Hopscotch overexpression in virgin adult female fat body decreases survivability and alters metabolic parameters in the high fat diet obesity model of *Drosophila melanogaster*

Obesity-promoted aberrant cell signaling can lead to insulin resistance and is associated with elevated Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway activity in humans. An obesity-like state can be mimicked in *Drosophila* by providing media with high saturated fat content. In *Drosophila* hopscotch (hop) encodes a kinase similar to mammalian JAK, which acts to phosphorylate STAT92E, the sole STAT transcription factor in flies. *Drosophila* fat body tissue was specifically studied because it is analogous to mammalian liver and adipose tissue.
The present study utilizes virgin female flies were placed on a high fat diet (HFD) or a normal diet (NMD). To activate Hop-Stat92E pathway in the fat body we utilized the yolk-GAL4 driver (BDSC#58814), which induces expression in specifically adult female fat bodies and was backcrossed into the *w*[1118] (BDSC#5905) background to match that of a line carrying UAS- $Hop^{Tum\text{-}1}$. $Hop^{Tum\text{-}1}$ is a hyperactive form of hopscotch. Virgin female flies utilizing a yolk-GAL4 driver to overexpress hopscotch in their fat body show a decrease in lifespan on both HFD and NMD. Flies expressing hopscotch also show elevated glycogen levels and an increase in feeding quantity on HFD that is not seen in controls. They also express differences during oral glucose tolerance tests. Finally, while HFD increases starvation resistance relative to NMD, virgin females expressing hopscotch in the fat body show a reduction in survival during starvation relative to controls. Results from these experiments implicate the fat body as an important site of JAK/STAT pathway activity in obesity pathogenesis.

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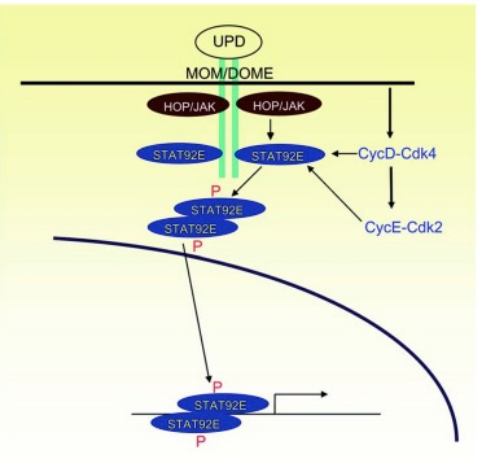
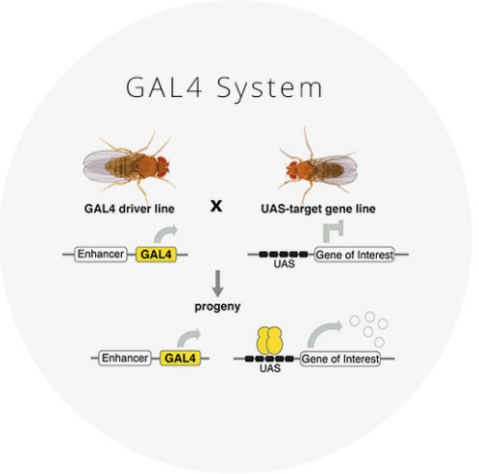
Background

- The driver line yolk-GAL4 and the UAS *Hop*$^{Tum\text{-}1}$ flies together use this system to overexpress hopscotch in adult female fat bodies
- Using this system to create an overactive Hop-Stat92E pathway allows for the study of insulin resistance which is often accompanied by type 2 diabetes which is often accompanied by obesity.



Singh, S., Chen, X., and Hou, S. JAK/STAT signaling regulates tissue outgrowth and male germline stem cell fate in *Drosophila.* 2005



Andrea H. Brand and Emma-Louise Dormand. The GAL4 system as a tool for unravelling the mysteries of the *Drosophila* nervous system. 1995

Objective

- Utilize a HFD that will lead to an obesity like state in *Drosophila.*
- Utilize GAL4-UAS expression system to drive the overexpression of $Hop^{Tum\text{-}1}$ in the female adult fat body.
- Evaluate the impact of overexpression of $Hop^{Tum\text{-}1}$ on virgin adult female; lifespan, starvation resistance, feeding quantity, glycogen content, hemolymph glucose content on an HFD and an NMD. In order to determine insulin sensitivity

Hypothesis

- Overexpression of Hop will influence insulin sensitivity and through insulin sensitivity it will also influence overall tolerance of an HFD.

