIXImus (β=0.39±0.10) vs. lower-fat animals for the PARAMETER cabinets (β=-0.62±0.12, P3D; β=-0.72±0.15, P2D). The DXA systems showed modest agreement with qNMR for lean mass, but PIXImus over-estimated lean mass ($r_{PIXImus}$=0.99, MD=5.52±2.17), with the discrepancy more substantial for leaner animals (β=0.30±0.02), whereas the next-generation DXA machines under-estimated lean mass (r_{P3D}=0.97, MD= -1.71±2.81); r_{P2D}=0.97, MD= -2.52±1.67; r_{X80}=0.94, MD= -4.21±2.17) with directionality that varied between machines. For rats, the XPERT 80 cabinet showed modest agreement with qNMR for lean mass (r_{X80}=0.99, MD= -106.1±25.2), but under-estimated lean mass more for leaner animals (β=-0.39±0.03), and showed weaker agreement and over-estimation of fat mass as compared with qNMR (r_{X80}=0.57, MD= 34.7±12.5), although the over-estimation was similar across fatter and leaner animals (β=0.29±0.22).

When chemical carcass analysis was considered the benchmark modality for body composition, scans of mice on either qNMR or DXA systems consistently showed strong linear agreement but over-estimated lean mass as compared to chemical carcass composition, where the over-estimation was more substantial for leaner animals (r_{qNMR}=0.995, MD= 11.4±3.5, β=0.88±0.02; $r_{PIXImus}$=0.995, MD= 16.9±5.6, β=1.11±0.01; r_{P3D}=0.97, MD= 9.7±6.0, β=1.15±0.04; r_{P2D}=0.97, MD= 8.8±4.3, β=0.98±0.04; r_{X80}=0.93, MD= 7.2±2.6, β=0.70±0.07). For fat mass, interestingly, qNMR showed strong linear agreement with chemical carcass analysis (r_{qNMR}=0.98, MD=0.074±).25), whereas all four DXA cabinets showed moderate linear agreement but over-estimated fat mass as compared to carcass analysis ($r_{PIXImus}$=0.89, MD= 2.2±0.9; r_{P3D}=0.84, MD=1.5±0.8; r_{P2D}=0.78, MD= 1.7±0.9; r_{X80}=0.68, MD= 1.0±0.9), where the over-estimation was more substantial for higher-fat animals for the PIXImus (β=0.34±0.1)and more substantial for lower-fat animals for the PARAMETER cabinets (β_{P3D}=-0.67±0.11; β_{P2D}=-0.76±0.13; β_{X80}=-0.33±0.18). For body composition analyses of rats, both qNMR and the XPERT 80 cabinet showed modest linear agreement with chemical carcass analysis for lean mass (r_{qNMR}=0.998, MD= 208±53, β=1.11±0.01; r_{X80}=0.992, MD= 99±29, β=0.82±0.02), but over-estimated lean mass more substantially for leaner animals, and showed slightly weaker agreement with carcass analysis for fat mass with qNMR under-estimating and XPERT 80 over-estimating (r_{qNMR}=0.89, MD= -7.2±5.3; r_{X80}=0.84, MD=27.8±8.2) and the over-estimation was worse for higher fat rats (β=0.32±0.12).

DISCUSSION: These data suggest that the next-generation DXA systems tested here generally show moderate agreement in measurement of BMD and body composition with existing benchmark analyses, supporting their measurement validity. While agreements in BMD between the XPERT 80 and PIXImus were weaker for rats, it is important to note that the PIXImus system was designed to analyze mice (not rats), and therefore lack of agreement should not be construed as lack of accuracy in data; further testing against other benchmark technology more appropriate for rats, such as in vivo microCT, is needed in future studies. SIGNIFICANCE/CLINICAL RELEVANCE: Next-generation small animal DXA machines may show good agreement with established benchmark technology, but further studies demonstrating rigorous validation of these instruments via comprehensive statistical comparisons are still needed.

REFERENCES: [1]. Coulombe J.C. et al., Bone 2024. [2] Reed D.R., et al., Physiol Behav 2007. [3] Bland J.M. and Altman D.G. Lancet 1986. [4] Bland J.M. and Altman D.G. Stat Methods Med Res 1999. [5] Pierce J.L. et al, JBMR 2022. ACKNOWLEDGEMENTS: This work was supported by the NIA P01AG036675 (Core B; Functional Outcomes). Rodents used in this study were provided by Kubtec Scientific, but Kubtec was not involved in the collection, analysis, or interpretation of data resulting from this study.