

Metabolic Disorder: An Underlying Driver in Mitochondrial Dysfunction and Osteoarthritis Pathogenesis

Sher Bahadur Poudel¹, Gozde Yildirim¹, Mercedes Del Rio-Moreno², Thorsten Kirsch³, Rhonda D Kineman², Shoshana Yakar¹

¹Department of Molecular Pathobiology, David B. Kriser Dental Center, New York University College of Dentistry, New York, NY, USA

²Department of Medicine, Section of Endocrinology, Diabetes, and Metabolism, University of Illinois at Chicago and Research and Development Division, Jesse Brown VA Medical Center, Chicago, IL 60612, USA.

³Department of Orthopaedic Surgery, NYU Grossman School of Medicine, New York, NY, USA

INTRODUCTION: Metabolic disorders, a group of conditions rooted in disrupted energy homeostasis and characterized by factors like obesity, insulin resistance, and hypertension, have become global health epidemics of considerable concern. Simultaneously, osteoarthritis (OA), the most prevalent form of arthritis, affects millions worldwide, causing pain, disability, and a substantial healthcare burden. These two seemingly distinct health issues share a surprising connection that extends beyond mere coincidence. Recent scientific investigations have uncovered a complex relationship between metabolic disorders and OA, shedding light on shared mechanisms that contribute to the pathogenesis of the latter. People with compromised metabolism develop OA and have more pervasive pathology, including increased inflammation, articular cartilage (AC) degeneration and increased intensive pain in the joints as compared to people with normal metabolism [1]. However, mechanism of OA pathogenesis in non-alcoholic fatty liver disease (NAFLD) is not clearly understood. Thus, this study aims to elucidate the mechanism by which chondrocyte metabolism is altered by NAFLD inducing the cartilage loss and OA development.

METHODS: All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of the NYU School of Medicine. **In vitro studies:** The murine primary chondrocytes derived from the knee joints AC were treated with different concentrations of palmitic acid (PA) and examined the cell viability in 24 and 48 hours. The mitochondrial structure of cells treated with PA or control (CTL) was observed under the confocal microscope after staining with mito-tracker orange. RNA was isolated from the cells after 6 hours culture in PA or CTL media and mRNA levels of mitochondrial fusion and fission gene markers and Sirt3 were checked by real time qPCR. Mito-stress, real time oxygen consumption rate (OCR) and extra cellular acidification rate (ECAR) were measured with the seahorse analyzer. **In vivo studies:** C57BL/6J (B6) male mice fed with NAFLD or CTL diet for 9 months were sacrificed and confirmed the NAFLD with the liver histology. Knee joints were collected and fixed for 48 hours. Subchondral bone (SCB) and knee joints AC morphology were examined with micro-computed tomography and safranin O Red staining respectively. Immunohistochemistry (IHC) was performed to check the iNOS and NLRP3 protein expression in AC.

RESULTS: Cell viability was reduced on dose and time dependent manner indicating that PA is toxic to the chondrocytes. We stained the mitochondria of primary chondrocytes with mito-tracker orange dye and found that PA affects the mitochondrial structure. PA induced mitochondrial fragmentation resulting into shorter mitochondria (Figure 1). However, interestingly there was no change in the gene expressions of mitochondrial fission gene markers Fis1 and Drp1. While, PA treated cells expressed higher Sirt3 mRNA levels compared to CTL media. Furthermore, the PA treated cells showed increased levels of OCR as well as ECAR when checked after 30 mins of treatment. In our murine metabolic disorder model, NAFLD mice displayed reduced bone volume percentage, trabecular number and bone mineral density of knee joint SCB compared to the CTL mice indicating that NAFLD induces the bone loss in sub chondral region. Histological analysis of the knee joints showed AC degeneration and loss of extracellular matrix which are the characteristics of OA. The severity of OA was increased in NAFLD compared to CTL mice (Figure 2). Furthermore, NAFLD mice possessed with higher percentage of positive chondrocyte cells expressing iNOS and NLRP3 proteins.

