# The Effect of a Ketogenic Diet on Injury-induced Osteoarthritis Progression

Nole M. Robertson[3*], Nikola N. Jaworska[1,2*], Molly R. Gallop[3], Baifeng Yu[1,2], Peyton Mower[3], Nurlatifa Zahra Mohd Fuad[1,2], Renan Vieira[3], Isabella Y.Goh[1,2], Jie Zhang[4], Amandine Chaix[2,3#], Natalia S. Harasymowicz[1,2,3,#]

[1]Department of Orthopaedics, [2] Molecular Medicine Program, [3]Nutrition and Integrative Physiology, [4] Anesthesiology Department
University of Utah College of Health University of Utah, Salt Lake City, UT, USA

*co-first authors, [#]co-corresponding authors

Email: natalia.harasymowicz@utah.edu **Disclosures**: NMR, NNJ, MG, BY, PM, NZMF, RN, IYG, AC (none), NSH (Immunis - 1),

**INTRODUCTION:** Osteoarthritis (OA) is a disease of the entire joint, characterized by degeneration of the articular cartilage and remodeling of the underlying bone, leading to the development of pain. Obesity, predicted to affect one in two individuals by 2050, is a major risk factor for the development of osteoarthritis (OA). The impact of obesity on OA progression has long been attributed to increased weight on the joints but later refuted by the presence of OA in non-weight bearing joints. Obesity is a complex disease for which the contribution of associated changes in metabolic and inflammatory parameters are emerging as key OA contributors. Previous studies demonstrated that obesity and dietary fatty acids (FAs) play a critical role in the development of osteoarthritis (OA) following joint injury[1, 2], pointing to the detrimental role of dietary lipids. To start disentangling the effect of increased weight versus hyperlipidemia – a common complication of obesity –we compared the progression of OA under two high-fat diets: a 60% HFD that leads to obesity and metabolic disease, and a 90% fat KD that leads to hyperlipidemia without obesity.

**METHODS:** Six-week-old male mice (n=11-15/group) were fed: (i) a Low Fat Diet (**LFD**; 10%/70%/20% kcals from fat (F), carbohydrates (C), protein (P) respectively; (Research Diets #D12450K); (ii) a High Fat Diet (**HFD**; 60%/20%/20% kcals from F/C/P resp.; Research Diets #D1292); or (iii) a Ketogenic Diet (**KD**; 89.9%/0.1%/10% kcals F/C/P resp. ; Research Diets #D16062902). A destabilization of the medial meniscus (DMM) surgery was performed at 16 weeks of age to introduce OA into the left hind limb. Body weight, body composition (NMR), and serum parameters (Triglycerides, Cholesterol, Non-esterified fatty acids (NEFA), Insulin) were measured to evaluate metabolic health before collection (week 27). Mechanical and thermal nociceptive sensitivities of the hind paws were assessed using an electronic von Frey aesthesiometer and the hot plate test. At the experimental endpoint, joints were collected, embedded, sectioned, and stained with fast green and Safranin-O (SafO), or hematoxylin and eosin (H&E) to assess OA and synovitis severity. Sections were graded using a modified Mankin scoring system and Synovitis scoring by three independent, blind scorers. Bone microarchitecture was analyzed by microCT. Statistical analyses were performed using one or two-way ANOVA with Tukey's correction at $p<0.05$. All animal procedures were approved by the University of Utah IACUC.

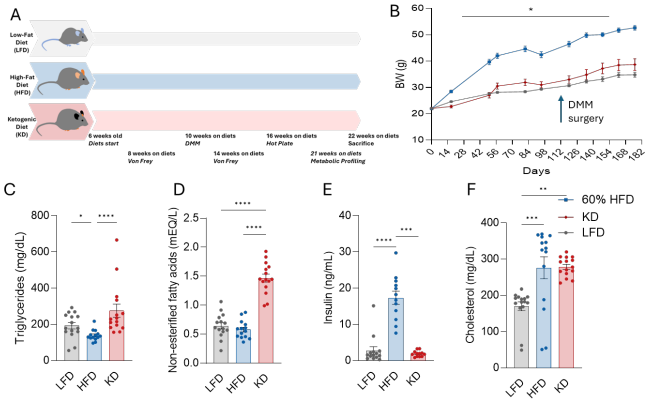


***Fig.1. Ketogenic Diet affects metabolic parameters without increasing body weight or insulin levels*** *(A) Study Design (B) Longitudinal Body Weight Assessment (C) Serum Triglyceride concentration (D) Serum Non-esterified Fatty Acids Concentration (E) Serum Insulin (F) Level Serum Cholesterol Level in mice fed either Low Fat Diet (LFD), High-Fat Diet (HFD), or Ketogenic Diet (KD). DMM-destabilization of medial meniscus. n=11-15 mice used. One Was Anova used to assess statistical significance, p<0.05 indicated as *, p<0.005 indicated as **, p<0.001 indicated as ***.*