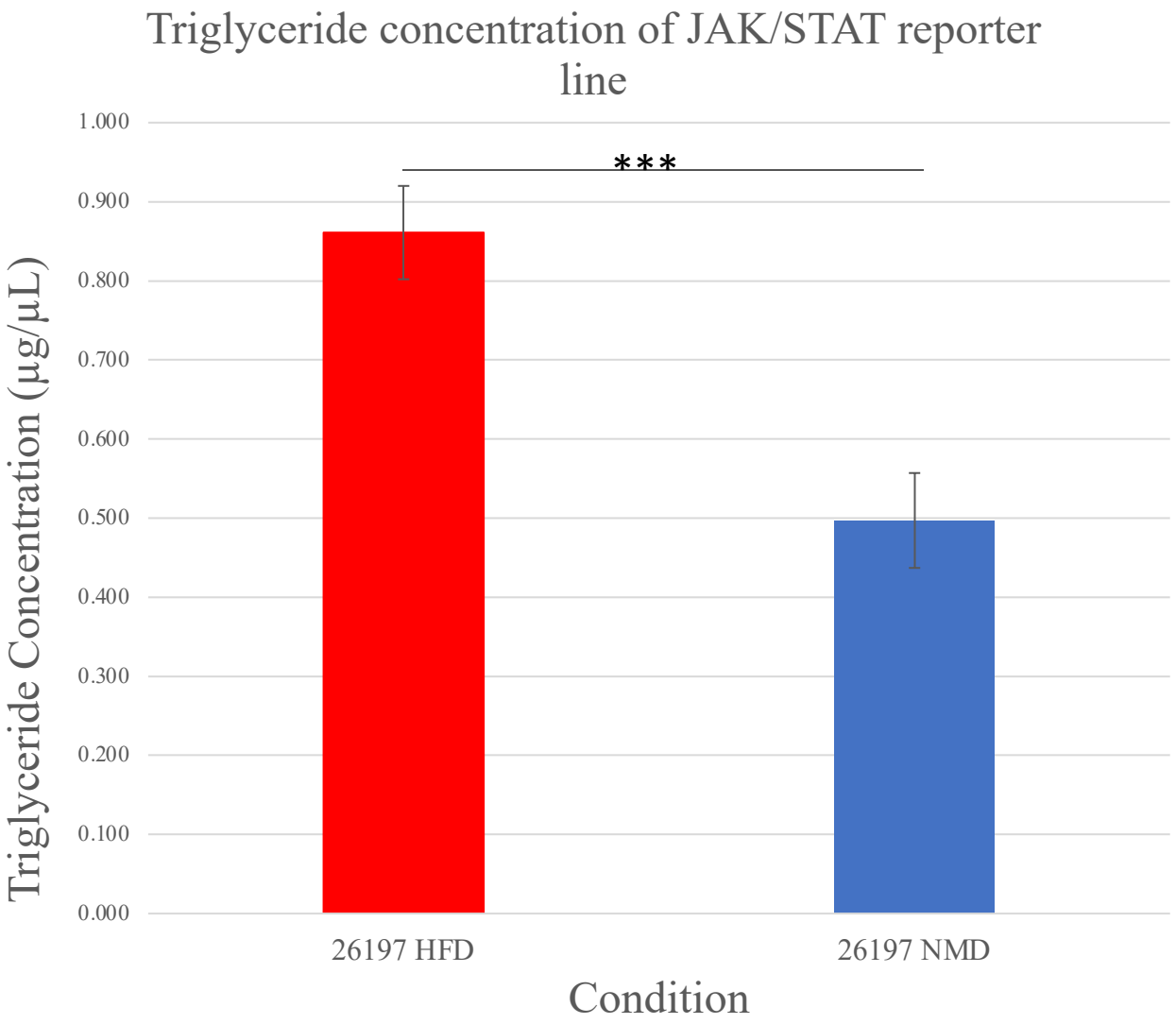


Fig 1. Exposure to HFD led to a significantly higher triglyceride concentration relative to NMD. Error bars represent standard error of mean (SEM). N=15 male flies per diet. *P<0.05, **P<0.01,***P<0.001. P-values are derived from TTEST.

