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Determination of baseline bone mineral density using dual energy X-ray absorptiometry in Suffolk-Dorset hybrid ewes

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ABSTRACT

Dual energy x-ray absorptiometry (DEXA) has the ability to rapidly and non-invasively measure bone mineral density and is the most widely accepted method for quantitative assessment of bone mineral status *in vivo*. There is scarce information available on the baseline bone mineral density (BMD) values in adult intact Suffolk Dorset ewes (5–7 years), a frequently used animal model for the study of post-menopausal osteoporosis. The objective of the present study was to determine the baseline bone mineral density values in 26 adult intact Suffolk-Dorset hybrid ewes using a Lunar Prodigy DPX x-ray bone densitometer. The DEXA scans of the femur, lumbar spine (L3-L6) and calcaneus were obtained. Because of the low variability between the scans for the lumbar vertebrae and calcaneus in the first two animals, only two scans were obtained for the remaining animals of the study. The femoral scans were rejected due to high variability between the scans. The BMD was calculated using the standard GE antero-posterior human spine acquisition software. The bone mineral densities of regions of interest (ROIs) were compared by the Bonferroni significant difference technique. The results of the study demonstrated that the BMD progressively reduced from L3 to L6 and a strong correlation was found between the BMD values for the ROIs from L3 to L6. The present study provided a precise and rapid method for measuring the BMD of the lumbar spine in Suffolk-Dorset breed of sheep and recorded reference values in adult sheep.

1. Introduction

The most widely used techniques of assessing bone mineral density are dual-energy x-ray absorptiometry (DEXA) and quantitative computerized tomography (CT) [1]. DEXA is the established standard for measuring bone mineral density and allows rapid and non-invasive measurements of BMD and bone mineral content (BMC) providing greater resolution at a low radiation dose [2,3]. Quantitative CT is the most sensitive method presently available, but results in substantially greater radiation exposure than DEXA. Further, CT is less accessible, technically more difficult to perform and a software package appropriate for sheep has not been developed as yet. DEXA is the most widely accepted method for quantitative assessment of bone mineral status *in vivo*, and currently serves as an operational definition of osteoporosis by the World Health Organization (WHO) [4] and is used most frequently in measuring the spine and proximal femoral bone densities to estimate the current or future risk of bone fracture or bone loss [5]. DEXA is considered as a gold standard for diagnosing osteoporosis [6].

Although, DEXA has been adapted for use in animals, there is scarce information on the BMD and BMC values in adult sheep, reported to be a viable animal model for the study of post-menopausal osteoporosis in humans [7–10]. The size of the animal, gross skeletal and vascular anatomy, hormonal profiles, bone histology and skeletal kinetics of bone turnover approximate human skeletal system thus making them a suitable model for osteoporosis research [11–14]. Finally, yet importantly, societal and ethical implications are low as compared to other large animal models [15,16]. Sheep are docile, inexpensive, easy to handle and have hormonal profiles similar to women [17].

Although the BMD values have been previously reported for lumbar spine in 3 to 5 year old ewes using the Hologic QDR 1000/W-X ray bone densitometer, the breed of sheep was not reported [18]. Ovariectomised three year old Merino sheep have been validated as a large animal model for the study of vertebral osteoporosis [19]. In humans, each manufacturer of densitometry

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equipment has his own set of reference values especially for the caucasian female population and hence the BMD differs depending on the equipment used [20]. Therefore, it is possible that the BMD values reported for sheep previously are not uniformly valid for all sheep breeds and for different types of densitometers. Moreover, baseline BMD values in Suffolk-Dorset hybrid ewes (age range 5–7 years, body weight range 138–185 lbs) have not been established as yet. The objective of the present study was to determine the baseline BMD values in sheep of specified breed and age. In this study, we determined the BMD of lumbar spine (L3-L6), femur and calcaneus in 26 adult intact Suffolk-Dorset hybrid ewes using a GE Lunar Prodigy DPX x-ray bone densitometer. The results of this study may be serve as reference values for BMD measurements in Suffolk-Dorset hybrid sheep models used for human osteoporosis research. The creation of standardized reference data must be an important priority in order to harmonize patient management using standardized BMD measurements [21].

2. Materials and methods

2.1. Anaesthesia

All procedures were approved by the Purdue animal care and use committee. Twenty six healthy intact Suffolk-Dorset hybrid ewes (mean age 5.7 ± 0.96, Age range: 5–7 years and body weight range: 138–185 pounds) were included in the study. Feed was withheld for 12 h prior to the scanning procedure. Anaesthesia was induced with Thiopentone sodium[1] at a dose rate of 12 mg/kg IV and maintained with 2–3% Isoflurane[2] in a closed system.

