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An approach for determining quantitative measures for bone volume and bone mass in the pediatric spina bifida population

Rachel E. Horenstein [a,*], Sandra J. Shefelbine [b], Nicole M. Mueske [c], Carissa L. Fisher [c], Tishya A.L. Wren [a,c]

[a] Department of Biomedical Engineering, University of Southern California, Los Angeles CA, United States
[b] Department of Mechanical and Industrial Engineering, Northeastern University, Boston MA, United States
[c] Children's Orthopaedic Center, Children's Hospital Los Angeles, Los Angeles CA, United States

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ABSTRACT

Background: The pediatric spina bifida population suffers from decreased mobility and recurrent fractures. This study aimed to develop a method for quantifying bone mass along the entire tibia in youth with spina bifida. This will provide information about all potential sites of bone deficiencies.
Methods: Computed tomography images of the tibia for 257 children ($n = 80$ ambulatory spina bifida, $n = 10$ non-ambulatory spina bifida, $n = 167$ typically developing) were analyzed. Bone area was calculated at regular intervals along the entire tibia length and then weighted by calibrated pixel intensity for density weighted bone area. Integrals of density weighted bone area were used to quantify bone mass in the proximal and distal epiphyses and diaphysis. Group differences were evaluated using analysis of variance.
Findings: Non-ambulatory children suffer from decreased bone mass in the diaphysis and proximal and distal epiphyses compared to ambulatory and control children ($P \le 0.001$). Ambulatory children with spina bifida showed statistically insignificant differences in bone mass in comparison to typically developing children at these sites ($P > 0.5$).
Interpretation: This method provides insight into tibial bone mass distribution in the pediatric spina bifida population by incorporating information along the whole length of the bone, thereby providing more information than dual-energy x-ray absorptiometry and peripheral quantitative computed tomography. This method can be applied to any population to assess bone mass distribution across the length of any long bone.

1. Introduction

Spina bifida is a birth defect that results from incomplete closure of the spinal column during fetal development. Children with spina bifida suffer from decreased mobility secondary to weakness or paralysis of lower extremity muscles, leading to atypical loading of the legs. As a result, reduced bone mineral density (BMD) is among the more common complications in children with spina bifida (Quan et al., 1998). Reduced bone density may cause recurrent fractures of the lower extremities that are more numerous and frequent than those in typically developing children (Quan et al., 1998). Children with spina bifida often undergo repeated surgeries and immobilizations, which in turn decrease bone density and increase fracture risk (Drummond et al., 1981; Marreiros et al., 2010). It has been reported that children with higher lesion levels and lower ambulatory ability have a higher risk of fractures than more functional children with spina bifida (Marreiros et al., 2010). The high risk of fracture in children with spina bifida appears to be due to decreased muscle activity in their paralyzed lower extremities and the resulting insufficient axial loading of these limbs (Parsch, 1991). The distal tibia and femur are the most common fracture sites in children with spina bifida, with fractures in the proximal tibia and femur occurring less commonly (Parsch, 1991).

To date, there are no commonly used analytic methods for providing information about distribution of bone mass across the entire length of a long bone. Current techniques for measuring BMD rely most commonly on dual-energy x-ray absorptiometry (DXA) and, to a lesser extent, peripheral quantitative computed tomography (pQCT). DXA provides a projected areal measure of BMD and therefore cannot provide a complete adjustment for bone size (Marreiros et al., 2012). This limitation has consequences for comparing BMD across individuals of different physical sizes (Bachrach, 2005, 2007; Specker and Schoenau, 2005), including children with spina bifida who typically have short stature in comparison to typically developing peers (Rosenblum et al., 1983). This short stature may result in a biased DXA result since lower BMDs are anticipated in shorter individuals due to their inherently smaller bones (Sheridan, 2009). A further limitation of DXA is that it cannot differentiate between cortical and trabecular bone (Bachrach, 2005). As an alternative to DXA, pQCT can provide three-dimensional information, but it can only be used to image distal sites due to the small size of the gantry, which cannot accommodate larger, more proximal sites. In

* Corresponding author at: Northeastern University, 360 Huntington Avenue, Egan 260, Boston, MA 02115, United States.
E-mail address: horenstein.r@husky.neu.edu (R.E. Horenstein).

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addition, the use of pQCT is limited because the field of view of current pQCT systems cannot accommodate the entire length of a long bone and therefore cannot provide a means to measure the whole bone for deficiencies in the pediatric population, including children with spina bifida. Furthermore, current standards for analyzing acquired BMD datasets do not necessarily include analysis of BMD information across the entire length of a bone, which may lead to incomplete results and conclusions.

The purpose of this study was to assess bone mass and bone area (BA) along the entire length of the tibia in children with spina bifida. The ability to examine an entire long bone is advantageous because it provides information about all potential sites of bone deficiencies.

2. Methods

2.1. Participants

A total of 257 children between the ages of 6–17 years were included in this analysis. Children were divided into three groups: typically developing children (control group, $n = 167$), ambulatory children with spina bifida (AmbSB group, $n = 80$), and non-ambulatory children with spina bifida (Non-AmbSB group, $n = 10$). All children in the AmbSB and Non-AmbSB groups had been diagnosed with myelomeningocele, the most common and severe type of spina bifida in which the spinal cord protrudes outside the spinal column during fetal development. The average age, height, weight, sex and race characteristics for each group can be found in Table 1.

