foxo gene was upregulated by 1.234-fold in female flies compared to males

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Summary

- Study done on gene expression in female *Drosophila melanogaster* (fruit flies) when compared to males.
- Flies were fed food + acetone.
- *foxo* gene is upregulated by 1.234-fold in females compared to male fruit flies.

Introduction

D. melanogaster

- Established model organism.
- Shares 60% of human genes.[8]
- Exhibits sexual dimorphism.[1]
- Easily replenishable, low cost, short lifespan.[3]

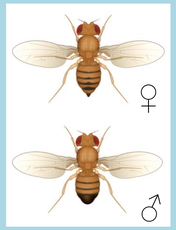

Figure 1: Female and male fruit flies.

foxo

- FOXO proteins are a subgroup of the Forkhead family of transcription factors.[3]
- Regulate insulin signaling pathway and growth.[3]

Fly food

- Flies exposed to acetone in food to later be used as vehicle control in future B[a]P experiments.

B[a]P

- Polycyclic aromatic hydrocarbon (PAHs) found in tobacco smoke, coal tar, grilled foods.[11]
- Important to study its toxicity and effects on organisms.[11]

Males vs Female fruit flies

- Different genetic makeup.[7]
- React differently to environmental stressors.[7]
- Gene expression is different.[7]

Gene Expression

- Allows us to find out how different environmental stressors affect organisms.[9]

Hypothesis

- The *foxo* gene will be downregulated by 2-fold in females compared to males.

Methodology

Fly Keeping
Breeding flies were housed in stable environment and grown on negative control food (cornmeal/ agar-based food).

F0 Generation
10 males & 10 females per vial from the breeding population made up the F0 generation & were fed vehicle control food (food + acetone) for 7 days.

Sample Collection
After 7 days, F0 fruit fly tissue samples (3 replicates of 20 heads/sex) were collected and frozen so RNA did not degrade.

RNA Extraction
Head tissue was homogenized with a BeadMill using glass beads. RNA was extracted from tissue using the Qiagen RNeasy Mini Kit for male and female samples separately.

qRT-PCR
qRT-PCR was performed using iTaq™ Universal SYBR® Green One-Step Kit between sexes to find expression levels of transcribed RNA for a gene. Reverse transcription to synthesize cDNA from RNA & quantitative PCR to amplify identified segment of DNA.

Data Analysis
Data from the experiments were analyzed. For qRT-PCR we removed outliers and performed t-test. We looked at orthologs of *foxo* to predict similarities in gene expression. Analyzed protein interactions to see which proteins interacted the most with *foxo* and what it means.

Figure Generation
Figures created to represent the results from the study. Figures were created for qRT-PCR results, orthologs of *D. melanogaster* genes and STRING figure of interactions of proteins with *foxo*.

Results

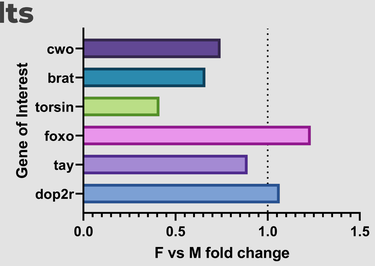

Figure 2: Female vs male qRT-PCR fold changes for *foxo* and other genes of interest. There were no statistically significant differences. Values under 1.0 indicate downregulation and values over 1.0 indicate upregulation. Average female vs male fold change for the *foxo* gene is 1.234. This figure was generated in GraphPad Prism 9.

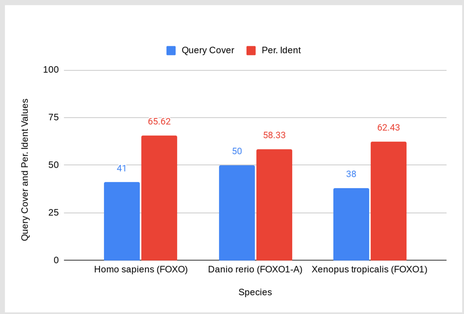

Figure 3: *foxo* gene sequence comparisons with orthologs in other species. Graph represents the query cover and percent identity of three orthologs of the *foxo* gene in different organisms. This figure was generated using Google Sheets.

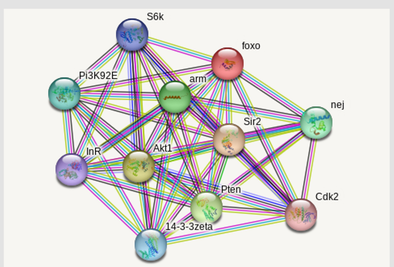

Figure 4: STRING figure of *foxo* interactions with other proteins. The nodes represent proteins. Colored nodes represent the query proteins and the first shell of interactors. The different colors of lines represent the kind of interactions that are occurring between the proteins. This figure was generated using STRING [https://string-db.org/]

Discussion & Conclusion

- Our hypothesis was incorrect. The *foxo* gene is upregulated by 1.234-fold in female compared to male fruit flies that were fed vehicle control food (+ acetone). (Figure 2)
- *foxo* is not significantly expressed more in females than male *Drosophila melanogaster* (p-value = 0.639 > 0.05). (Figure 2)
- Apart from *foxo* the only other gene that was upregulated in females compared to males was *dop2r* & the gene least expressed was *torsin.* The other genes had fold changes that ranged from 0.66-0.89. (Figure 2)

- *foxo* had orthologs in *H. sapiens* (*FOXO*), *D. rerio* (*FOXO1-A*) & *X. tropicalis* (*FOXO1*). The highest % Identity was found in *H. sapiens* (65.62%) and the highest query cover was found in *D. rerio* (50%).
- Since *foxo* is upregulated in female vs. male fruit flies & all the orthologs have high query covers and % identities, it's possible that there is similar gene expression in organisms with orthologs like humans or zebrafish. (Figure 3)

- *foxo* gene interacts with many other proteins that jointly perform its functions like insulin signaling. The protein that is predicted to interact most with *foxo* is *Sir2,* which is involved in sex determination & regulation of lifespan. (Figure 4)

Limitations/Future Directions

- The qRT-PCR data had outliers which had to be removed. To generate more accurate and consistent results the experiment needs to be repeated.
- The flies were not controlled for age. There is a chance that the varying ages of the flies might have affected the results.
- This experiment was based on flies that were fed food + acetone. This data could serve as a vehicle control for future studies about the effects of B[a]P on flies and their gene expression.

Sources