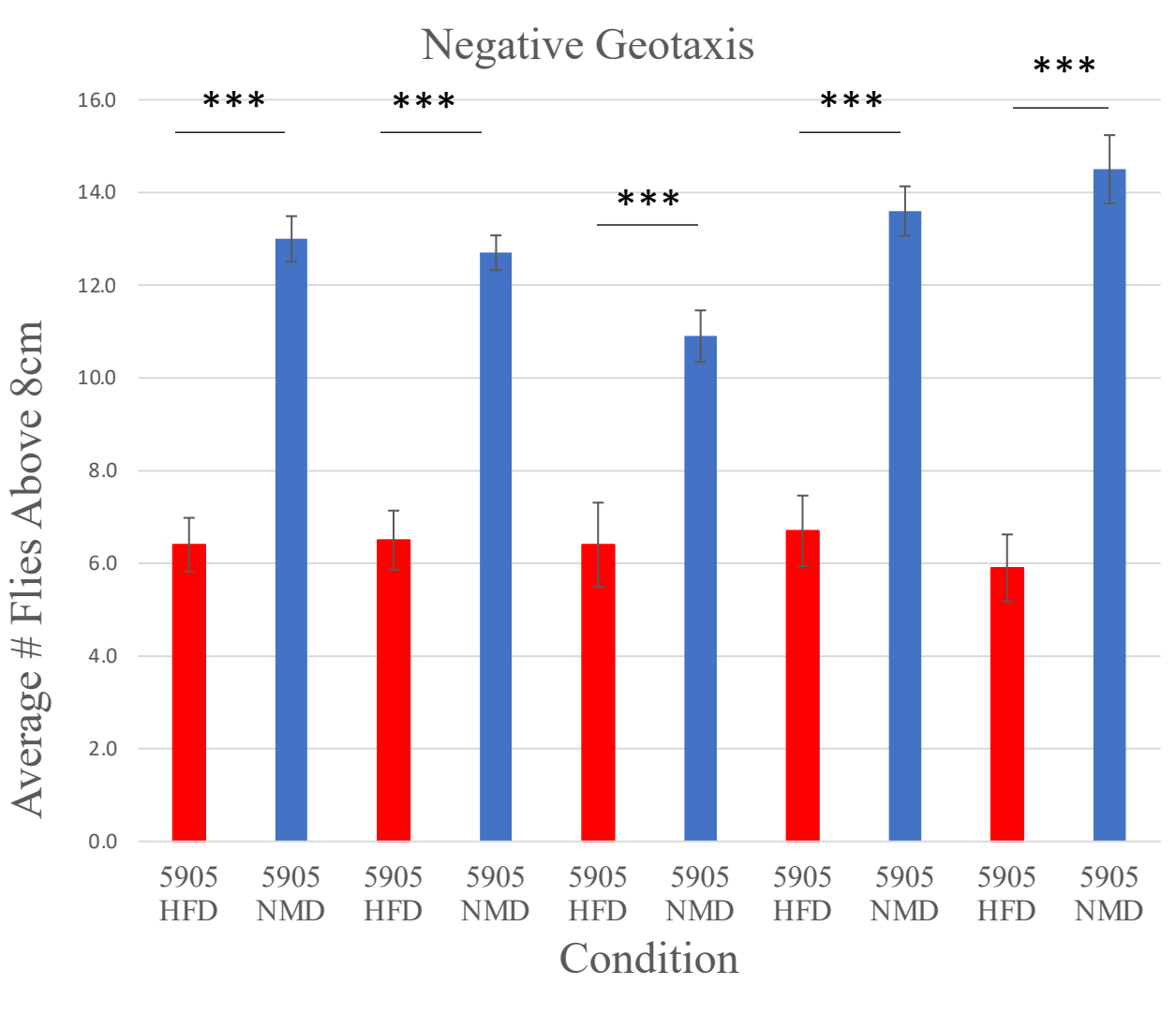


Fig 2. Exposure to HFD led to significantly decreased climbing ability in a given time compared to those exposed to NMD. Error bar represent standard error of mean (SEM). N=100 flies per condition. *P<0.05, **P<0.01,***P<0.001. P-values are derived from TTEST.

