**Christopher Infranco**

**Honors Thesis Proposal:**

# Effects of Vitamin D3 on Ferroptotic Cell Death in *C. elegans*

## INTRODUCTION:

Ferroptosis is a form of regulated cell death previously demonstrated through research on *C. elegans*, a species of nematode worm (Perez et al., 2020), as well as human cancer cells (Dixon, 2012). A proposed mechanism for ferroptosis outlines that cell death is induced by oxidation of polyunsaturated fatty acids (PUFAs) within a cell and subsequent membrane disruption (Mortensen, 2023). Research conducted by Dr. Jennifer Watts at Washington State University has demonstrated that treating C. *elegans* with the 20-carbon fatty acid di-homo-gamma-linolenic acid (DGLA) can induce germ cell death within the worms. It is thought that such germ cell death is due to the induction of ferroptosis of the germ cells based on subsequent studies showing reduction in cell death after treatment with ferroptosis inhibitors (Perez et al., 2020). Separate research at the University of Central Arkansas found that Vitamin D3 can promote longevity in *C. elegans* and potentially improve their ability to withstand oxidative stresses (Huggins, 2022). Given the basis of oxidative stress as a means of cell death in ferroptosis, it is natural to wonder what role Vitamin D3 may have in potentially conferring resistance to ferroptosis in *C. elegans* specimens having been treated with exogenous DGLA. My preliminary data in the Watts lab suggests that co-treating *C. elegans* with both DGLA and Vitamin D3 can reduce sterility levels within germ cells compared to treatment with DGLA

alone, further substantiating the hypothesis that Vitamin D3 can confer resistance to ferroptosis. This study seeks to examine how further how Vitamin D3 can impact the sensitivity of *C. elegans* to germ cell death by DGLA-induced ferroptosis. Given that ferroptosis has been demonstrated to occur in human cancer cells (Perez, 2020), further knowledge of this fascinating process and its nuances can offer insights into the treatment of human cancers in the future.

**QUESTION (PROPOSED ACTIVITY):**

This project seeks to discover what role Vitamin D3 has in increasing or decreasing the sensitivity to DGLA-induced ferroptosis in *C. elegans* mutants already genetically disposed to a sensitivity or resistance to ferroptosis.

**METHODOLOGY:**

In this study, we will examine the role of Vitamin D3 on impacting sensitivity to DGLA-induced ferroptosis by co-treating both ferroptosis-sensitive and ferroptosis-resistant mutant strains of *C. elegans* with both Vitamin D3 and DGLA. For each mutant, and for wild-type worms, we will prepare 20 plates (populations), with 4 plates for each treatment of 0 DGLA and 0 Vitamin D3 (intended to be a negative control), 0.125 mM DGLA and no D3 (intended to be a positive control), 50 μM of Vitamin D3 and no DGLA, and 0.125 mM DGLA and 50 μM of Vitamin D3. The use of a 50 μM concentration of Vitamin D3 in our trials is motivated by a similar concentration utilized in work on Vitamin D3 and *C. elegans* by Huggins (2012). The doses not treated with Vitamin D3 will instead be given around 10 mL of ethanol per 5 plates poured, which will serve as a vehicle in accounting for confounding effects potentially introduced from the process of adding the ethanol-dissolved Vitamin D3.After allowing time for

growth and development of the worms, we will visually score 50 worms on each plate for fertility and sterility and use a DAPI staining protocol to analyze the individual gonads of a random subset of specimens from each plate. We will also run samples from each treatment through a gas chromatography analysis to identify the actual fatty acid uptake of the worms from the various treatment groups and validate successful uptake of the treated fatty acids. We will compare the levels of sterility and/or fertility in the worms treated with DGLA and Vitamin D3 with those treated only with DGLA to identify any potential protective effect of Vitamin D3. We will use a paired, two-tailed t-test, with a significance threshold of $p < 0.05$ to compare the significance of our results between our various treatment groups. The experiment conducted will be replicated three times.

