IN VIVO OVEREXPRESSION OF HOPSCOTCH IN THE GUT MODULATES INSULIN-LIKE PEPTIDE SIGNALING IN VIRGIN FEMALE *DROSOPHILA MELANOGASTER* UNDER VARIOUS NUTRIENT CONDITIONS

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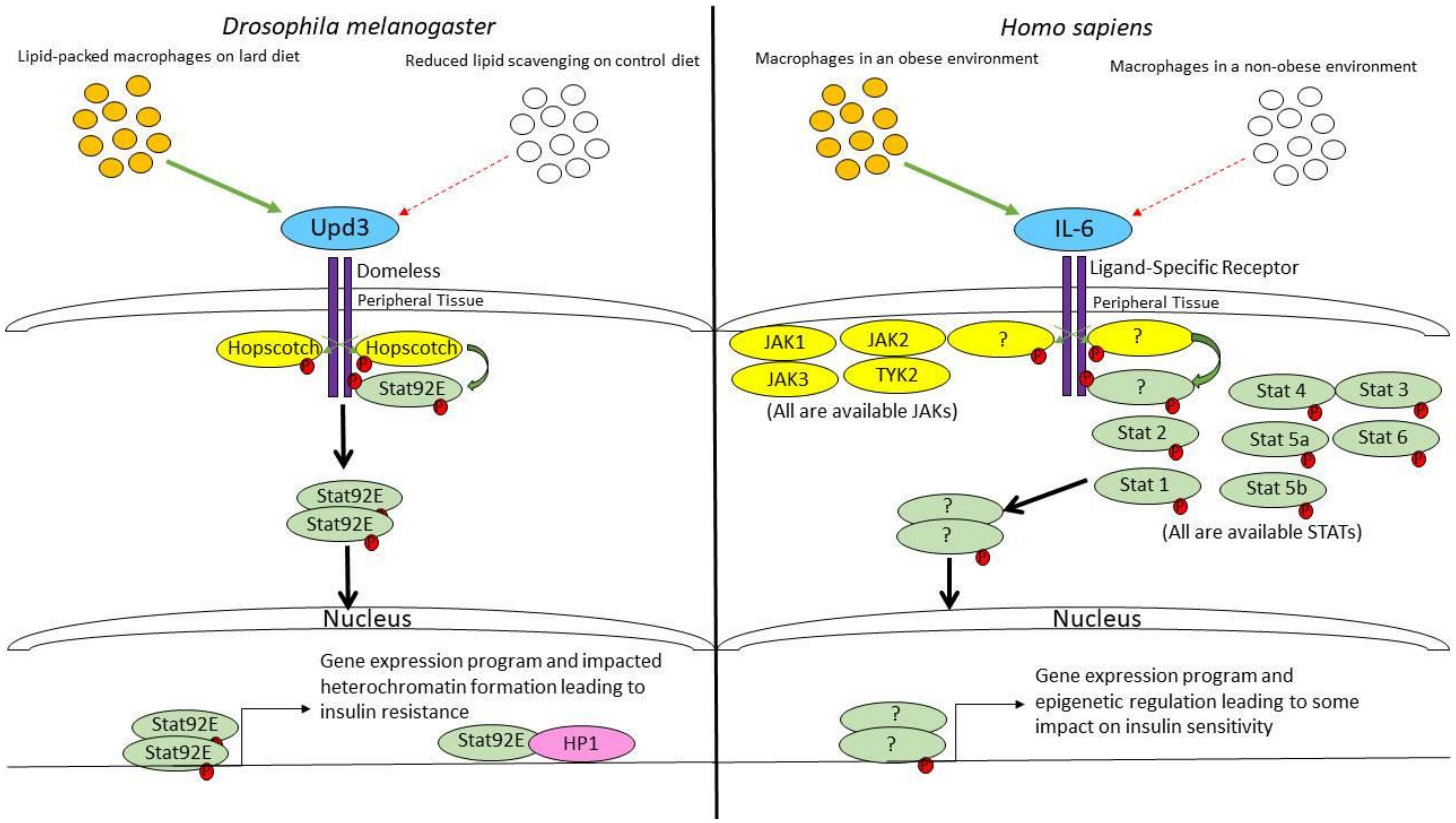
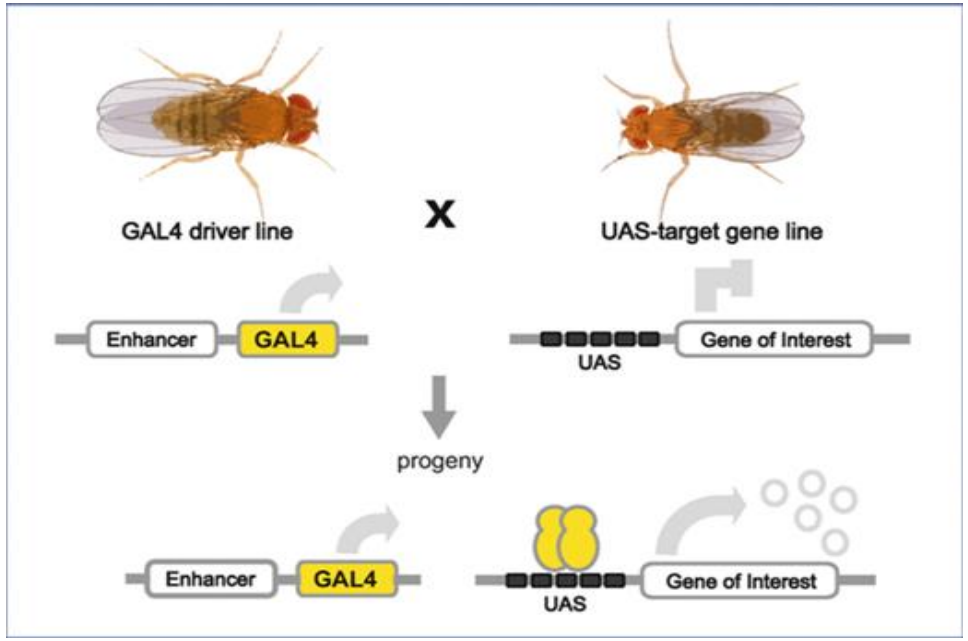
- Diet-induced obesity in *Drosophila* has been linked to decrease in lifespan, feeding behavior alteration, altered energy storage and altered expression of Dilp2, which plays a role in nutrient uptake and energy storage. Flies analogous physiological system with mammals makes them a good model for the study of the JAK/STAT pathway in obesity and insulin resistance. Obesity is linked to Type 2 diabetes via insulin resistance, which is linked to elevated JAK/STAT pathway activity in mammals. In this study, an obesity-promoting diet was utilized (high fat diet, HF; 20% w/v, saturated fat). Overexpression of $Hop^{Tum\text{-}1}$ in the gut, which is *Drosophila* JAK, was achieved by using the drm-GAL4 driver. Midgut is a major site of energy sensing in flies and is analogous to the small intestine of mammals. The flies were exposed to normal (NM) and HF for five days prior to conducting experiments. Overexpression in the midgut led to a decrease in lifespan, glycogen content, triglyceride content, reduced feeding quantity and reduced starvation resistance. Overexpression also led to reduced insulin signaling if fed NM or fed NM and starved overnight, but increased insulin signaling if previously fed a HF diet regardless. Additionally, flies were starved overnight and exposed to either 10% glucose or water to induce Dilp2 release. Here, overexpression led to increase in insulin signaling on water treatment regardless of prior diet, but decrease insulin signaling if fed glucose regardless of prior diet. However, the $Hop^{Tum\text{-}1}$ construct showed signs of leaky expression or transvection and further study is needed.