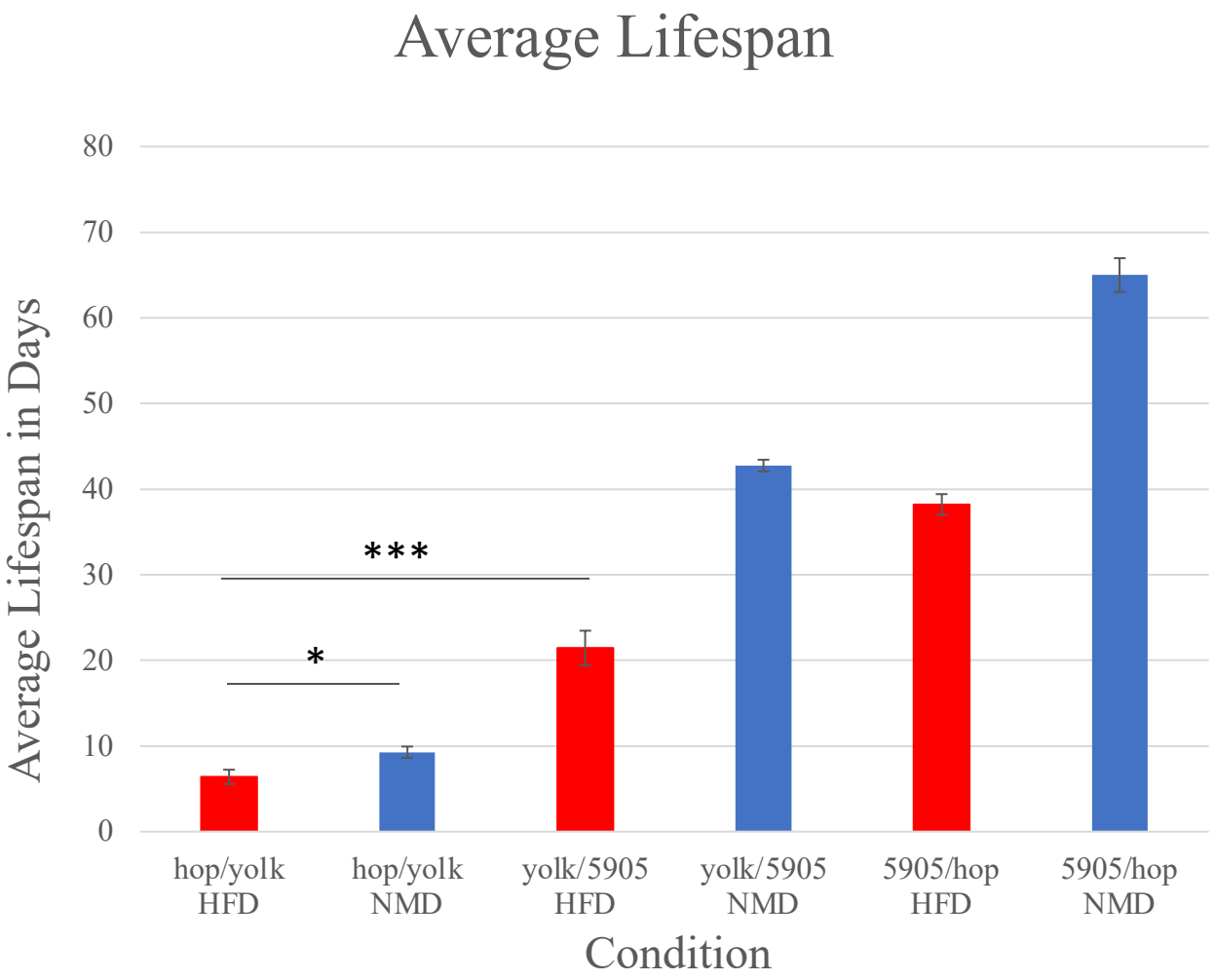


Fig 3. Exposure to HFD led to significantly shortened lifespan regardless of genotype. Overexpression of *Hop*$^{Tum\text{-}1}$ in the virgin female fat body led to significantly shortened lifespan of flies compared to both control genotypes. Error bar represent standard error of mean. N=100 flies per condition. . *P<0.05, **P<0.01,***P<0.001. P-values are derived from TTEST.