**EXPECTED RESULTS AND CONCLUSIONS:**

From this study, we expect to see a decrease in relative germ cell death between *C. elegans* populations treated with both DGLA and Vitamin D3 compared to those only treated with DGLA. Preliminary trials have shown no impact of Vitamin D3 alone on sterility, so I am predicting no influence of only Vitamin D3 on sterility levels in the nematodes in this experiment. If we find a difference in sterility in worms treated with only DGLA compared to those treated with both DGLA and Vitamin D3, more research will be conducted into a potential biochemical mechanism behind this action. Preliminary data has suggested a protective effect of Vitamin D3 on sterility in *C. elegans,* so a consistent result with the preliminary trials is to be expected from this study. Given that Dixon (2012) and Perez et al. (2020) have shown the relevance of ferroptosis to human cancers, hopefully continued study into the nuance of this

fascinating process can offer insight into treatment of this and other human diseases in the future.

**ANNOTATED BIBLIOGRAPHY**


Dixon, Scott J., et al. "Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death." *Cell*, vol. 149, no. 5, May 2012, pp. 1060–72. *PubMed*, https://doi.org/10.1016/j.cell.2012.03.042.

This paper outlines the basis of ferroptosis as a form of cell death distinct from more familiar processes such as apoptosis, and reveals the possibility for ferroptosis in human cancer cells.

Gao, M., Yi, J., Zhu, J., Minikes, A. M., Monian, P., Thompson, C. B., & Jiang, X. (2019). Role of mitochondria in ferroptosis. *Molecular Cell*, *73*(2), 354-363.e3. https://doi.org/10.1016/j.molcel.2018.10.042

Study demonstrating the role of mitochondria in promoting cysteine-deprivation ferroptosis in *c. elegans,* therefore highlighting some of the nuances of the process that could impact this project.

Huggins, B., & Farris, M. (2022). Vitamin D3 promotes longevity in Caenorhabditis elegans. *GeroScience*, *45*(1), 345–358. https://doi.org/10.1007/s11357-022-00637-w

Research in this source demonstrates the effect of Vitamin D on promoting longevity in *c. elegans,* thus motivating further research into Vitamin D and this nematode.

Mark, K. A., Dumas, K. J., Bhaumik, D., Schilling, B., Davis, S., Oron, T. R., Sorensen, D. J., Lucanic, M., Brem, R. B., Melov, S., Ramanathan, A., Gibson, B. W., & Lithgow, G. J. (2016). Vitamin d promotes protein homeostasis and longevity via the stress response pathway genes skn-1, ire-1, and xbp-1. *Cell Reports*, *17*(5), 1227–1237. https://doi.org/10.1016/j.celrep.2016.09.086

This study also implicates the role of Vitamin D in extending the lifetimes of *c. elegans,* giving more credence to the previously listed source's findings.

Mortensen, M. S., Ruiz, J., & Watts, J. L. (2023). Polyunsaturated fatty acids drive lipid peroxidation during ferroptosis. *Cells*, *12*(5), 804. https://doi.org/10.3390/cells12050804

Review article showing that dietary fatty acids can induce ferroptosis in *c. elegans.* Such a finding is the basis for the use of DGLA to stimulate ferroptosis in this study, and to then investigate whether Vitamin D3 can impact the results.

Perez, M. A., Clostio, A. J., Houston, I. R., Ruiz, J., Magtanong, L., Dixon, S. J., & Watts, J. L. (2022). Ether lipid deficiency disrupts lipid homeostasis leading to ferroptosis sensitivity. *PLOS Genetics*, *18*(9), e1010436. https://doi.org/10.1371/journal.pgen.1010436

This study demonstrates that ether lipids have a role in protecting against ferroptosis, showcasing that certain chemical species can be protective against this process and spurring interest into the discovery of more protective agents, such as potentially Vitamin D3.

Perez, Marcos A., et al. "Dietary Lipids Induce Ferroptosis in Caenorhabditiselegans and Human Cancer Cells." *Developmental Cell*, vol. 54, no. 4, Aug. 2020, pp. 447-454.e4. *DOI.org (Crossref)*, https://doi.org/10.1016/j.devcel.2020.06.019.

Paper suggesting that ferroptosis in *c. elegans* germ cells is responsible for sterility in response to treatment with DGLA. The paper's findings with regards to DGLA form the basis of the use of this compound as a sterility-inducing agent in the proposed study to be conducted. The paper's finding also imply that ferroptosis can be induced in cancer cells.