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Evaluation of TAG and Glucose Concentrations to Determine the Degree of Adaptation to a High-Sugar Diet in *Drosophila melanogaster*

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Introduction

Background

- High-sugar diets have been shown to reduce the lifespan and healthspan in the fruit fly *Drosophila melanogaster,* inducing type II diabetes-like morbidities, including increased fat content, blood glucose, and insulin resistance(1)
- Experimental evolution on *Drosophila* has produced rapid phenotypic adaptation and enrichment within the genome for beneficial loci (2)
- This project aims to select large fly populations for adaptation to HS to enrich the *Drosophila* genome and identify loci protective against morbidities associated with a high-sugar diet
- We hypothesize that the HS-adapted populations will increase tolerance to HS feeding over time, whereas the control populations will not
- We also hypothesize that populations will shift towards more control-like phenotypes after adaptation to high-sugar feeding

Objectives

- To quantify tolerance to HS to determine if adaptation has occurred, weights will be recorded from flies from each of the populations
- Triacylglyceride (TAG) and glucose concentrations will also be quantified

Methods

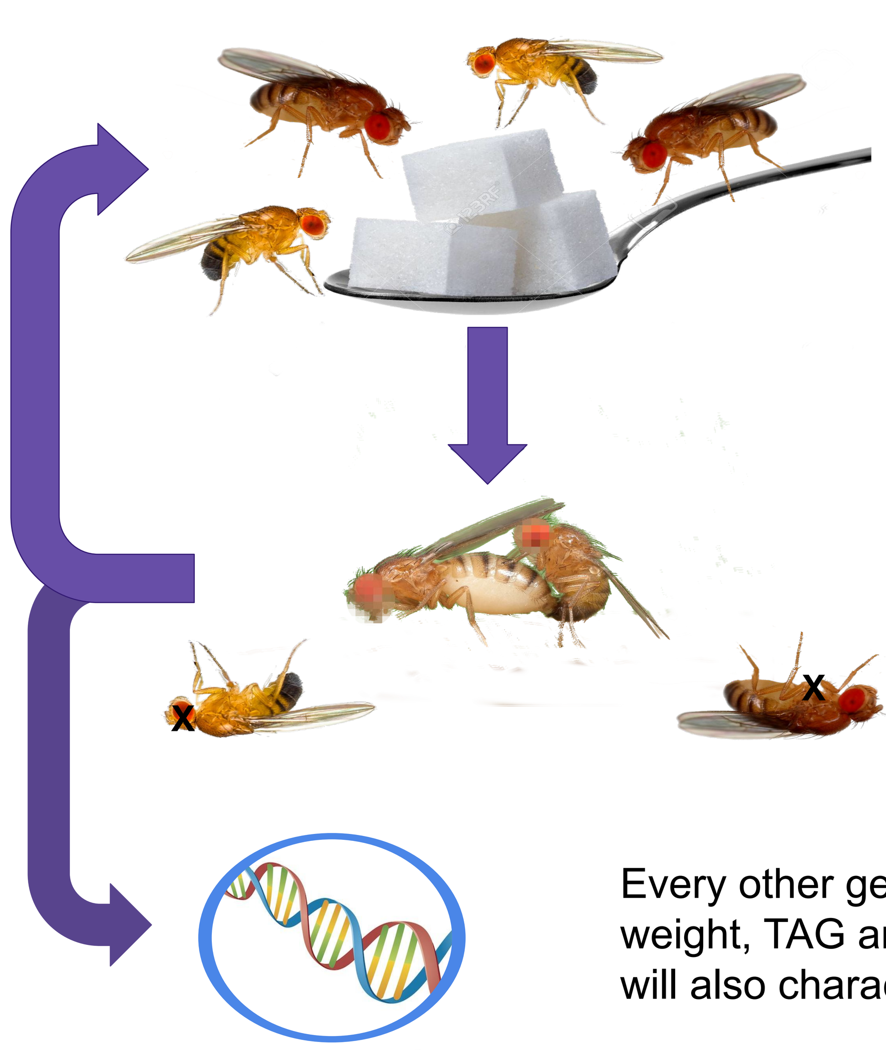

Figure 1. Model: Fly Husbandry
Populations are separated into ~6000 male and female flies who are aged separately on either control or high-sugar diets

Once 50% of the high-sugar population has died, males and females will be allowed to mate, producing the next generation

Every other generation, flies will be collected for weight, TAG and glucose analysis. Ultimately, we will also characterize the genomes of these flies.

This will be repeated for ~20 generations. **4 replicate populations** fed a high-sugar diet as adults will be run in parallel to **4 control populations** fed a standard, low-sugar diet.

Flies were collected from each population and fed either 1 M sucrose, also called high-sugar (HS) or 0.15M sucrose (control) diets for a week before sacrificing flies for the assays below.

Weight Collection

Male or female flies were weighed with 5 flies/tube and frozen at -80°C.