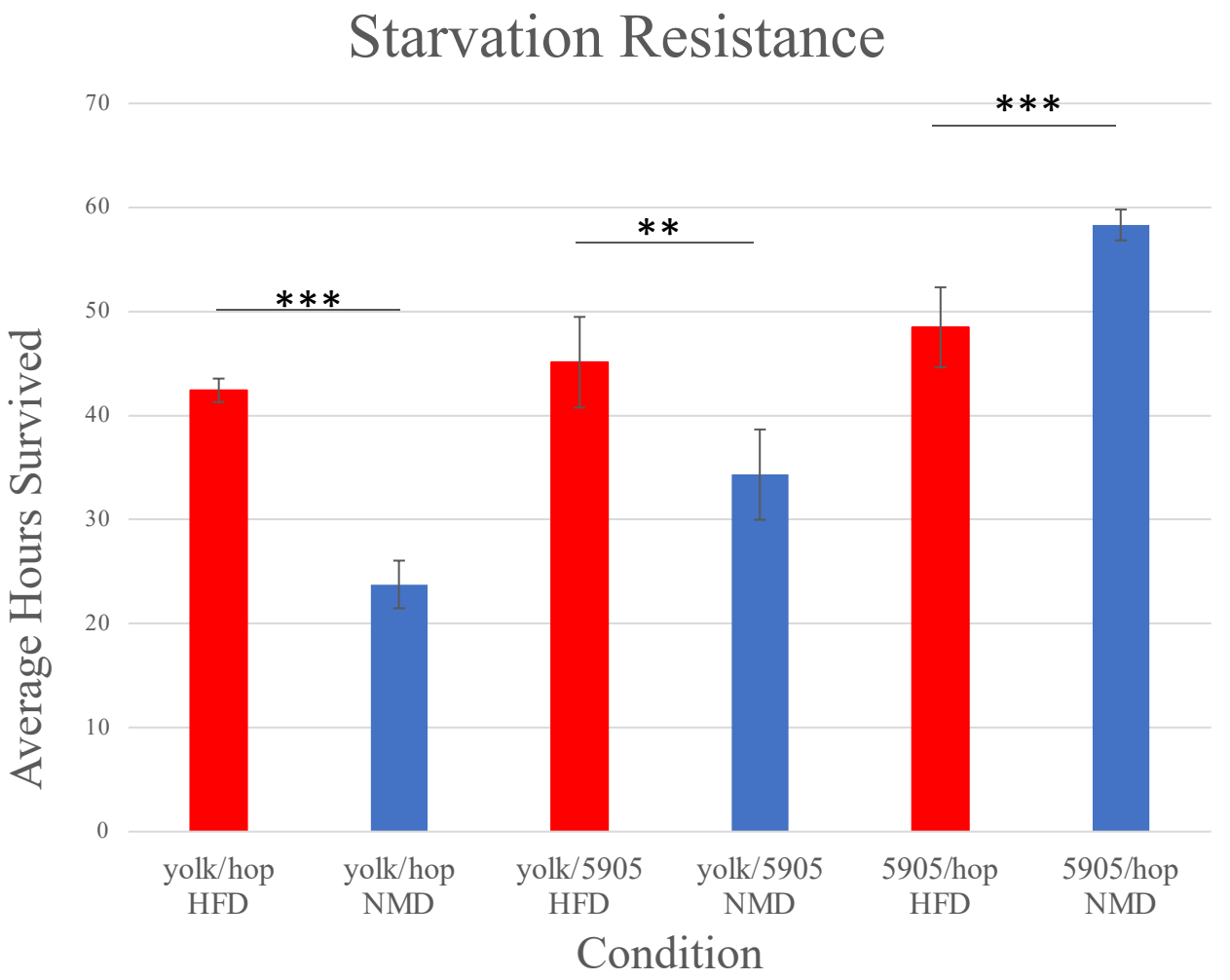


Fig 4. Exposure to HFD in flies overexpressing *Hop*$^{Tum\text{-}1}$ in virgin female fat body led to significantly more hours survived during starvation. Error bar represent standard error of mean. N=100 flies per condition. . *P<0.05, **P<0.01,***P<0.001. P-values are derived from TTEST.

