The function of intermediate filaments in intracellular mechanical stress in lipid-loaded hepatocytes

Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) affects an estimated ~33% of the US population, yet remains vastly underdiagnosed.[1] Left untreated, progressive lipid accumulation within hepatocytes can lead to inflammation, fibrosis, and liver dysfunction.[1-2] Our lab previously demonstrated that lipid droplets (LDs) act as internal mechanical stressors causing nuclear deformation and increased chromatin condensation.[3] These results indicate a pathological role, however, how these lipid droplets lead to nuclear changes remains unclear.

One hypothesis suggests direct compression by LDs whereas another implicates the cytoskeleton and its connection to the nucleus via the LINC complex.[4] Our lab observed LD-induced actin and microtubule disorganization, and pharmacological disruption of actin polymerization partially reversed nuclear abnormalities, suggesting cytoskeletal involvement.[3]

While actin is well studied in mechanotransduction, intermediate filaments (IFs) are increasingly recognized as crucial mechanical integrators due to their structural diversity and association with the nuclear envelope.[5-6] Thereby, we investigated the role of IFs in transmitting LD-induced mechanical stress to the nucleus in hepatocytes.

Materials and Methods

Primary Human Hepatocytes (PHHs) were seeded on 250-Pa collagen-coated polyacrylamide gels. Cells were treated with 5% fatty-acid free BSA with or without the addition of 400uM sodium oleate to induce lipid loading. After 48 hours of lipid-loading cytoskeletal components were perturbed using 5uM Latrunculin A (actin), 10uM Nocodazole (microtubules), 5uM Blebbistatin (myosin), and 5uM Withaferin A (intermediate filaments) for 4 hours.

An MTT analysis was performed to test Withaferin A's cytotoxicity.

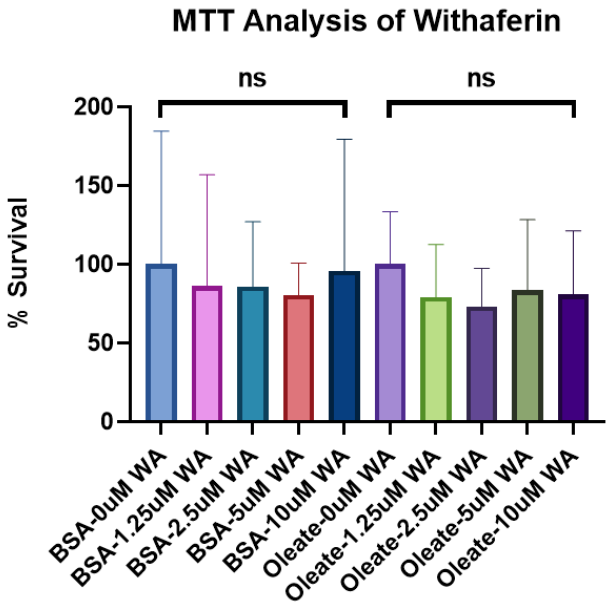
Cells were fixed with 4% paraformaldehyde and stained for cytoskeletal elements (actin, tubular, cytokeratin 8, myosin IIa) and histone methylation marker (H3K27Me3). They were imaged by confocal microscopy. Image analysis included fluorescence intensity, ridge detection, fibril orientation, and chromatin condensation using FIJI and MATLAB.

