Calcif Tissue Int (1991) 49:438–440

Calcified Tissue International
© 1991 Springer-Verlag New York Inc.

Comment

Accuracy and Precision of Lumbar Bone Mineral Content by Dual-Energy X-Ray Absorptiometry in Live Female Monkeys

M. J. Jayo,[1] S. E. Rankin,[1] D. S. Weaver,[1,2] C. S. Carlson,[1] and T. B. Clarkson[1]

[1]Comparative Medicine Clinical Research Center, Bowman Gray School of Medicine, Winston-Salem, North Carolina 27103; and [2]Anthropology, Wake Forest University, Winston-Salem, North Carolina 27103, USA

Received November 26, 1990, and in revised form January 11, 1991

Summary. Dual-energy X-ray absorptiometry (DXA) was used to determine the *in vivo* bone mineral content (BMC) of lumbar vertebrae in 20 feral adult female cynomolgus macaques (*Macaca fascicularis*). The ash weight of the third lumbar vertebra (L3) was compared to the measured L3BMC of the *in vivo* DXA analyses. Correlation between the estimated L3BMC by DXA and the actual ash weight was significant ($r = 0.965$, $P < 0.01$); however, DXA methodology underestimated ash weight on the average of 6.2%. Correlation was significant between two sequential *in vivo* DXA scans ($r = 0.988$, $P < 0.001$). Noninvasive *in vivo* DXA was a fast, precise, and effective method for measuring the lumbar BMC in female cynomolgus macaques.

Key words. Dual-energy X-ray absorptiometry — Monkeys.

Noninvasive measurement of bone mineral is an important tool in the evaluation of bone mineral status in experimental studies. In human and nonhuman primate subjects, various types of noninvasive measurements have been used to obtain estimates of current or future relative risk of bone fracture by assessing the amount of bone present at a given time or the rate of bone loss (serial *in vivo* monitoring) [1–6].

Dual-energy X-ray absorptiometry (DXA) is the newest, most effective, and accurate tool for noninvasive determination of bone mineral status in human primates [5, 7]. Use of DXA in laboratory animals has been limited, and there are no reports regarding its accuracy and precision in nonhuman primates [8]. We have reported data using dual photon absorptiometry (DPA) in nonhuman primates [9]. We now report the use of *in vivo* DXA to determine the bone mineral content (BMC) of the lumbar spine in female cynomolgus macaques. These data provide a technical basis for the use of DXA in studies using our nonhuman primate animal model of surgical menopause [6, 10].

Materials and Methods

Twenty feral adult female cynomolgus macaques (*Macaca fascicularis*) imported from Jakarta, Indonesia, were used in this study. The animals were part of an osteoporosis/atherosclerosis experiment and were surgically menopausal (bilaterally ovariectomized) for approximately 22 months. The animals were given water *ad libitum* and fed controlled amounts of semipurified diets (mimicking the diet typically consumed by North American human beings) containing 0.44 mg of cholesterol/Cal. The calcium to phosphorus ratio was 1.03 (805 mg of calcium and 778 mg of phosphorus/100 g of diet). The average age of the test animals was 10.7 years (standard error of the mean (SEM) = 0.32) as estimated by dentition. The mean body weight (BW) of the test animals was 3.49 kg (SEM = 0.12). The animals were housed in groups of 5–6 monkeys in indoor pens (14.5 cubic meters) with outdoor runs of similar size. State and federal laws, standards of the Department of Health and Human Services, and guidelines established by our institutional Animal Care and Use Committee were followed.

The DXA apparatus was calibrated daily using a dual step wedge standard constructed of acrylic and aluminum (as provided and recommended by the manufacturer [11]). Also, as a quality control index, an anthropomorphic spine phantom made of calcium sulfate and cast polyester of known mineral content was scanned daily for evaluation of *in vitro* accuracy and precision [11]. The scans were performed on a DXA scanner (Norland model XR26, Norland Corp., Fort Atkinson, WI) designed for human patients and approved for laboratory animal use. Each scan yielded the cortical plus trabecular BMC in the path of the energy beams in g. All DXA scans and their analyses were made by one of us (SER).

Methodology for DXA lumbar spine scans in macaques was similar to that previously described for DPA [9]. After a 12-hour fast, the animals were sedated using ketamine hydrochloride (15 mg/kg, Vetalar, Parke-Davis, Morris Plains, NJ) and acepromazine maleate (0.05 mg/kg, PromAce, Fort Dodge Laboratories Inc., Fort Dodge, IA) given intramuscularly. The DXA table pad was removed and replaced with a sheet of plywood with a central T-shaped plexiglas patient window. Another window was made for the calibration box. The animals were placed supine on the central $152.0 \times 38.0 \times 0.5$ cm plexiglas board and secured in place with velcro straps that were glued to the plexiglas surface. The tail was secured with tape and velcro to the plexiglas. The spine scans were taken at a 1.5 mm point resolution, 1.5 mm line spacing, 6 cm scan width, and a speed of 60 mm/sec. Scans were taken from approximately 1 cm below the inferior xiphoid process to approximately 2 cm below the cranial ventral iliac spine. In order to determine the precision of *in vivo* DXA, two sequential lumbar spine scans were performed per animal without repositioning.

