C. Elegans as a Model Organism for Investigating the Relationship Between Uric Acid and Lipid Metabolism

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Introduction | Methods | Results | Discussion

- Obesity and metabolic syndrome are associated with elevated levels of uric acid (UA) and several studies have reported a correlation between UA levels and cardiometabolic risk factors. A causative role for UA in the development of obesity and insulin resistance has been proposed. This line of research has been impeded by the fact that, unlike commonly used model systems, humans lack uricase which catabolizes UA.
- *C. elegans* also lacks uricase but it does contain the gene for the UA-producing enzyme xanthine dehydrogenase/xanthine oxidase (XDH/XO) and has been shown to excrete UA. We have previously confirmed that a XDH/XO knockout mutant has undetectable XDH/XO activity and does not produce UA.
- The specific aim of this work was to determine if the effects of allopurinol could be seen with *in vivo* treatment and optimizing methods for assessing lipid levels in nematodes.
- We hypothesized that introduction of allopurinol to *C. elegans* would decrease UA accumulation. We also hypothesized that lipids could be visualized in the wild type and mutant strains along with triglycerides present at high enough levels to be reproducibly detected in both strains.

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- Wild type and XDH/XO knockout worms were grown at 25°C under standard conditions and fed with *Escherichia coli* strain Na22 in the presence or absence of allopurinol.
- Young adult worms were collected by centrifuged and lysed into pellets to be utilized for the assays and imaging.
- Protein concentration of the extracts was determined using the ThermoScientific Coomassie Plus Protein Assay.*
- UA concentration was quantified using the Stanbio LiquiColor assay reagent.*
- Triglyceride concentration was determined using the triglyceride colorimetric assay kit from Cayman Chemical.*
- All assays were done at least three times with each data point done in triplicate within experiments.
- Nile red staining was performed using the Abcam Nile Red Staining Kit (cat# ab228553). Imaging was performed using the Leica Aperio Versa 8 Scanning System at 20x magnification.

*Data Analysis: For all assays, standard curves were generated and used to calculate unknown concentrations. UA and triglyceride levels were corrected for protein concentration. Intra-assay variability (%CV) was calculated for each sample using the triplicate data points within each assay. Inter-assay %CV was calculated using the values from separate experiments.

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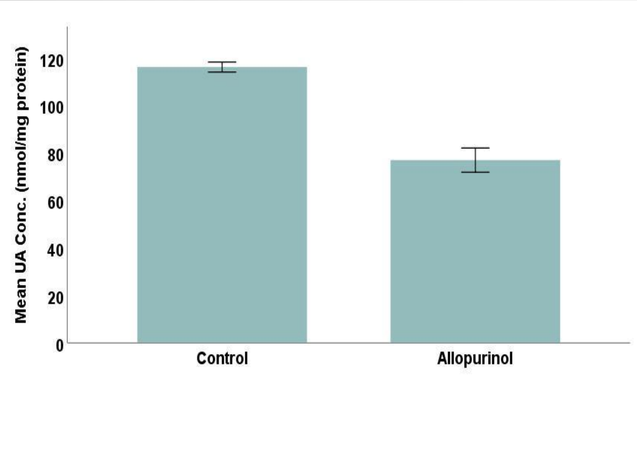

Figure 1. Representative allopurinol *in vivo* efficacy results. Values represent the mean ± SD of three experiments with each data point done in triplicate within experiments. Intra and inter-assay %CV for UA determination were less than 5% and less than 10% respectively. As shown previously, UA could not be detected in extracts of the XDH/XO knockout strain.

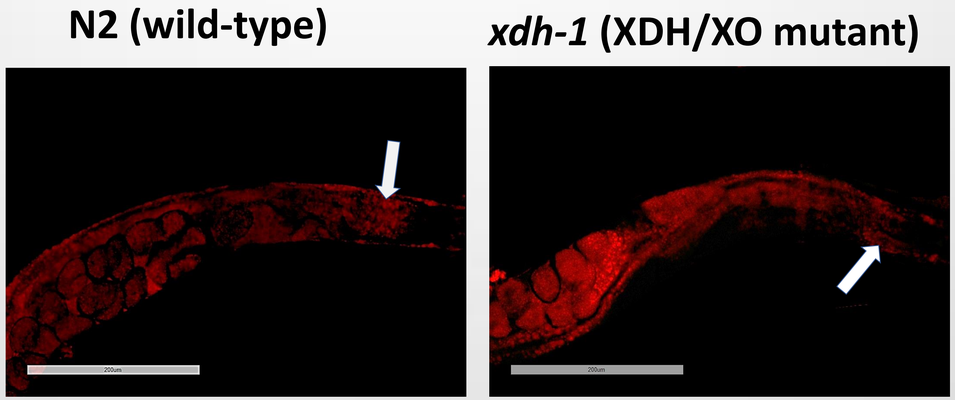

Figure 2. Nile red staining of wild-type and mutant strains. Lipid droplets aggregates designated by arrows.

Figure 3. Crawling *C. elegans*
Courtesy of Bob Goldstein lab

Strain	Triglyceride Conc. (μg TG/mg protein)
Wild Type (N2)	89.3 ± 8.8
XDH/XO mutant (*xdh-1*)	76.4 ± 3.6

Table 1. Triglyceride concentration in C. elegans extracts. Results represent the mean + SD of three experiments. Intra and inter-assay %CV for triglyceride determination were both less than 10%.

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These results demonstrate that *C. elegans* is responsive to *in vivo* administration of allopurinol. In addition, triglycerides can be reproducibly measured and visualized in both wild-type and XDH/XO knockout strains. While additional studies are needed, these are important steps toward validating the use of *C. elegans* as a model organism for investigating the relationship between hyperuricemia and lipid metabolism and storage.

References

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