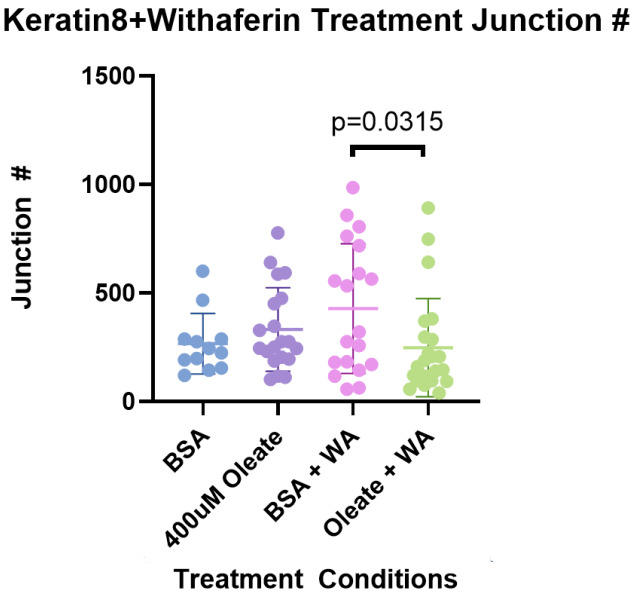
Results, Discussion & Conclusions

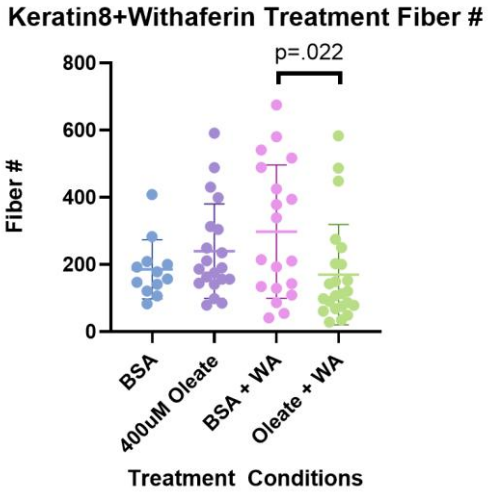
MTT assays confirmed that Withaferin A (WA) concentrations of up to 10uM were not cytotoxic. We found that LDs alone did not significantly disrupt keratin-8 filament structure, suggesting IFs form a stable network under mechanical load. In LD-loaded cells, Withaferin A increased keratin fiber width, possibly indicating compensatory filament bundling or stress-induced stabilization. Notably, Withaferin A significantly altered fiber number and branching in non-LD cells, implying that IFs may become mechanically engaged and resistant to disassembly under LD-induced stress. In these initial experiments, chromatin condensation seems to have increased an equal amount in both LD and non-LD cells when treated with Withaferin, suggesting keratin intermediate filaments do regulate chromatin condensation in a manner that is independent of internal stress.

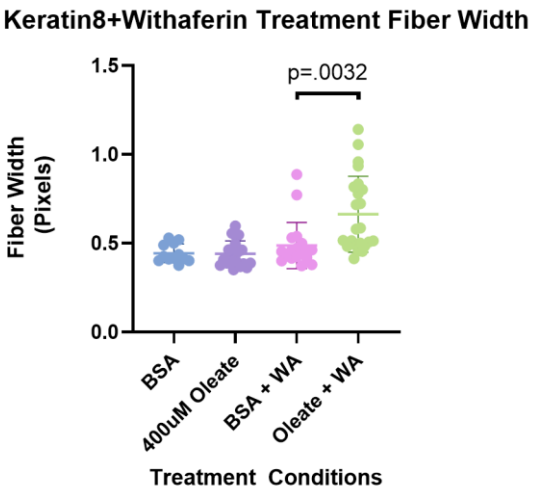
Given our findings, it is possible that, without mechanical stress, intermediate filaments are free to relax and branch more when exposed to WA. To test this further, higher concentrations of WA should be tested to see if phenotypes worsen, and if the effect of LD-presence can be overridden. Additionally, seeding cells on stiffer substrates could explore whether the phenotypes observed are unique to intracellular stress, or apparent with presence of any mechanical stress. For chromatin condensation, once more data have been collected, a more definitive claim can be made on the relation between intermediate filaments and chromatin condensation. Further testing will also include methylation and acetylation markers to see the effects of different histone condensing mechanisms in the presence of LDs. Additionally, the use of methyltransferase and deacetylase inhibitors could help to further explore those pathways.

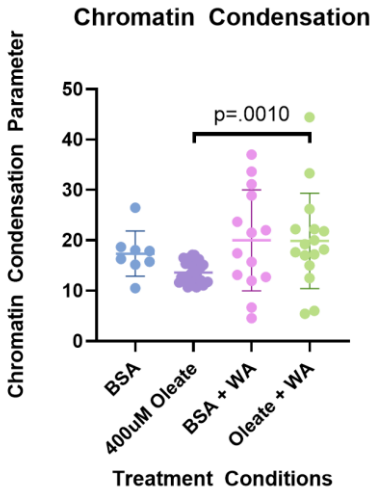
These findings support a structural role for IFs in buffering mechanical loads in steatotic hepatocytes and suggest their involvement in maintaining nuclear integrity during intracellular lipid accumulation.











A. MTT Analysis of Withaferin on regur and lipid loaded cells revealed no change in cell viability. **B.** Withaferin treatment on non-LD cells showed a significant increase in junction number compared to LD cells. **C.** Only withaferin treatment on non-LD cells significantly affected fiber #. **D.** Withaferin treatment on LD cells significantly affected fiber width. **E.** Chromatin condensation increased an equal amount in both non-LD and LD cells when treated with withaferin, significantly more than LD untreated cells.

1. Rivera W. How many people have liver disease? [Internet]. American Liver Foundation. 2023. Available from: https://liverfoundation.org/about-your-liver/facts-about-liver-disease/how-many-people-have-liver-disease/
2. Website N. Non-alcoholic fatty liver disease (NAFLD) [Internet]. nhs.uk. 2025. Available from: https://www.nhs.uk/conditions/non-alcoholic-fatty-liver-disease/
3. Loneker AE, Alisafaei F, Kant A, Li D, Janmey PA, Shenoy VB, et al. Lipid droplets are intracellular mechanical stressors that impair hepatocyte function. Proceedings of the National Academy of Sciences [Internet]. 2023 Apr 10;120(16). Available from: https://doi.org/10.1073/pnas.2216811120
4. Lityagina O, Dobreva G. The LINC between mechanical forces and chromatin. Frontiers in Physiology [Internet]. 2021 Aug 2;12. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8365421/
5. Strnad P, Stumptner C, Zatloukal K, Denk H. Intermediate filament cytoskeleton of the liver in health and disease. Histochemistry and Cell Biology [Internet]. 2008 Apr 28;129(6). Available from: https://doi.org/10.1007/s00418-008-0431-x
6. Ndiaye AB, Koenderink GH, Shemesh M. Intermediate filaments in cellular mechanoresponsiveness: mediating cytoskeletal crosstalk from membrane to nucleus and back. Frontiers in Cell and Developmental Biology [Internet]. 2022 Apr 11;10. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9035595/