BACKGROUND



- The figure on the left represents Hop-Stat92E pathway (JAK/STAT) in *Drosophila* where a lard enriched diet induces secretion of Upd3 by the action of macrophages. Upd3 then goes on to bind to its receptor, which homodimerizes to promote trans-phosphorylation of Hopscotch, which further phosphorylates Stat92E. Stat92E translocate into the nucleus and results in expression of gene program that contributes to insulin resistance.
- The figure on the right shows the same pathway in humans. However, unlike in Drosophila, the action of microphages results in secretion of IL-6 in obese environment, which then binds to its receptor. In mammals, there are four JAK proteins and seven STATs that may homo/heterodimerize and the process is more complex.



- GAL4-UAS system is widely used *in vivo* ectopic expression. Here, a "driver construct" (specifies the tissue of interest) is crossed into the genome of a fly that consists of an "expression construct" (contains gene of interest).
- The driver construct consists of a promoter and is fused to a yeast-derived transcriptional activator protein called GAL4. The expression construct consists of an upstream activating sequence (UAS) that binds to GAL4.
- Here, the expression of UAS-Hop in gut was driven by crossing them with gut specific drm-GAL4 flies.

Objective

- Utilize GAL4-UAS binary expression system to drive the overexpression of $Hop^{Tum\text{-}1}$ in the adult mid-gut.
- Evaluate the impact of overexpression of $Hop^{Tum\text{-}1}$ on lifespan, energy homeostasis, and insulin signaling on a high fat diet (HF) and a normal diet (NM) after a variety of nutrient manipulations.

Hypothesis

- *In vivo* overexpression of $Hop^{Tum\text{-}1}$ in midgut of flies exposed to HF will influence insulin sensitivity.
- Overexpression will also modulate metabolic phenotypes and insulin signaling in a diet and nutrient specific manner.

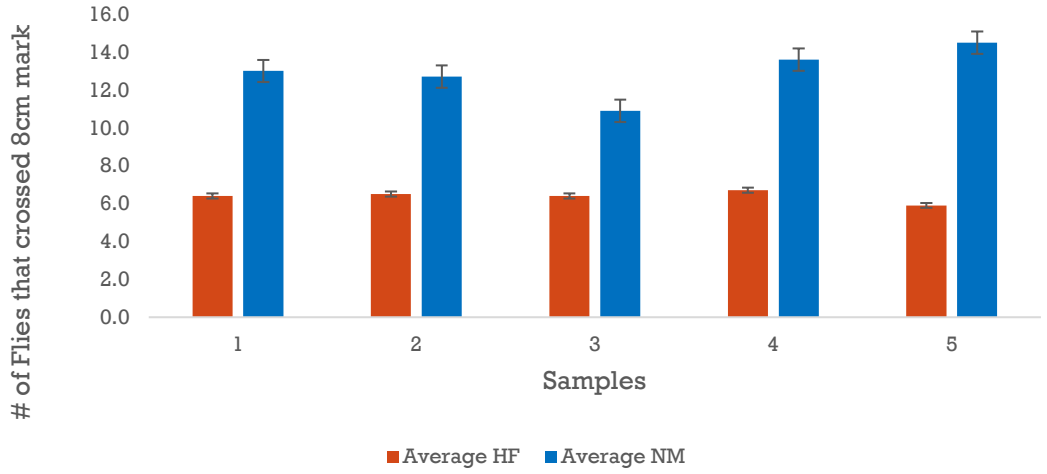


Fig 1: Effect of diet on 5905 females on their climbing ability: Flies exposed to HF showed significant decrease in ability to climb 8cm compared to those exposed to NM. Error bar represent standard error of mean. N=100 flies per condition.