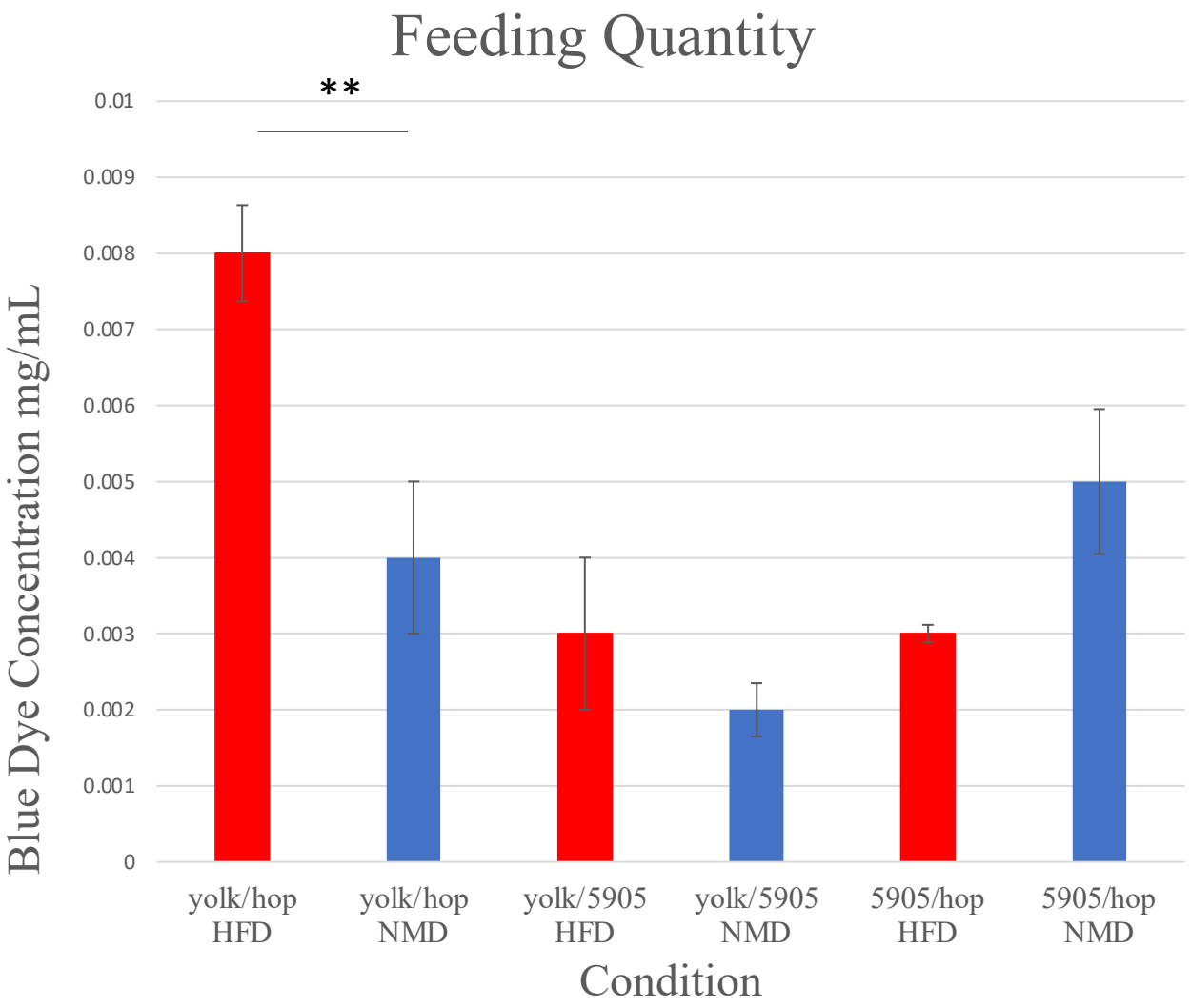


Fig 5. Exposure to HFD in flies overexpressing *Hop*$^{Tum\text{-}1}$ in virgin female fat body led to significantly increased feeding behavior**.** Error bar represent standard error of mean. N=100 flies per condition. . *P<0.05, **P<0.01,***P<0.001. P-values are derived from TTEST.

