High Phosphate Diet Decreases Quantity and Quality of Mouse Femur

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INTRODUCTION: More than 30% of the US population consumes inorganic phosphate approximately 2.5 times the estimated average requirement [1, 2]. Excessive dietary phosphate in flavor enhancers and preservatives for processed foods can cause cardiovascular and kidney diseases [2]. Clinical and preclinical rat studies also showed that a high phosphate (HP) diet had negative effects on bone health, with biomarkers of bone metabolism primarily indicating increased resorption (2). However, detailed characterization of bone alteration in association with the HP diet-induced bone modeling and remodeling has not been fully investigated.

We hypothesize that a HP diet changes the quantity and quality of bone, thereby increasing the risk of bone fracture. Thus, the objective of the current study was to determine the effects of HP diet on characteristics of mouse femur.

METHODS: Following IACUC approval, twenty C57BL6 male mice (20-week-old) were fed with a normal phosphate (NP) diet containing 0.6% inorganic phosphate (Pi) for 12 weeks (n=10) and with HP diets containing 2.0% Pi for 14 weeks (n=10). A femur was randomly dissected from each mouse. The femurs (n=10 for each group) were thawed at room temperature and scanned by micro-CT (Skyscan 1122-D) with 20×20×20 µm³ voxel size. The micro-CT images, volumetric parameters were obtained by counting bone voxels that were segmented from non-bone voxels using a heuristic algorithm (Fig. 1a). The voxel counts were multiplied by the unit volume of voxel. A total volume (TV) includes volumes of bone (BV) and bone marrow, which was used to compute the bone fraction (BV/TV). Cortical bone (CB) and trabecular bone (TB) regions were separated using a compartmentalizing method. Mineral density parameters were measured using a gray level of each bone voxel that was converted to a value of tissue mineral density (TMD) using a calibration curve. A bone mineral density (BMD) was obtained by dividing the total mineral content (TMC) by the TV. Mean, standard deviation (SD), low and high (Low5 and High5) values at lower and upper 5th percentile values of TMD histogram were obtained (Fig. 1b). CB morphological parameters including thickness (Ct.Th) were also computed at a region centered at 55% (CB5) of the femoral length from the head, which is the location that 3-point bending load was applied. TB morphological parameters were determined at the region of interest (0.72 – 0.72 × 1.5 mm³) above growth plates at the distal condyle of femur. Trabecular bone fraction (BV/TV), surface-to-volume ratio (BS/BV), number (TB.N), thickness (Tb.Th), and separation (Tb.Sp) were measured. Following the non-destructive micro-CT scanning, the femurs were compressed at 55% length of the femur from the femoral head in the anterior-posterior direction using a 3-point bending jig with 5 mm span length (Fig. 2a). Non-destructive displacements with 0.01 mm were loaded and unloaded to obtain a slope of the static load-displacement curve to obtain a static stiffness (K), and a hysteresis (W) was computed as a difference of loading and unloading areas to measure amount of energy loss during loading (Fig. 2b). Dynamic mechanical analysis (DMA) used non-destructive bending oscillatory displacements (0.01±0.005 mm) at 0.5, 1, 2, and 3 Hz (Fig. 2c). Dynamic complex (K*) and tangent delta (tan δ) for the NP group (p<0.03). For the HP group, the Fmax had significantly positive correlations with K, K*, MeanCB, and Tb.Th (p<0.02) (Fig. 4). The Fmax had significantly positive correlations with K, K*, and Ct.Th but a negative correlation with tan δ for the NP group (p<0.03). For the HP group, the Fmax had significantly positive correlations with K, K*, MeanCB, and Ct.Th (p<0.01). DISCUSSION: The HP diet likely stimulates active osteoclastic bone resorption to decrease Ct.Th and Tb.Th but increase BS/BV, resulting in reduction of BV/TV. Active osteoblastic new bone formation follows the osteoclastic bone resorption due to the HP diet to remove more mineralized existing bone tissues and depot less mineralized new bone tissues. As a result, TMD parameters decrease in the HP group. The HP diet also aggravates mechanical characteristics of the femur, which increase the risk of fracture. The newly formed less mineralized bone tissues of cortical bone (TMD Low5CB) play an important role in determining the fracture strength (FMAX).

SIGNIFICANCE/CLINICAL RELEVANCE: The current findings provide detailed information on how excessive dietary phosphate substantially alters characteristics of bone increasing the fracture risk.


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