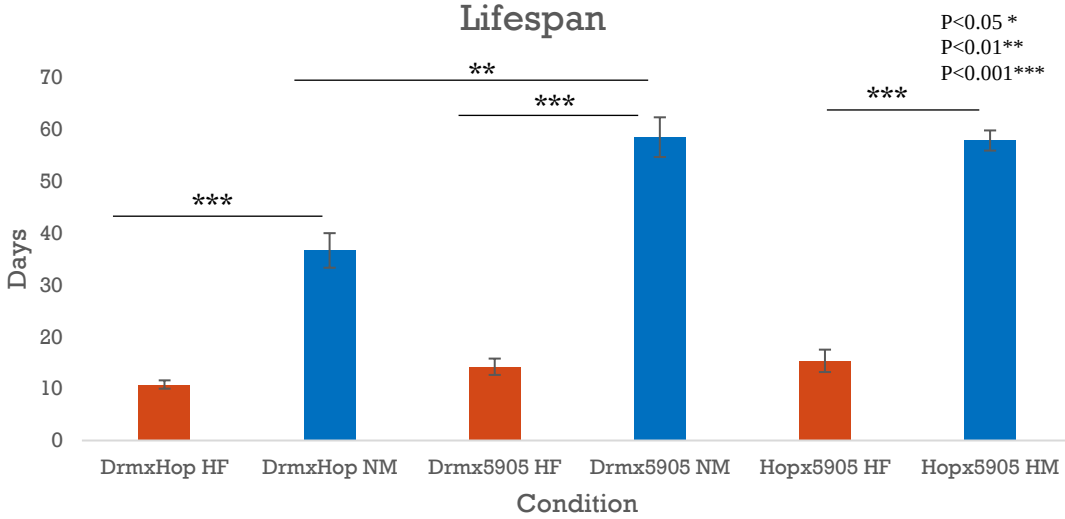


Fig 2: Effect of overexpression of *Hop^{Tum-1}* in the midgut on lifespan: Flies exposed to HD led to significantly shortened lifespan compared to those exposed to NM regardless of the genotype. Overexpression in the gut led to shortened lifespan of flies compared to both control genotypes. Genotype*Diet P=0.0009, Diet P<0.001 and Genotype P<0.0001. Error bar represent standard error of mean. N=100 flies per condition. P-values are derived from ANOVA analysis (Student T)

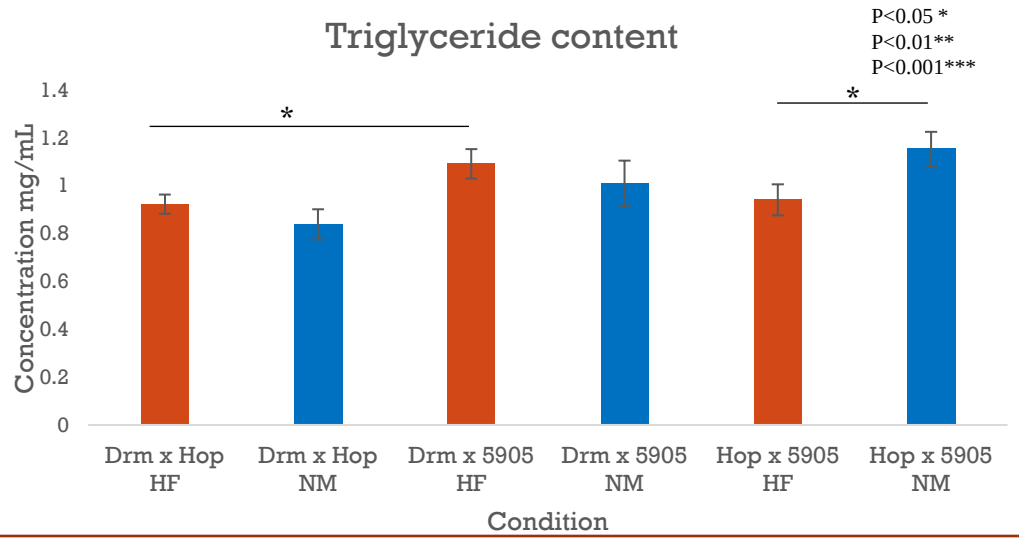


Fig 3: Effect of overexpression of *Hop^{Tum-1}* in the midgut on triglyceride content: Overexpression led to decrease in stored triglyceride content relative to the control flies. Genotype*Diet P=0.0222, Diet P=0.7882 and Genotype P=0.0493. Error bar represent standard error of mean. N= 13 flies per condition. P-values are derived from ANOVA analysis. (Student T)