Immediately following the spine scans, each monkey was euthanized with an intravenous injection of sodium pentobarbital and necropsied. The lumbar spine segment (L1–L5) was excised and the soft tissue was removed. The L3 vertebra of each spine segment was removed, ashed overnight in a laboratory box furnace at 650°C, and weighed to determine bone ash weight (true bone mineral). The

Offprint requests to: M. J. Jayo

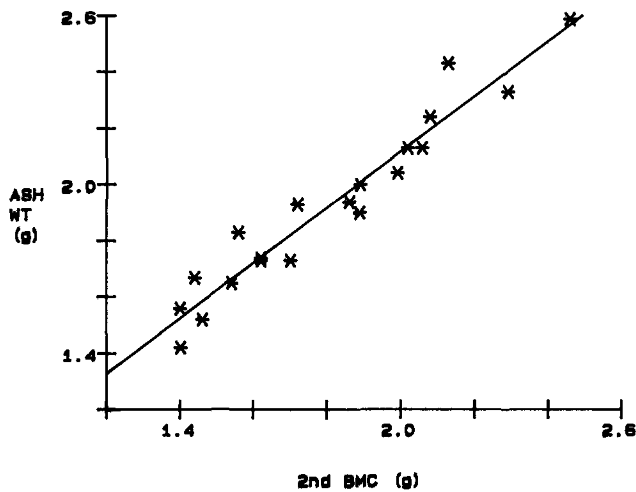

Fig. 1. Significant correlation between L3BMC of the second consecutive *in vivo* lumbar spine scan measured by DXA and the actual L3 ash WT in 20 female cynomolgus macaques (ash WT = [BMC × 0.984] + 0.148, for which r = 0.965 and SEE = 0.084).

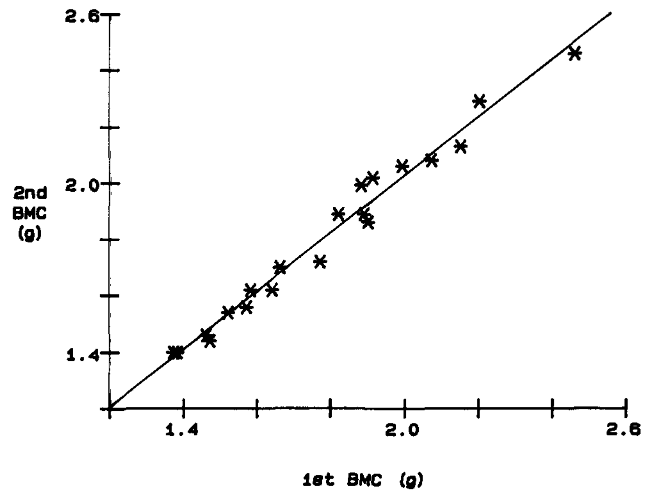

Fig. 2. Significant correlation between L3BMC of the first and second consecutive *in vivo* spine scan measured by DXA in 20 female cynomolgus macaques (second BMC = [1st BMC × 1.027] − 0.027, for which r = 0.988).

L3BMC of the second *in vivo* DXA scan was compared to the L3 vertebral bone ash weight as a test of accuracy.

Means, SEM, standard error of the estimate (SEE), and coefficients of variation (CV) were calculated for BMC. Percent differences, correlations, and analyses of variance were done as required. All tests of significance were two-tailed.

Results

Daily DXA spine scans (n = 16) of the phantom standard provided by the manufacturer yielded an *in vitro* average % difference for BMC of 1.55% (SEM = 0.14) with a CV of 0.55%, slightly underestimating the mineral content.

The total working time for *in vivo* DXA was approximately 15–20 minutes per animal, including sedation, preparation, and scanning time (two spine scans per animal). Each lumbar spine was approximately 4.5 minutes (including scanning and analysis).

The mean L3BMC by DXA for the 20 *in vivo* scans was 1.81 g (SEM = 0.07), and the mean bone ash weight was 1.93 g (SEM = 0.07), resulting in an absolute average difference of 6.20% (range of 0.53–14.75%). Figure 1 is a scatter plot with linear regression analysis demonstrating the significant correlation between L3BMC and the L3 ash weight. L3BMC by DXA consistently and significantly ($P < 0.01$) underestimated the actual bone ash weight. For the regression model comparing ash weight and L3BMC, the y intercept was significantly different from 0 ($P < 0.05$) due to underestimation of L3BMC by DXA, and the slope was not significantly different from 1 ($P > 0.05$). The animal's BW correlated significantly with L3BMC by DXA (r = 0.552, $P < 0.02$).

L3BMC precision by *in vivo* DXA was excellent. Figure 2 is a linear regression scatter plot for the first and second *in vivo* DXA scans. The correlation between these two scans was significant (r = 0.988, $P < 0.001$ and SEE = 0.048).

