

High Fat Diet Exacerbates Loss of Bone Fracture Toughness with Aging in C57BL/6 Mice

Kenna Brown¹, Ghazal Vahidi¹, Maya Moody¹, Ramina Behzad², Hope Welhaven¹, Brady Hislop¹, Kat Paton¹, Dr. Lamyia Karim², Dr. Ronald June¹, Dr. Stephen Martin¹, Dr. Chelsea Heveran¹

¹ Montana State University Bozeman, MT

² University of Massachusetts Dartmouth Dartmouth, MA

Email of presenting author: kennabrown@montana.edu

Disclosures: RKJ owns stock in Beartooth Biotech which was not involved in this study

INTRODUCTION:

The elderly population is the fastest growing worldwide and is at a higher risk of eating high fat diets (HFD) [1,2]. Both diet-induced obesity (DIO) and aging alter metabolism and increase fracture risk [3,4]. Aging and DIO are known to change matrix and mineral properties of the bone that are thought to contribute to increased fracture risk, such as accumulation of advanced glycation end products (AGEs) [4,5]. The similarities in systemic and matrix changes caused by both DIO and aging pose the question of whether HFD could exacerbate aging-related loss of bone fracture resistance. Further, a separate question is whether HFD itself, or obesity produced from HFD, decreases bone fracture resistance. We hypothesized that a short-term diet of 45% dietary fat would exacerbate the loss of bone fracture toughness in aging C57BL/6 mice.

METHODS:

We assigned HFD (45% fat) or low-fat diet (LFD, 10% fat) for 8 weeks to male and female C57BL/6 mice (n = 7-11/ group). Mice were euthanized at 5 and 22 months of age. Study procedures were approved by the University IACUC. Multilevel analyses of bone properties included, weight tracking, intraperitoneal glucose tolerance testing (ipGTT), quantitative histomorphometry, microCT, fracture toughness testing, fluorescent AGE (fAGE) quantification, metabolomics from flushed cortical bone [6], RT-PCR for lacunar canalicular system (LCS) turnover markers, and histology for adipocytes, osteoclast activity, and osteocyte death. 3-way ANOVA analyses were done on all data, except for metabolomics (ECCO, Mummichog) [7]. ANOVA results were performed with and without outliers identified by Grubb's test and corrected for multiple comparisons by Bonferroni tests. Correlations were performed with Spearman's method. Significance was defined as $p < 0.05$.

RESULTS:

HFD and aging disrupted glucose metabolism regardless of weight gain. The resistance to initial crack propagation, $K_{c,yield}$, decreased with HFD (-15%, $p = 0.023$) and aging (-16%, $p = 0.017$) in an additive manner (Figure 1.A). HFD did not change any cortical microarchitecture. Cancellous measures were mostly unchanged, except for sex-diet interactions with BMD (-16% $F < M$ for both diets, $p = 0.010$) and trabecular thickness (+9.7% $F > M$ HFD only, $p = 0.048$). The shared metabolic signatures of aging and HFD identified by ECCO include pathways relevant to matrix changes by increased glycosylation, decreased mineralization, altered turnover activity, and increased cellular stress and death (Figure 1.B).

HFD combined with aging increased osteocyte death (+75%, $p = 0.024$) and decreased osteoclast and bone marrow adipocyte (bMAT) densities (-81%, -62% respectively, both $p < 0.05$). The *Opg:Rankl* ratio declined in aging mice (-52%, $p = 0.015$) while HFD increased expression of other LCS turnover markers, such as *Atp6v1g1* (+38%, $p = 0.014$). Diet but not aging decreased endocortical mineralizing surface (-33%, $p = 0.019$).