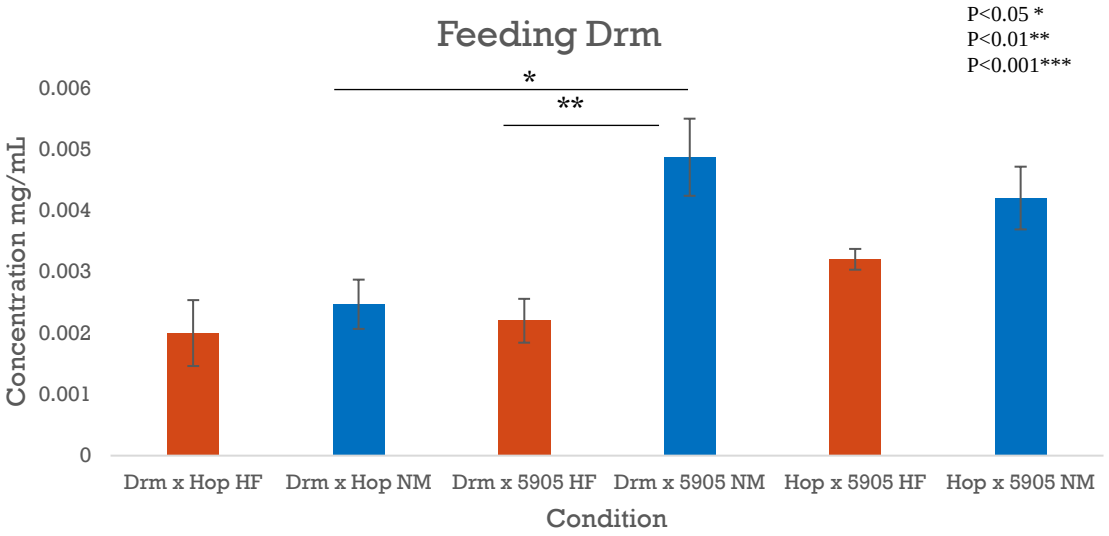


Fig.4 : Effect of overexpression of *Hop^{Tum-1}* in the midgut on feeding behavior: Flies overexpressing *Hop^{Tum-1}* in the midgut display reduction in feeding quantity compared to both controls on NM. There is generally a significant reduction in feeding quantity on HF relative to NM. This is particularly true for the controls, but not experimental flies. Genotype*Diet P=0.0625, Diet P=0.0012 and Genotype P=0.0072. Error bar represent standard error of mean. N=25 flies per condition. P-values are derived from ANOVA analysis. (Tukey)

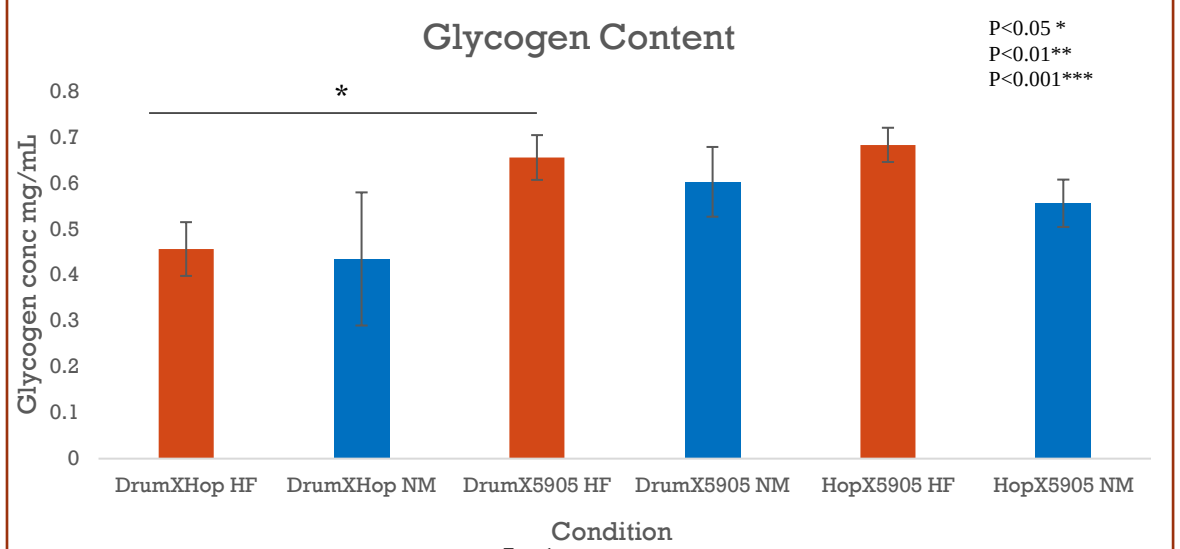


Fig.5 : Effect of overexpression of *Hop^{Tum-1}* in the midgut on glycogen content: *drm*-GAL4 driven overexpression of *Hop^{Tum-1}* resulted in less glycogen storage relative to the control flies, which is especially prominent on HF. Genotype*Diet P=0.1505, Diet P=0.6763 and Genotype P= 0.0065. Error bar represents standard error of mean. N = 25 flies per condition. P-values are derived from ANOVA analysis. (Tukey)