Discussion

In the present study there was excellent correlation between the estimated L3BMC by DXA and the actual ash weight of L3, as well as between the two sequential DXA scans. Norland DXA methodology consistently underestimated actual bone ash weight by 6.2%. These results are similar to those from a study of human cadaver lumbar spines, in which the Hologic DXA underestimated the ash weight by 8.9% [7]. Although not tested here, the DXA precision may be affected by repositioning of the subject.

Lumbar spine scans by DXA in female macaques compare favorably with other reported absorptiometry techniques, particularly DPA [9]. The reported accuracy of DPA is 1–2% using pelvic phantoms and 5–12% using human lumbar spine [7, 11, 12–15]. Accuracy, by DXA with the XR26, was similar to that obtained with the Norland 2600 DPA, which also underestimates the actual bone ash weight [9]. For both DPA and DXA, the accuracy for *in vivo* measurements can be degraded further by attenuation due to extraosseous calcification in osteophytes or vascular structures, or by vertebral crush fractures [8]. At necropsy, no gross skeletal abnormalities were observed over the scanned area. Because calcification of abdominal aortas due to the atherogenic diet fed was an experimental concern, a thoracic and abdominal aorta segment with grossly evident atherosclerotic lesions was excised and scanned *ex vivo* by DXA. The scans revealed no mineral content within the vessels.

An advantage of DXA over DPA is that the time required for the DXA lumbar spine scans is substantially less. Time is particularly important in *in vivo* studies using laboratory animals, in which appropriate sedation during the scanning process is required.

DXA is a precise and rapid method for noninvasive measurements of lumbar spine bone mineral status in live female cynomolgus macaques, and is a promising tool for assessment of bone loss in our nonhuman primate model for osteoporosis research [6, 10, 15].

Acknowledgments. This work was supported in part by Grants HL38964 from the National Heart, Lung, and Blood Institute and RR00919 from the National Center for Research Resources. The authors thank Jean Gardin, Dianna Swaim, Ann Chappelle, and Kim Long for their technical assistance. The Norland XR26 Bone Densitometer was generously donated by Glaxo Laboratories, Research Triangle Park, NC.

References

1. Cann C, Genant H, Kolb F, Ettinger B (1985) Quantitative computed tomography for prediction of vertebral fracture risk. Bone 6:1–7
2. Riggs B, Wahner H (1982) Changes in bone mineral density of the proximal femur and spine with aging. J Clin Invest 70:716–723
3. Riggs BL, Melton LJ (1986) Involutional osteoporosis. N Engl J Med 314:1676–1677
4. Elders PJM, Netelenbos JC, Lips P, van Ginkel FC, van der Stelt PF (1988) Accelerated vertebral bone loss in relation to the menopause: a cross-sectional study on lumbar bone density in 286 women of 46 to 55 years of age. Bone Miner 5:11–19
5. Mazess RB, Barden HS (1988) Measurement of bone by dual-photon absorptiometry (DPA) and dual-energy x-ray absorptiometry (DEXA). Annal Chir Gynaecol 77:197–203
6. Jayo MJ, Weaver DS, Clarkson TB, Carlson CS, Welles EG, Rankin SE (1990) Surgical menopause causes a significant decrease in lumbar bone mineral and serum HDL-cholesterol in female cynomolgus macaques (abstract #431) J Bone Miner Res (suppl 2)5:S181
7. Ho CP, Kim RW, Schaffer MB, Sartoris DJ (1990) Accuracy of dual-energy radiographic absorptiometry of lumbar spine: cadaver study. Radiology 176:171–173
8. Mazess RB (1983) Noninvasive bone measurements. In: Kunin AS, Simons DJ (eds) Skeletal research: an experimental approach. vol 2. Academic Press, Orlando, pp 277–343
9. Jayo MJ, Weaver DS, Rankin SE, Kaplan JR (1990) Accuracy and reproducibility of lumbar bone mineral status determined by dual photon absorptiometry in live male cynomolgus macaques (*Macaca fascicularis*). Lab Anim Sci 40:266–269
10. Miller LC, Weaver DS, McAlister JA, Koritnik DR (1986) Effects of ovariectomy on vertebral trabecular bone in the cynomolgus monkey (*Macaca fascicularis*). Calcif Tissue Int 38:62–65
11. Norland Corporation (1990) Operator's manual for the Norland XR-26 x-ray bone densitometer
12. Isaia G, Musetta M, Salamano G, Carlevatto V, Molinatti GM (1988) Diagnostic notes—lumbar BMC reproducibility as evaluated by means of dual photon absorptiometry. Maturitas 10:59–63
13. Peppler W, Mazess RB (1981) Total body bone mineral and lean body mass by dual-photon absorptiometry. Calcif Tissue Int 33:353–359
14. Wahner HW, Dunn WL, Mazess RB, Towsley M, Lindsay R, Markhard L, Dempster D (1985) Dual-photon Gd-153 adsorptiometry of bone. Radiology 156:203–206
15. Mazess RB, Vetter J, Weaver DS (1987) Bone changes in oophorectomized monkeys: CT findings. J Comput Assist Tomogr 11:302–305