Chemotaxis Behavior Reinforces KLF Mutation Characteristics in *C. elegans*

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ABSTRACT: Diabetes is a common and life-threatening illness that is pervasive in our society today. The onset of diabetes and obesity has been associated with mutations in the transcription factor protein family of KLF, which has been identified in metabolic processes in both humans and the model organism *C. elegans.* Research suggests that in *C. elegans*, obesity due to KLF mutations is the result of the inability of the organism to metabolize intestinal adipose, which instead accumulates in the intestine. Until now there has been no conclusive evidence verifying that the intestinal fat is not completely utilized, rather this was deductively speculated. In this experiment, it is demonstrated through the vehicle of chemotaxis behavior, that *C. elegans* hosting KLF mutations do not metabolize intestinal fat. This experiment examines food-seeking behavior in *C. elegans* KLF mutants when compared to wild-type (N2) organisms after each groups had been subjected to both normal conditions (well-fed) and stress conditions (starved). We also examined the strain AG50 which causes a physiological mutation that makes it 'obese' and hinders its movement. Upon comparison, it is expected that the KLF mutants that are well-fed will display the same heightened food-seeking behavior as wild-type organisms that are starved. This behavior is due to the incapability of the mutants to effectively utilize their intestinal fat, prompting the organism to continue to seek out food aggressively despite being in a satiated state.

Background

- The *C. elegans* nematode worm is a model organism that is ideal for study as it reproduces rapidly and shares much homology with humans. This homology makes *C. elegans* a fantastic tool for studying the effects of novel drugs as well as gene mutations **(Figure 1).**
- The vehicle of chemotaxis provides a simple means of determining the various behavioral effects genetic mutations may have on the nematode worms by allowing for observation of their food-seeking patterns.
- Krüppel-like transcription factors (KLF) genes are a set of zinc-finger DNA binding proteins that regulate gene expression. Worms with mutations in KLF genes have been shown to be unable to properly utilize their body fat (Zhang et al., 2013) **(Figure 1).**
- **We hypothesize that satiated *C. elegans klf*-3(ok1975) will continue to seek out food as aggressively as their starved wildtype (N2) counterparts.**

Figure 1: (A) The model organism *C. elegans.* **(B)** zinc finger KLF protein representation

GOAL

To determine if *C. elegans klf*-3(ok1975) can metabolize intestinal fat using chemotaxis behavioral assays.

Materials & Methods

***C. elegans* Chemotaxis Assay Procedure:**

- Worms are housed on NGM plates at 20°C
- Worms are "egg synced". This is done by lysing the worms in order to release their eggs and clean the stock.
- Worms to be used for starvation are starved for 24 hours prior to assay.
- Assay plates are sectioned into four quadrants with 1µL 1M sodium azide placed at each test and control site to act as a paralytic agent (**Figure 2)**.
- 1µL 10% Diacetyl in ethanol is placed on each test site as an odorant to mimic a food source. 1µL 95% ethanol is placed on each control site as a non-attractant control.
- 150-250 Worms are placed at the center (origin) of each of the corresponding plates (starved or satiated). Once done, the worms are placed at 20°C for one hour before being placed in the refrigerator to halt any further movement.
- Worms are then counted to determine the numbers on each test, control, and origin site.
- Chemotaxis Index is determined using the equation:

$$\frac{(\text{total \# at test} - \text{total \# at control})}{(\text{total worms-total \# at origin})}$$

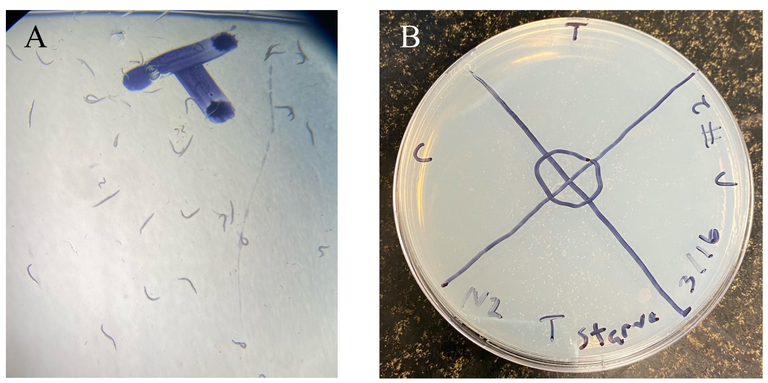

Figure 2: (A) shows the test site of the results of a trial. This site contains the pseudo-food source, diacetyl. **(B)** Chemotaxis assay plate. Quadrants are labeled as either T (test) or C (control)

Results

- Starved N2 worms sought out food more than their satiated counterparts.
- Starved N2 worms have a higher chemotaxis index (generally between 0.8 & 1)
- Satiated N2 worms have a significantly lower chemotaxis index (generally between 0.4 & 0.7)
- The KLF-2 & KLF-3 mutations in starved worms did not have a significant effect on chemotaxis rates towards a food source
- The starved AG50 had significantly impaired rates of chemotaxis towards a food source **(Figure 3)**

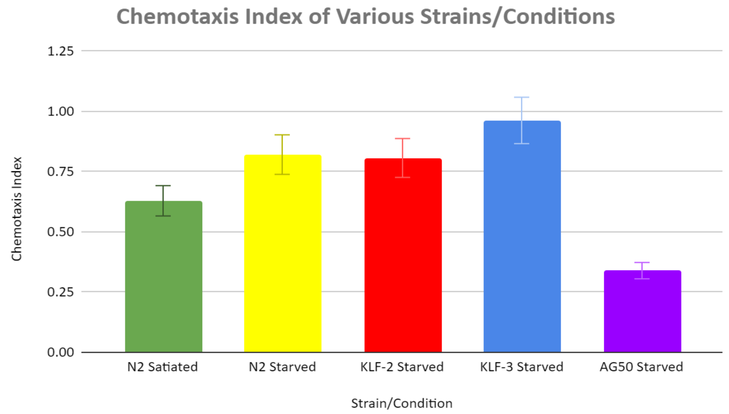

Figure 3: Chemotaxis indexes for Wildtype (N2) as well as various other mutations in differing conditions.

Conclusion

- This preliminary data supports the claim that *C. elegans klf*-3(ok1975) will seek out food despite being in a satiated state.
- Due to many of the restrictions surrounding COVID-19, much of our research was significantly slowed or brought to a halt altogether.

Future Direction

- Perform chemotaxis assays using worms with mutated *klf* genes.
- Perform generational chemotaxis assays using the progeny of previously assayed worms.
- Perform chemotaxis assays to determine the food seeking behavior of satiated *klf* mutants.

ACKOWLEDGEMENTS

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Reference

Zhang, J., Hashmi, S., Cheema, F., Al-Nasser, N., Bakheet, R., Parhar, R. S., . . . Hashmi, S. (2013). Regulation of Lipoprotein Assembly, secretion and fatty ACID B-OXIDATION BY Krüppel-Like Transcription Factor, klf-3. *Journal of Molecular Biology, 425*(15), 2641-2655. doi:10.1016/j.jmb.2013.04.020