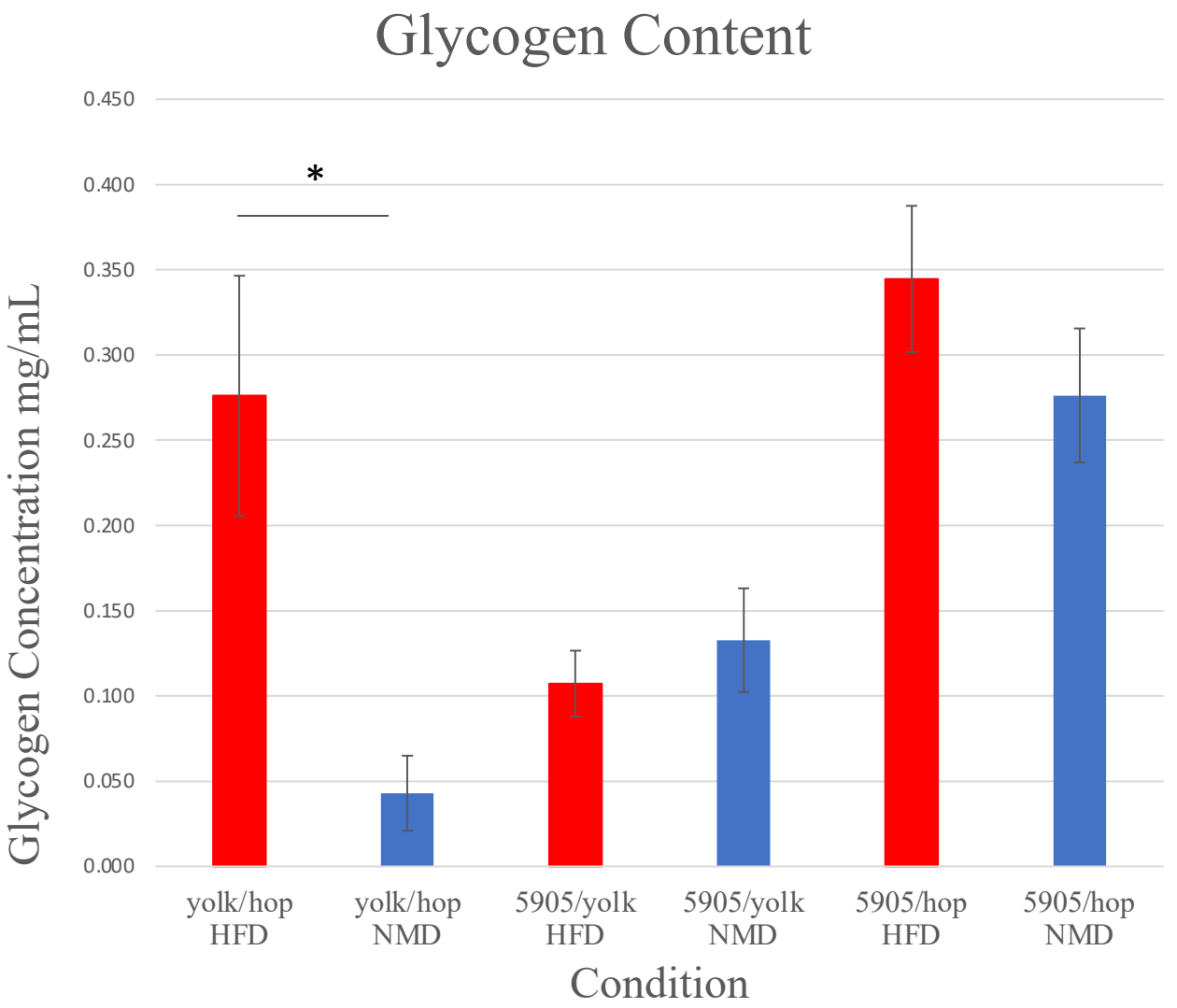


Fig 6. Exposure to HFD in flies overexpressing *Hop*$^{Tum\text{-}1}$ in virgin female fat body led to significantly increased glycogen storage. Error bar represent standard error of mean. N=25 flies per condition. . *P<0.05, **P<0.01,***P<0.001. P-values are derived from TTEST.