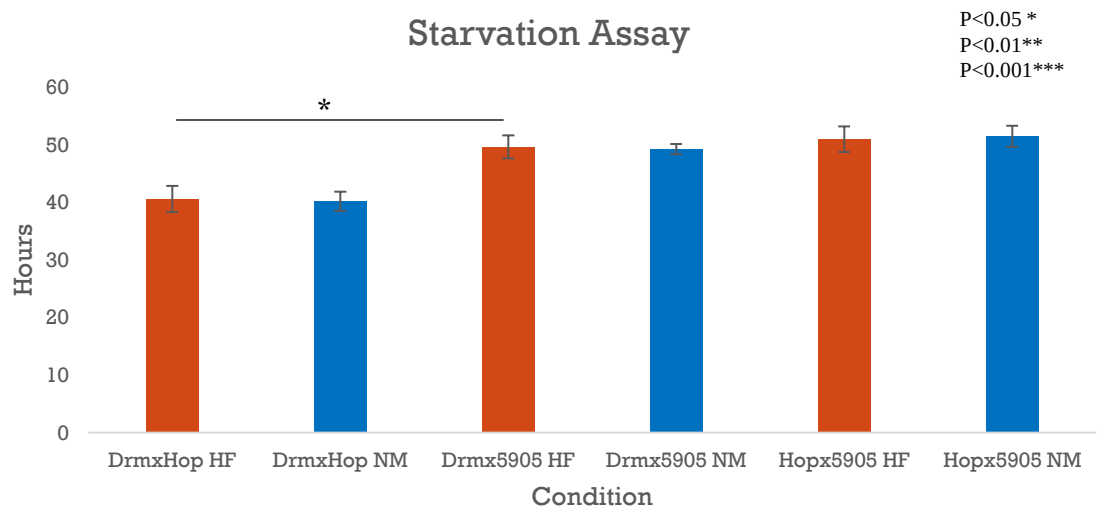
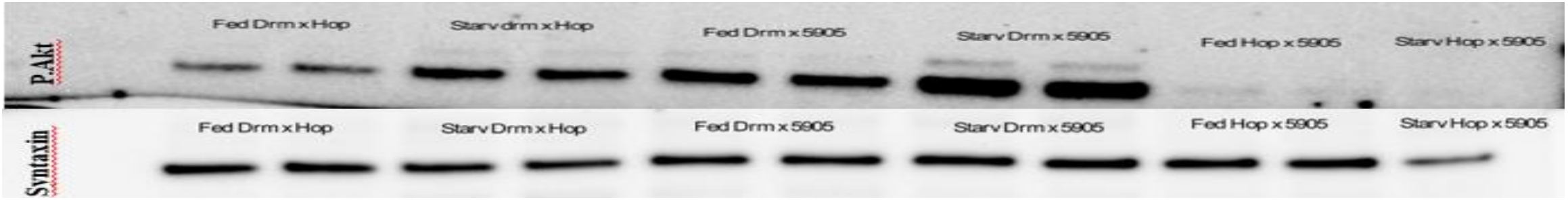


Fig.6 : Effect of overexpression of *Hop^{Tum-1}* in the midgut on starvation: *drm*-GAL4 driven overexpression of *Hop^{Tum-1}* resulted in less energy storage relative to the control lines. Genotype*Diet P<0.0001, Diet P=0.9691 and Genotype P= 9625. Error bar represents standard error of mean. N = 25 flies per condition. P-values are derived from ANOVA analysis (Student T).

WESTERN BLOTS

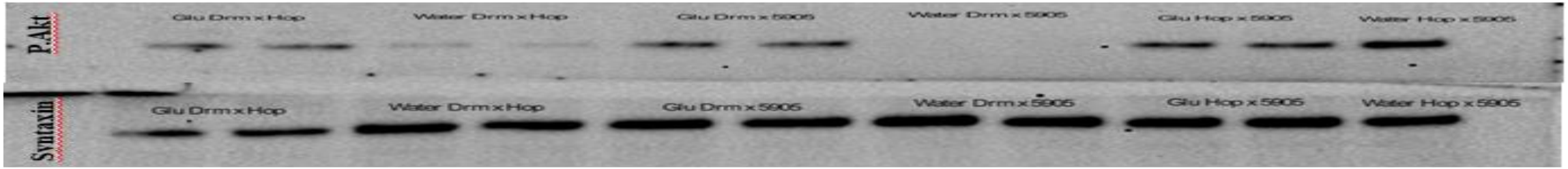
(A) Western blot results for Fed and Starved p-Akt exposed HF with the respective genotypes labelled on top of the bands. Here, each genotype and diet condition has a duplicate except for Starved Hop x 5905 (Lane 12). The first lane was loaded with a protein ladder and is not visible on the blot.