DISCUSSION: Chondrocyte metabolism is vital for the synthesis, maintenance, and repair of the cartilage matrix, ultimately contributing to the overall health and function of cartilage in joints. NAFLD is characterized with dyslipidemia associated with increased levels of free fatty acids (FFAs) [2]. In previous studies, it was found that elevated FFAs level was primary cause for OA onset in dyslipidemia. FFAs exerted lipotoxicity and chondrocyte apoptosis resulting to severe cartilage degradation in mice fed with high fat diet [3]. FFAs increased oxidative stress in human articular chondrocytes mediated by inflammatory cytokines (IL-6 and IL-8) and triggered extracellular matrix degradation [4]. Our study also showed that long chain fatty acid, PA exerted chondrocyte toxicity resulting the increased cell death associated with mitochondrial alterations. The preliminary data from this study indicate that NAFLD increases the metabolic dysfunction affecting the mitochondrial structure and function of AC chondrocytes that induces the cartilage degradation and SCB loss leading to OA. Further experiments are planned to understand the molecular mechanism by which mitochondrial dysfunctions are occurred by the NAFLD that increases the OA pathogenesis.

SIGNIFICANCE: Current interventions aimed to slow down the progression or regeneration of cartilage loss are limited as the molecular mechanisms involved in OA pathogenesis are not completely understood. Findings from this study is expected to provide a base for understanding how metabolic disorder associated with NAFLD damages mitochondrial metabolism in AC chondrocyte and promote OA development, offering the potential to provide new therapeutic targets for OA treatment.

ACKNOWLEDGEMENTS: This study was supported by “Department of Molecular Pathobiology Accelerator Award (P01) 2023”, Department of Molecular Pathobiology, New York University College of Dentistry, New York.

REFERENCES:

- Wang, H., Cheng, Y., Shao, D., Chen, J., Sang, Y., Gui, T., Luo, S., Li, J., Chen, C., Ye, Y., Yang, Y., Li, Y., & Zha, Z. (2016). Metabolic Syndrome Increases the Risk for Knee Osteoarthritis: A Meta-Analysis. Evidence-based complementary and alternative medicine : eCAM, 2016, 7242478.
- Mocciaro G, Allison M, Jenkins B, Azzu V, Huang-Doran I, Herrera-Marcos LV, Hall Z, Murgia A, Susan D, Frontini M, Vidal-Puig A, Koulman A, Griffin JL, Vacca M. Non-alcoholic fatty liver disease is characterised by a reduced polyunsaturated fatty acid transport via free fatty acids and high-density lipoproteins (HDL). Mol Metab. 2023 Jul;73:101728. doi: 10.1016/j.molmet.2023.101728. Epub 2023 Apr 19. PMID: 37084865; PMCID: PMC10176260.
- Lee, S. W., Rho, J. H., Lee, S. Y., Chung, W. T., Oh, Y. J., Kim, J. H., Yoo, S. H., Kwon, W. Y., Bae, J. Y., Seo, S. Y., Sun, H., Kim, H. Y., & Yoo, Y. H. (2018). Dietary fat-associated osteoarthritic chondrocytes gain resistance to lipotoxicity through PKCK2/STAMP2/FSP27. Bone research, 6, 20.
- Medina-Luna, D., Santamaría-Olmedo, M. G., Zamudio-Cuevas, Y., Martínez-Flores, K., Fernández-Torres, J., Martínez-Nava, G. A., Clavijo-Cornejo, D., Hernández-Díaz, C., Olivos-Meza, A., Gomez-Quiroz, L. E., Gutiérrez-Ruiz, M. C., Pineda, C., Blanco, F., Reginato, A. M., & López-Reyes, A. (2017). Hyperlipidemic microenvironment conditionates damage mechanisms in human chondrocytes by oxidative stress. Lipids in health and disease, 16(1), 114.

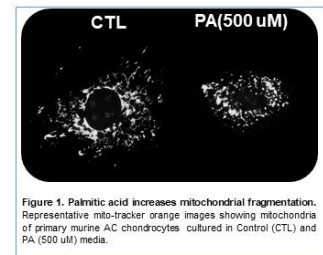


Figure 1. Palmitic acid increases mitochondrial fragmentation. Representative mito-tracker orange images showing mitochondria of primary murine AC chondrocytes cultured in Control (CTL) and PA (500 uM) media.

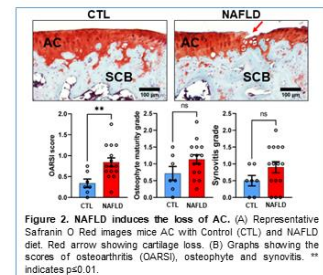


Figure 2. NAFLD induces the loss of AC. (A) Representative Safranin O Red images mice AC with Control (CTL) and NAFLD diet. Red arrow showing cartilage loss. (B) Graphs showing the scores of osteoarthritis (OARSI), osteophyte and synovitis. ** indicates p<0.01.