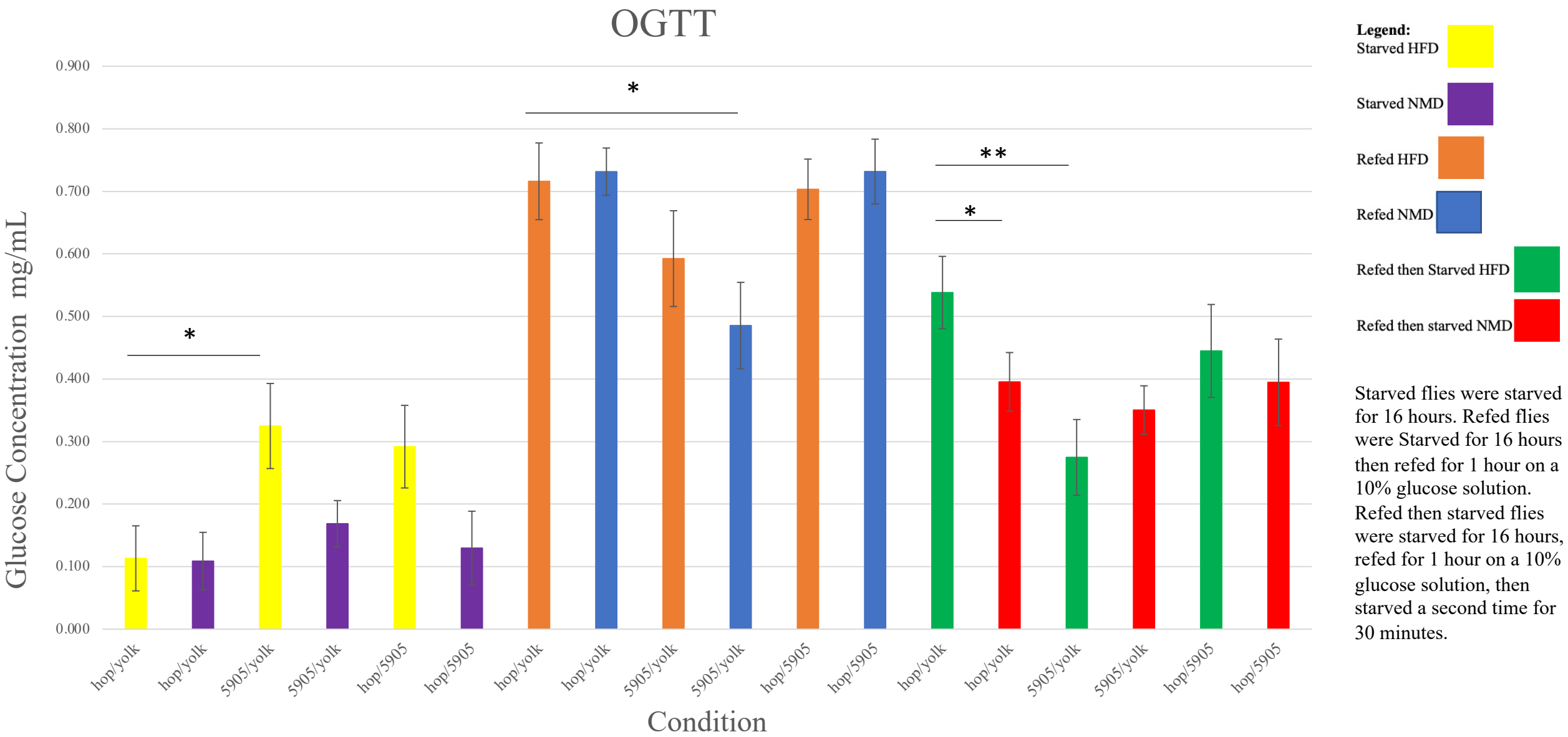


Fig 7. Between all feeding conditions (starved, refed, refed then starved) there is a significant difference ($p < .001$). Overexpression of *Hop*$^{Tum\text{-}1}$ in the experimental line in combination with a HFD led to significant differences within feeding conditions ($p < .05$ *, $p < .01$ **). Error bar represent standard error of mean. N=150 flies per condition. P-values are derived from TTEST.

Conclusions

- The overexpression of $Hop^{Tum\text{-}1}$ in the virgin female fat body is reducing lifespan and possibly insulin sensitivity, this can be seen in feeding quantity, glycogen content, and OGTT.
- OGTT shows a reduced ability to clear glucose from hemolymph after acute exposure to glucose, as well as reduced glucose in hemolymph during starvation. This is especially apparent when combined with an HFD.
- The $Hop^{Tum\text{-}1}$ construct shows some evidence of leak and/or transvection. Transvection is a process where a heterozygous genotype can lead to transcription on an allele enhancing transcription on the other allele.

Future Studies

- Through RNA-Seq identify genes associated with the alteration of JAK/STAT pathway activity specifically in response to exposure of HFD.
- Use qRT-PCR in order to monitor changes in expression of genes involved in Hop-Stat92E activity.
- Conduct Western Blots in order to identify changes in Akt phosphorylation, which would signal an increase in insulin signaling.