2.2. Positioning of animal and scanning procedure

Under general anaesthesia, the DEXA scans of the lumbar spine (L3-L6) were obtained using GE Lunar Prodigy DPX x-ray bone densitometer, with the sheep positioned in ventro-dorsal recumbency (Fig. 1). A part of the sacrum incorporated in the images when L6-L7 was scanned was manually removed with edge manipulation. The last four lumbar vertebrae were identified from the DEXA scan images and designated as L3, L4, L5 and L6 (Fig. 2). The regions of interest (ROIs) were defined as the bone areas between two parallel lines located in the inter-vertebral spaces between L3-L4, L4-L5, L5-L6 and L6-S1.

The iliac crest and the sacral crest were identified to locate the lumbosacral junction in sheep. The scanning started by positioning the laser light indicator on the animals' midline at the level of the iliac crest, commenced and progressed proximad until all the vertebrae were visible on the display screen. The scanning was stopped at this time to centre the spines such that the spaces on the right and left of the lumbar vertebrae were symmetrical. Lumbar scanning was repeated at the ROI four and three times, respectively, in the first two animals, with repositioning after each scan. To test for precision, consecutive scans were performed. Because of the low variability between the scans per animal for the first two animals, only two scans were obtained for the remaining animals of the study. The scans were repeated if the variability between the successive scans exceeded 2%. The BMC and BMD of the lumbar spine were measured in a standard ventro-dorsal view. Out of the 26 animals, in seven animals the femoral scans were performed on the lateral aspect of the right distal femur and in seven animals, the calcaneal scans were performed on the medial aspect of right calcaneus by restraining the limb securely to the table with sand bags.

[1] Pentothal TM, Hospira Inc, IL, USA.
[2] IsoFlo, Abbott Animal Health, IL, USA.

Fig. 1. DEXA scan of the lumbar spine (L3-L6) using GE Lunar Prodigy DPX X-Ray bone densitometer Scanning procedure with anaesthetized sheep positioned in ventro-dorsal recumbency.

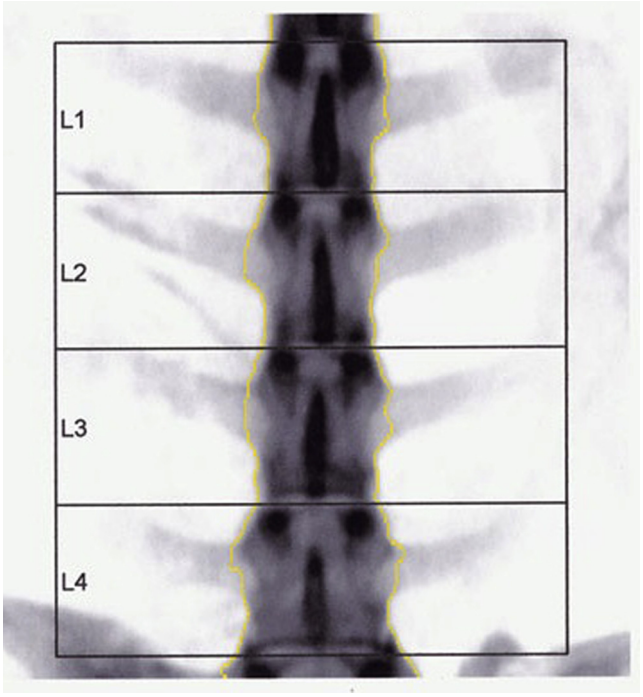

Fig. 2. DEXA scan image of the last four lumbar vertebrae. Segment designated L1 in the scan image corresponds to L3, L2 corresponds to L4, L3 corresponds to L5 and L4 corresponds to L6 vertebra in the study.

2.3. Image acquisition and statistical analysis

The right calcaneal scans were performed using a standard left hip human protocol (Fig. 3). The BMD (g/cm^2) was calculated using the standard GE antero-posterior human spine acquisition software (version 3.60). Default settings for the lumbar spine as recommended by the manufacturer were used for the study. All scans were performed by the same operator. The final images were then acquired and used for BMD calculations. The BMD means and standard deviations as well as correlation of the BMD of all ROIs between L3 and L6 were calculated using Minitab software for the Macintosh (version 9.0). Analysis of the same ROI width was carried out, as a small systematic increase in the BMD was seen

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