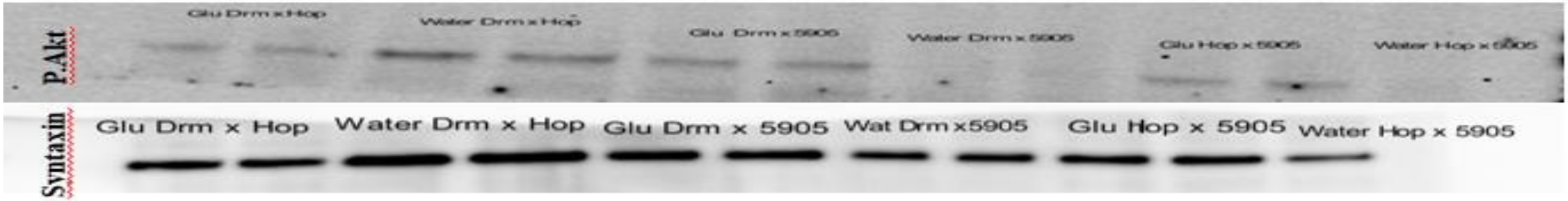
(B) Western blot results for Fed and Starved p. Akt exposed NM with the respective genotypes labelled on top of the bands.

Fig 7 : Effect of overexpression of *Hop^{Tum-1}* on insulin signaling under fed and starved conditions. (A): Here, overexpression of *Hop^{Tum-1}* in the midgut under fed and starved conditions when previously exposed to HF show an increase in insulin signaling. **(B)**: Flies exposed to NM show decreased insulin signaling due to overexpression of *Hop^{Tum-1}* in the midgut on both fed and starved conditions. Compared to flies exposed to NM, flies exposed to HF display an overall decrease in phosphorylation of Akt for all genotypes except Hopx5905. The Hop construct shows evidence of leak/transvection given the signal in the Hopx5905 lanes relative to Drmx5905.

WESTERN BLOTS (continue…)



(C) Western blot results for p-Akt of flies exposed to glucose and water re-fed flies that were previously exposed to HF after overnight starvation. Here, each genotype and diet condition has a duplicate except for water refeeding Hop x 5905 (Lane 12).



(D) Western blot results for p-Akt of flies exposed to glucose and water re-fed flies that were previously exposed to NM after overnight starvation.

Fig 8 : Effect of overexpression of *Hop^{Tum-1}* on insulin signaling under starvation or acute glucose exposure. (C): For HF pre-exposed flies, the overexpression of *Hop^{Tum-1}* in the midgut results in increased signaling under starvation conditions and slightly reduced signaling during acute glucose feeding. **(D)**: For NM pre-exposed flies (5 days), overexpression in the midgut results in increased signaling under starvation conditions and slightly reduced signaling during acute glucose feeding. Compared to NM, flies exposed to HF experience increased insulin signaling after acute feeding on glucose. The Hop construct shows evidence of leak/transvection given the signal in the Hopx5905 lanes relative to Drmx5905.

Conclusion

- The results obtained corroborates our hypothesis where *in vivo* overexpression of $Hop^{Tum\text{-}1}$ in midgut of flies exposed to HF influenced insulin sensitivity.
- In virgin females, exposure to HF reduced climbing ability.
- Overexpression led to reduced lifespan when exposed to HF for 5-7 days. However, triglyceride storage is not impacted.
- Overexpression modulated metabolic phenotypes and insulin signaling in a diet and nutrient environment specific manner as observed in figure 7(A) and 7(B) where flies exposed to HF show increased insulin signiling and those exposed to NM show decreased insulin signaling .
- The construct $Hop^{Tum\text{-}1}$ showed some evidence of leak/transvection. Transvection alludes to the phenomenon where expression of some genes are impacted by immediacy of homologous genes.

Future direction

- Use qRT-PCR in order to monitor changes in expression of genes involved in Hop-Stat92E activity.
- Conduct oral glucose test to observe the effect of $Hop^{Tum\text{-}1}$ on hemolymph glucose content.
- Through RNA-Seq Identify genes associated with the alteration of JAK/STAT pathway activity specifically in response to exposure of HFD.

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