

# High Fat Diet Induces Deleterious Macroscopic And Microscopic Changes In The Rabbit Femur

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**INTRODUCTION:** Metabolic dysfunction caused by a high fat diet (HFD) has been shown to have detrimental effects on the musculoskeletal system including fracture, tendon pathology and inflammation, contributing to significant health care burden. Overall, there remains a paucity of data regarding characterisation of HFD effects on bone in a large animal model. In addition, the effect of sex has not previously been investigated. Our study therefore aimed to investigate the macroscopic and microscopic effects of a HFD on bone in a mature-aged rabbit animal model and to elucidate any sex-linked differences.

**METHODS:** 24 retired breeder New Zealand White rabbits were approved and used to represent mature age animals (Mayo Clinic IACUC A5660). For each gender, the animals were randomly divided into four groups: the Control group males (n=6) and females (n=6) received a standard rabbit diet for 6 weeks, whereas the HFD group males (n=6) and females (n=6) were fed the standard diet supplemented with 0.5% cholesterol and 4% peanut oil as previously reported for 12 weeks to first induce metabolic dysfunction, followed by treatment with the same HFD for a further 6 weeks. At the end of the 6 week timepoint, the rabbits were sacrificed and femurs were harvested for analysis. This included biomechanical testing with three-point-bend to failure (n=6/group) and nanoindentation (n=6/group) of cortical and trabecular bone. Bone volume, bone mineral density analysis and micro-architecture were assessed using X-ray (n=3/group), microCT imaging (n=6/group) and histology (n=3/group). One-way ANOVA with a Tukey post-hoc for multiple comparisons and simple t tests were used for determining statistical significance in histomorphometry and biomechanical tests with a  $p \leq 0.05$  considered to represent statistically significant differences.

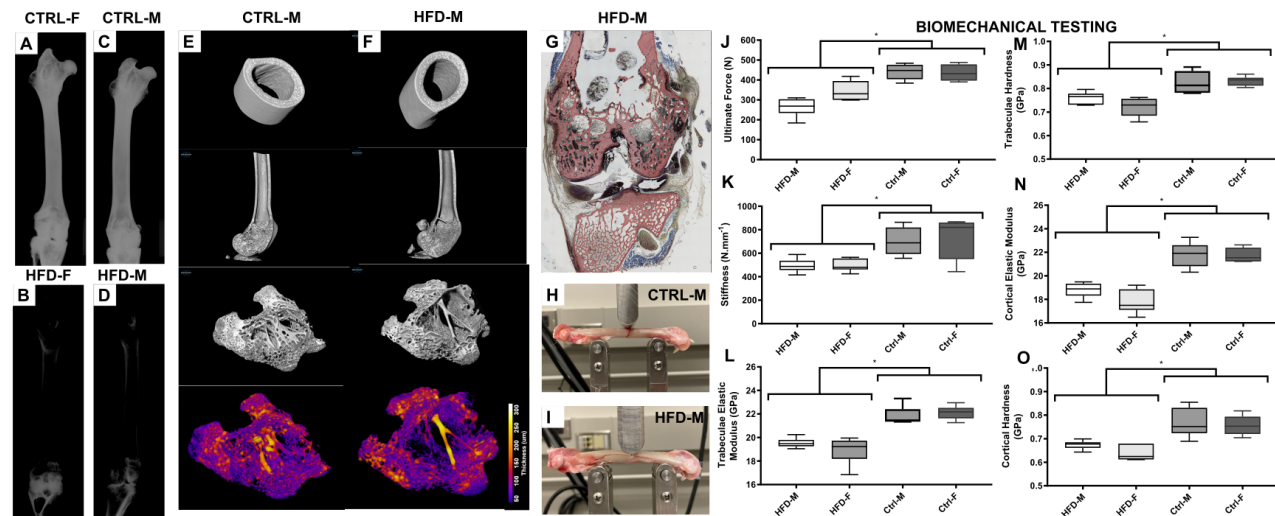
**RESULTS:** No difference in bone volume was noted between Control and HFD animals in both trabeculae and cortical bone during microCT analysis ( $p > 0.05$ ; Figure 1E-F). However, bone mineral density was lower in HFD animals with trabecular bone being most affected (10% reduction in BMD;  $p < 0.05$ ). Analysis of structural thickness of the trabeculae demonstrated a composition of more narrow trabeculae in HFD bone with significant difference between the HFD-M and Ctrl-M group ( $p < 0.05$ ). X-rays further corroborated this finding and illustrated the stark contrast in bone density between HFD and Ctrl animals with the HFD-M group being most affected (Figure 1A-D). Histomorphometry analysis exhibited increased osteoclast activity and reduced microvasculature in the bone of HFD animals. In the three-point bend test of the femoral shaft, there was a 30% reduction in ultimate force and stiffness of HFD femurs when compared to controls ( $p < 0.05$ ; Figure 1J) and contrasting fracture patterns at failure (Figure 1H-I). The mean reduction in ultimate force and stiffness of HFD-M femurs were significantly greater than that observed in the HFD-F group ( $p < 0.05$ ). There were no significant differences between the groups in maximum deflection prior to failure ( $p > 0.05$ ). During nanoindentation analysis, there was a 13% and 11% reduction in elastic modulus and hardness respectively of trabeculae bone ( $p < 0.05$ ; Figure 1L-M). Similarly, in the cortical bone of HFD animals, there was a significant 11% and 14% reduction in elastic modulus and hardness respectively ( $p < 0.05$ ; Figure 1N-O).

**DISCUSSION:** The introduction of a HFD has been linked to increased trabecular bone loss and reduced bone mineral content in rodent animal models (1, 2). However, the impact of HFD on cortical bone remains unclear and studies have reported mixed results (2, 3). Rabbits have been identified as a superior alternative to rodents in bone studies due to their larger skeleton and phylogenetic similarities to primates. This study has characterized the deleterious effects of a HFD on bone biomechanical properties, bone architecture and bone mineral density as well as the microscopic changes on a cellular level, with a greater impact on males observed. While the elastic/hardness properties of the individual osteons and bone mineral density showed no significant difference between HFD-M and HFD-F groups, the reduction in macroscopic biomechanical properties (ultimate force and stiffness) and smaller trabeculae diameter observed on microCT indicate that HFD affects the macroscopic architecture of male bone greater than females.

**SIGNIFICANCE/CLINICAL RELEVANCE:** A HFD contributes to metabolic dysfunction and negatively impacts bone quantity and quality on a macroscopic and microscopic level. Males are affected more than females in this setting of metabolic dysfunction and may be at higher risk for fracture.

## REFERENCES:

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**Figure 1:** XR, microCT, histology and biomechanical analysis results. **A-B):** Ctrl-M vs HFD-M x-ray; **C-D):** Ctrl-f vs HFD-F x-ray; **E-F)** MicroCT 3D reconstructions; **G)** Example of resin-embedded histology of distal femur in Sanderson rapid bone stain; **H-I)** Differences in fracture pattern at failure during 3 point bend test; **J-K):** Ultimate force and stiffness from 3 point bend test; **L-O):** Elastic modulus and hardness of trabeculae and cortical bone during nanoindentation testing.