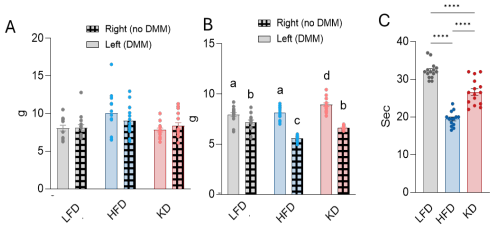


***Fig.2 Different diets affect pain sensitivity post-DMM.*** *Paw mechanical withdraw threshold (in g) measured by Von Frey (A) at two weeks before and (B)four weeks after DMM surgery. (C) Hot plate latency measured at 9 weeks post-DMM. DMM- destabilization of medial meniscus, 2-way Anova, different letters indicate p<0.05 between groups. * p<0.05 indicates group effect, n=11-15 mice used per group.*

**RESULTS:** After 6 weeks on the diet, HFD mice were significantly heavier than KD and LFD mice (Fig. 1B). At the experimental endpoint, HFD mice had significantly higher fat mass, fat mass percentage, and serum insulinemia than the KD and LFD mice (data not shown and Fig. 1E). Serum hypercholesterolemia (Fig. 1F) was similar in KD and HFD mice. KD mice on the other hand had higher serum triglycerides (Fig.1 C) and non-esterified fatty acids (NEFA) (Fig. 1D). Two weeks before DMM surgery, there was no significant difference in mechanical pain threshold between right and left hind limbs in any of the experimental groups. Interestingly, four weeks after DMM, mice on HFD had a significantly lower pain threshold while mice on KD displayed significantly higher pain threshold in the non-operated limb and no difference in the operated limb when compared to LFD mice. At nine weeks post-DMM, HFD mice had significantly lower hot plate latency compared to LFD and KD mice. Finally, we assessed the effect of high dietary fat intake with or without obesity and metabolic disease on the severity of OA and synovitis. SafO staining and Total Mankin Scoring confirmed that HFD mice had significantly higher OA severity compared to LFD. However, OA severity was not different between mice on LFD and KD (Fig.3A-B), nor did they display lower bone quality when compared to LFD. At the time of sacrifice, mice on HFD were the only ones to display significant changes in bone microarchitecture, including increased Trabecular Separation in the medial femoral condyles (Fig.3C-D).

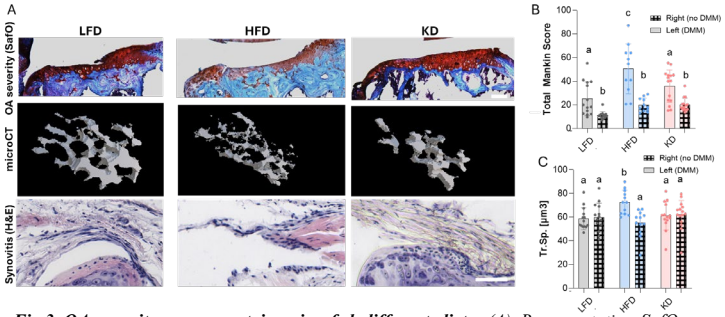


***Fig.3 OA severity assessment in mice fed different diets.*** *(A) Representative SafO staining, microCT analysis and H&E staining of medial tibial condyle (B) Total Mankin Score (C) Trabecular Spacing of medial femoral condyle as quantified by microCT. .2-way Anova, different letters indicate p<0.05 between groups. * p<0.05 indicates group effect, n=11-15 mice used per group. Scale bar = 50µm.*

**DISCUSSION:** The role of KD in the OA pathogenesis has not been fully established, with studies indicating both protective [3] and detrimental roles of KD in disease progression [4]. Importantly, the inconsistency in the dietary macronutrient composition of KDs and variations in food intake may help explain these discrepancies. Here, we present that OA progression was similar between mice fed LFD or a 90% fat KD in male mice. Hence, a KD, despite being richer in fat than HFD, did not mimic the severity of OA in diet-induced obese mice. These results suggest that beyond dietary fat and systemic lipidemia, metabolic alterations such as insulin resistance and inflammation may underlie obesity-accelerated OA. Future studies will clarify the more precise contribution of those factors. **SIGNIFICANCE:** Overall, this research carries implications for future therapeutic strategies, dietary interventions, and guidelines for managing OA, particularly in the context of dietary fat intake and metabolic health.

**REFERENCES:[1]**Wu, C.L. *et al. Ann Rheum Dis* **74**, 2076-083 (2015);**[2]**: Tang, R. *et al. Proc Natl Acad Sci U S A* **121**, e2402954121 (2024).**[3]**: Kong, G. *et al. Arthritis Res Ther.* **24**(1):113; **[4]** Dyson, G *et al. ACR Open Rheumatology*, **7**: e11794.