2.2. Image acquisition

All participants were assessed by CT using the same scanner (Philips Gemini GXL, Philips Medical Systems Inc., Cleveland, OH) and the same mineral reference phantom for simultaneous calibration (Mindways Model 3 CT Calibration Phantom, Mindways Software, Inc., Austin, TX). The phantom was scanned at the same time as the bone and extended the entire length of the tibia. The same certified radiology technologist carried out all scans. With the subject lying supine, contiguous 1 mm slices were acquired at 90 kVp, 32 mA (100 mA for scout scan), and 1 s rotation time from knee to ankle joints. The scan field of view was 25 cm and the matrix resolution was 512 × 512 pixels. All images were acquired with a sharp point filter for distortion compensation and artifact reduction and a level B resolution filter; both are standard filters from Philips scanner software. These scanning parameters were set much lower than standard clinical CT settings to minimize radiation exposure; the effective radiation dose was estimated to be <0.05 mSv. Each CT scan was completed in approximately 5 min.

2.3. Image processing

Each CT-image (DICOM format) sequence was imported into Osirix software (Rosset et al., 2004) (Fig. 1A) and a region of interest (ROI) containing only the right tibia was manually defined. For one non-ambulatory child with spina bifida, the left leg was analyzed instead of the right due to a previous fracture in the right leg. The tibial ROI was defined for each image in the stack between the proximal and distal tibia ends, and all pixel values outside the tibial ROI (including the fibula) were set to a background value of −1000 HU. The proximal end of the tibia was defined as the most proximal image slice where the intercondyloid eminence was first visible and the distal end was defined as the most distal image slice where the medial malleolus was visible. The isolated tibia images were imported into ImageJ software (1.47v). Noise was removed using a median filter with a radius of 1 pixel and a threshold of 50 for the raw pixel value (Fig. 1B, D).

Analysis of the bone was performed using an ImageJ plugin, BoneJ (version 1.3.11) (Doube et al., 2010). BoneJ uses a thresholding method to identify bone and calculate bone properties. The lower limit HU threshold used for bone was 206 HU, and the maximum was left at the BoneJ default of 4000 HU. The lower limit corresponds with a density of 126.5 mg/cm^3 K_2HPO_4 and was chosen to be low enough to capture both trabecular and cortical bone. A standard conversion equation was used to convert CT Hounsfield Unit (HU) values to density (equivalent aqueous K_2HPO_4 mg/cm^3) based on the phantom calibration:

$$\text{BMD} = \frac{\text{ROI Value (HU)} - \text{BMD Intercept}}{\text{BMD Slope}}$$

where BMD Intercept = 2.3 and BMD Slope = 1.6. This standard conversion equation was used in place of scan-specific conversions to facilitate integration with BoneJ. The BMD Intercept and BMD Slope constants represent the average slope and intercept determined from a sample of fifteen scans. To test for possible changes in the conversion due to scanner drift, the fifteen scans spanned the entire timespan of the study. Scan-specific conversion coefficients were calculated for each scan and used to calculate densities equivalent to the thresholds for trabecular bone (206 HU) and cortical bone (700 HU). The mean percent difference between the densities calculated using the standard and scan-specific calibrations was 1.4% and 1.0% for the 206 HU and 700 HU thresholds, respectively. This result indicated that variability in the scanner calibration throughout the time period of the study was reasonably low, and the use of a standard density conversion equation was appropriate.

To control for bone position relative to the scan plane, all tibias were aligned with their long axis using the moments of inertia function in BoneJ. A new stack was created by calculating the principle axes of inertia and aligning the tibia along the minimum axis. The realigned images retained the pixel height of the original image, and the voxel depth was adjusted to be equal to the pixel height. The aligned bone was re-sliced to a slice spacing equal to the image's pixel height. Pixel size varied minimally between images, with a difference of 0.486 mm between the maximum and minimum pixel height. The entire CT-image stack was then processed with the slice geometry function in BoneJ. This function computes the total area of all pixels with values above the 206 HU threshold chosen for bone. The resulting bone area (BA) is a measure of the total area of bone in each cross-section (Fig. 1C, E).

2.4. Calculating density weighted bone area along the length of the tibia

The BoneJ slice geometry results and the isolated tibia images were imported into MATLAB (Mathworks Inc., Natick, MA) to perform size-normalization and calculate density weighted BA (DWBA). Normalized BA (nBA = BA/*total bone length*^{2}) and normalized length were calculated to compare bones of different sizes.

In order to determine the DWBA across the entire normalized length of the tibia, custom MATLAB code was written to weight the area of each pixel above the bone threshold (206 HU) by its pixel density. Pixel area was determined using the pixel height and width from the DICOM

Table 1
Means and standard deviations of age, height, and weight for the three study groups, as well as percent Hispanic and gender characteristics of the participants.

Mean (SD)	Control group ($n = 167$)	AmbSB group ($n = 80$)	Non-AmbSB group ($n = 10$)
Age (years)	12.0 (3.1)	9.7 (2.6)	12.8 (2.2)
Height (cm)	150.4 (17.3)	129.7 (17.3)	133.0 (13.4)
Weight (kg)	47.8 (17.3)	37.4 (18.8)	53.5 (19.4)
Race, n (%) Hispanic	110 (65.9%)	75 (93.8%)	10 (100.0%)
Gender, n (%) male	90 (53.9%)	44 (55.0%)	8 (80.0%)