High Phosphate Diet Decreases Quantity and Quality of Mouse Femur

Do-Gyoon Kim[1], Kedryn K. Baskin[1], Kristin Nguyen [1], Momoko Karashima [1], Minji Kim[1,2], Yasmin Samanian [1], Olivia Jackson [1], Cynthia Murray [1], Daniel A. Branch [1], Beth S. Lee[1],
[1]Ohio State University, Columbus, OH, USA, [2]Ewha Women's University, Seoul, Republic of Korea
Kim.2508@osu.edu

Disclosures: Kim, DG (N), Baskin, K (N), Nguyen, K (N), Karashima, M (N), Kim, M (N), Samanian, Y (N), Jackson, O (N), Murray, C (N), Branch, D (N), Lee, B (N)

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 pre-clinical 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 1172-D) with 20×20×20 μm^3 voxel size. Using 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 (Low_5 and $High_5$) 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% (CB_{55}) 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 mm^3) above growth plates at the distal condyle of femur. Trabecular bone fraction (BV/TV_{TB}), 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) that accounted for energy dissipation ability were measured. The DMA values at each frequency were averaged. Following the non-destructive DMA testing, the same femur was fractured at a bending displacement rate of 0.5 mm/second. Maximum force (F_{max}), displacement (d_{max}), and toughness (U) were assessed at fracture using the load-displacement curve (Fig. 2d). A Student's t-test was performed to compare each parameter between NP and HP groups with a significance of $p<0.05$. Correlations of F_{max} with other parameters were examined using Pearson's correlation tests. Significance was set at $p<0.05$.

RESULTS SECTION: The HP group had significantly lower bone fraction (BV/TV), bone mineral density (BMD), and mean (TMD Mean), low and high (Low_5 and $High_5$) values of the TMD histogram, and Ct.Th ($p<0.02$) than the NP group (Fig. 1c, and Fig. 3). The Tb.Th was marginally lower, but the BS/BV was marginally higher for the HP group than the NP group ($p<0.062$). The HP group had significantly lower static stiffness (K), dynamic stiffness (K*), energy dissipation (tan δ), and maximum force (F_{max}) than the NP group ($p<0.05$) (Fig. 4). The F_{max} 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 F_{max} had significantly positive correlations with K, K*, $Mean_{CB}$, and Low_{5CB} ($p<0.02$) (Fig. 4).

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 deposit less mineralized new bone tissues. As a result, TMD parameters decrease in the HP group. Combination of the morphological and TMD changes gives rise to lower BMD in the HP group than the NP 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 Low_{5CB}) play an important role in determining the fracture strength (F_{Max}).

A limitation of this study may be that the detailed physiological change along with the alteration of bone characteristics was not investigated. Direct associations between these physiological changes and alteration of bone characteristics remains to be investigated.

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

REFERENCES: [1] Lee, A.W. and S.S. Cho, Nutr J, 2015;14:28. [2] Vorland, C.J., et al., Curr Osteoporos Rep, 2017;15(5):473-482.

ACKNOWLEDGEMENTS: OSU College of Dentistry student research program.

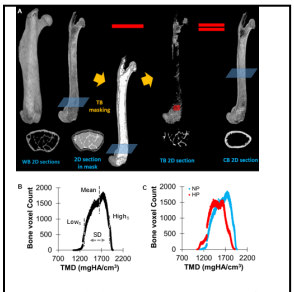

Fig. 1 (A) Compartmentation of 3D micro-CT image. (B) The tissue mineral density (TMD) parameters, (C) typical TMD histograms of WB in NP and HP groups. Red square for TB morphology.

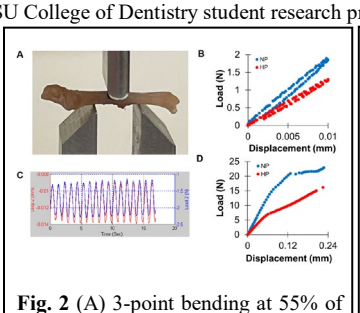

Fig. 2 (A) 3-point bending at 55% of length (B) non-destructive static loading for K and W, (C) DMA, and finally, (D) static fracture in NP and HP groups. All non-destructive static, DMA, and fracture testing were performed using the same specimen.

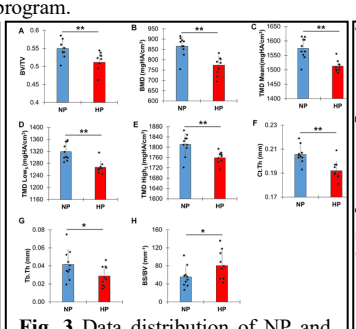

Fig. 3 Data distribution of NP and HP groups (n=10 for each group). (A) BV/TV, (B) BMD, (C) TMD Mean, (D) Low_5 and (E) $High_5$ values, (F) Ct.Th, and (G) Tb.Th, (H) BS/BV. *; p=0.06, **; p<0.005.

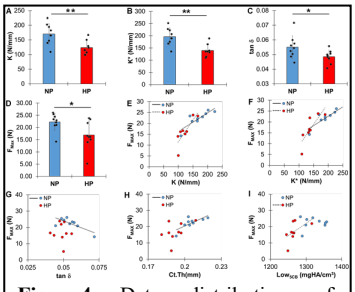

Fig. 4 Data distribution of mechanical parameters of NP and HP groups (n=9 for each group). (A) K, (B) K*, (C) tan δ, and (D) F_{MAX}. Significant correlations of F_{MAX} with (E) K, (F) K*, (G) tan δ, (H) Ct.Th, (I) Low_5 TMD of CB. *; p<0.05, **;