Whole bone fAGEs demonstrate complex sex, diet, and age interactions and relationships to material and mechanical properties. LFD animals did not demonstrate significant differences in fAGE content between sex or age groups. HFD females had decreased fAGE content for aged mice (-34%, $p = 0.043$) while HFD males had increased fAGE content for aged mice (+50%, $p = 0.043$). HFD males had weak to moderate negative relationships with fracture toughness measures ($K_{c,max}$ $r^2 = -0.287$, $K_{c,yield}$ $r^2 = -0.462$ respectively) and elastic modulus ($r^2 = -0.451$). HFD females, however, showed opposite relationships from the males (Figure 1.C). HFD females had positive, moderate to strong correlations with fracture toughness measures ($K_{c,max}$ $r^2 = 0.691$, $K_{c,yield}$ $r^2 = 0.727$ respectively) and a weak positive correlation with elastic modulus ($r^2 = 0.439$).

DISCUSSION:

This study finds that fracture toughness is reduced through short-term HFD, regardless of weight gain. In this study, some of the mice became obese, but most groups did not. Regardless, most mice fed HFD experienced disruption in glucose metabolism. Histological and metabolomics data add insight into these effects, demonstrating that HFD has impacts on bone cell abundance, viability, and metabolic products found in cortical tissue.

HFD reduced mineralizing surface in both ages and osteoclast density in aged mice only, suggesting that surface turnover is hindered by HFD in aged mice. These data, considered with decreased bMAT density, indicate HFD significantly reduces the differentiation ability of stem cells into multiple cell types critical to bone health in aged animals. Further, metabolic pathways affected by both aging and HFD implicate several known contributors to decreased bone matrix maintenance, including sphingolipid metabolism, N-glycan biosynthesis, and arachidonic acid metabolism.

HFD may have an important effect on the osteocyte. HFD and aging together decrease osteocyte viability. Further, several LCS turnover markers assessed by PCR are increased with HFD, which may reflect a different response of osteocytes in maintaining their local environment in the context of HFD. These data have implications for osteocyte maintenance of bone quality and potentially mechanosensitivity.

The reasons for decreased bone toughness are multifactorial and are not the same between aging and HFD. Furthermore, important sex differences are apparent. For example, for males, increased fAGE content in bone is associated with reduced toughness, while this relationship is inverted for females. Additional characterization of bone matrix is ongoing.

SIGNIFICANCE:

We show that HFD can exacerbate aging-related bone fragility. The shared metabolic signatures of aging and HFD add insights into the decrease in bone fracture resistance in both conditions. This information could help provide targets for improved therapies and quality of life in the elderly population.

REFERENCES:

[1] Choi+, Public Health Nutr., 2021; [2] Jarzebski+, Nature, 2021; [3] Ionova-Martin+, Osteoporos Int, 2011; [4] Zimmermann+, Proc Natl Acad Sci, 2011; [5] Mansur+, Front Endocrinol, 2019; [6] Welhaven+, JBMR+, 2022; [7] Hislop+, BioRxiv, 2022

ACKNOWLEDGEMENTS: NSF 2120239 and NIH AG068680

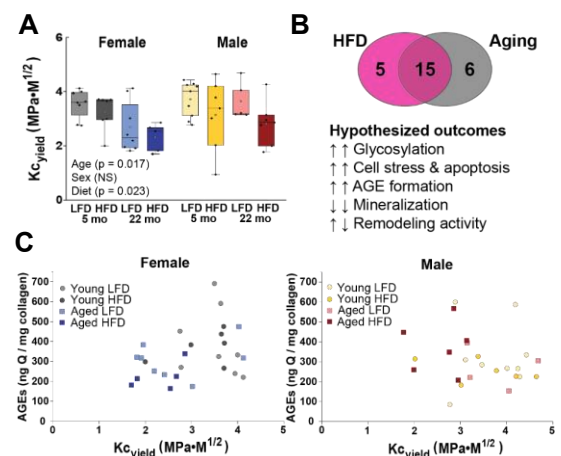


Figure 1 (A) $K_{c,yield}$ fracture toughness with additive aging and diet behavior. (B) Metabolomic signature overlap between aging and HFD, and some of the hypothesized outcomes of the overlapping signature. (C) fAGE content has inverse relationships with K_c fracture toughness measures between sexes.