TAG Assay

Groups of 5 flies were homogenized in 1% Tween in PBS. Two µl of homogenate was transferred to a 96 well plate with 198 µl Triglyceride reagent (Fisher TR22421) and compared to a standard curve. A microplate reader read absorbance at 540 nm to quantify TAG.

Glucose Assay

The homogenate was added to Glucose reagent (Fisher TR15321) and compared to a standard curve. A microplate reader was used to measure absorbance at 340 nm to determine the glucose/mg tissue

Results

Weight Analysis

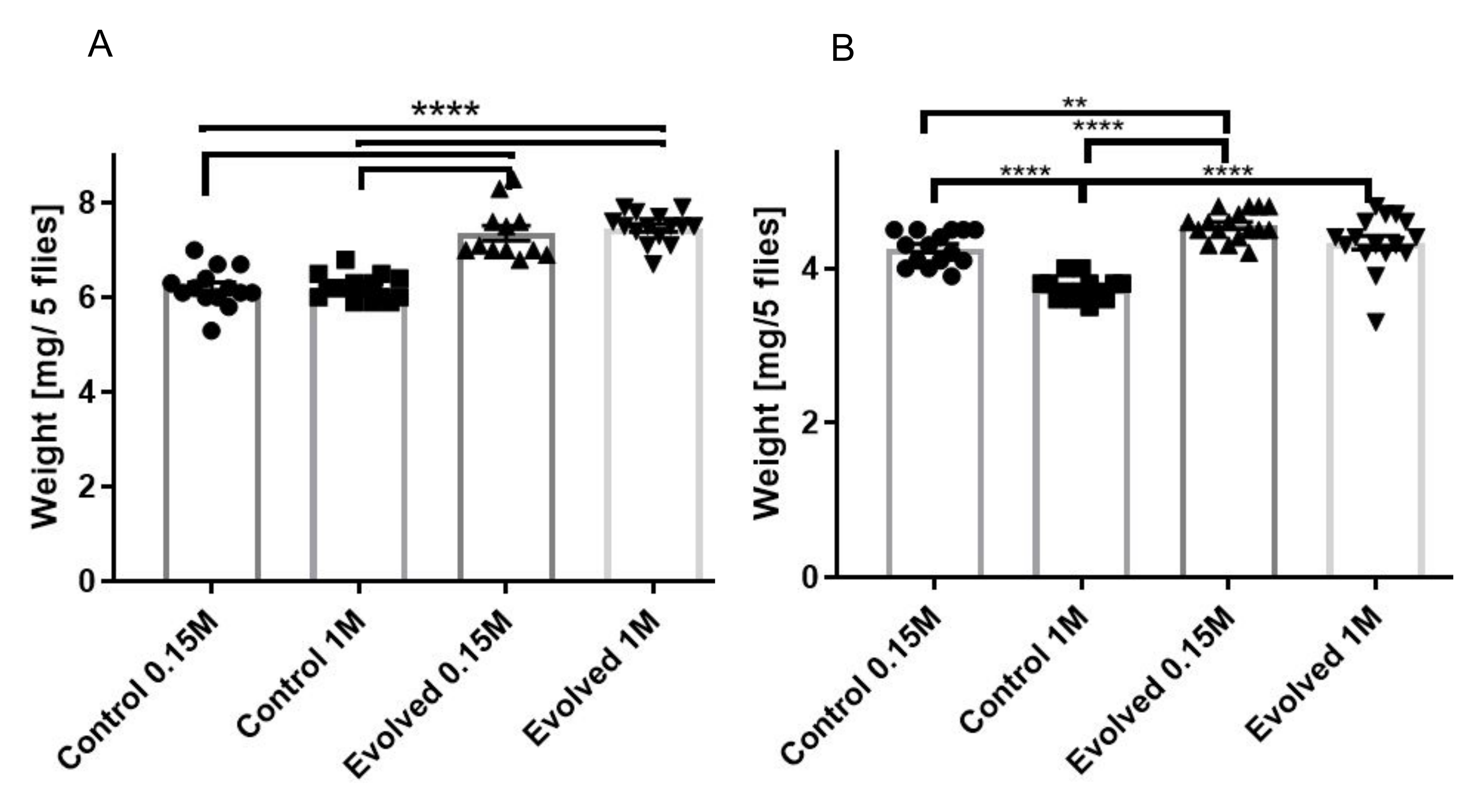

Figure 2. HS evolved flies weigh more than non-adapted control flies. (A) Female populations reared on 1M (HS) diet exhibited a significantly higher ($p<0.001$) weight compared to 0.15M (control) diets. Flies from the same population exhibited no difference in weight after being reared on 1M (HS) and 0.15M (control) diets for a week. (B) Male populations reared on 1M (HS) diets exhibited a significantly higher ($p<0.001$) weight than 0.15M (control) diets. Males from 0.15M (control) diets placed on 1M (HS) depict a significantly lower weight ($p<0.001$) compared to those maintained on a 0.15M (control) diet for a week. Significance was determined by ANOVA and Tukey's multiple comparison test. ** $p<0.01$ **** $p<0.0001$.

TAG Analysis

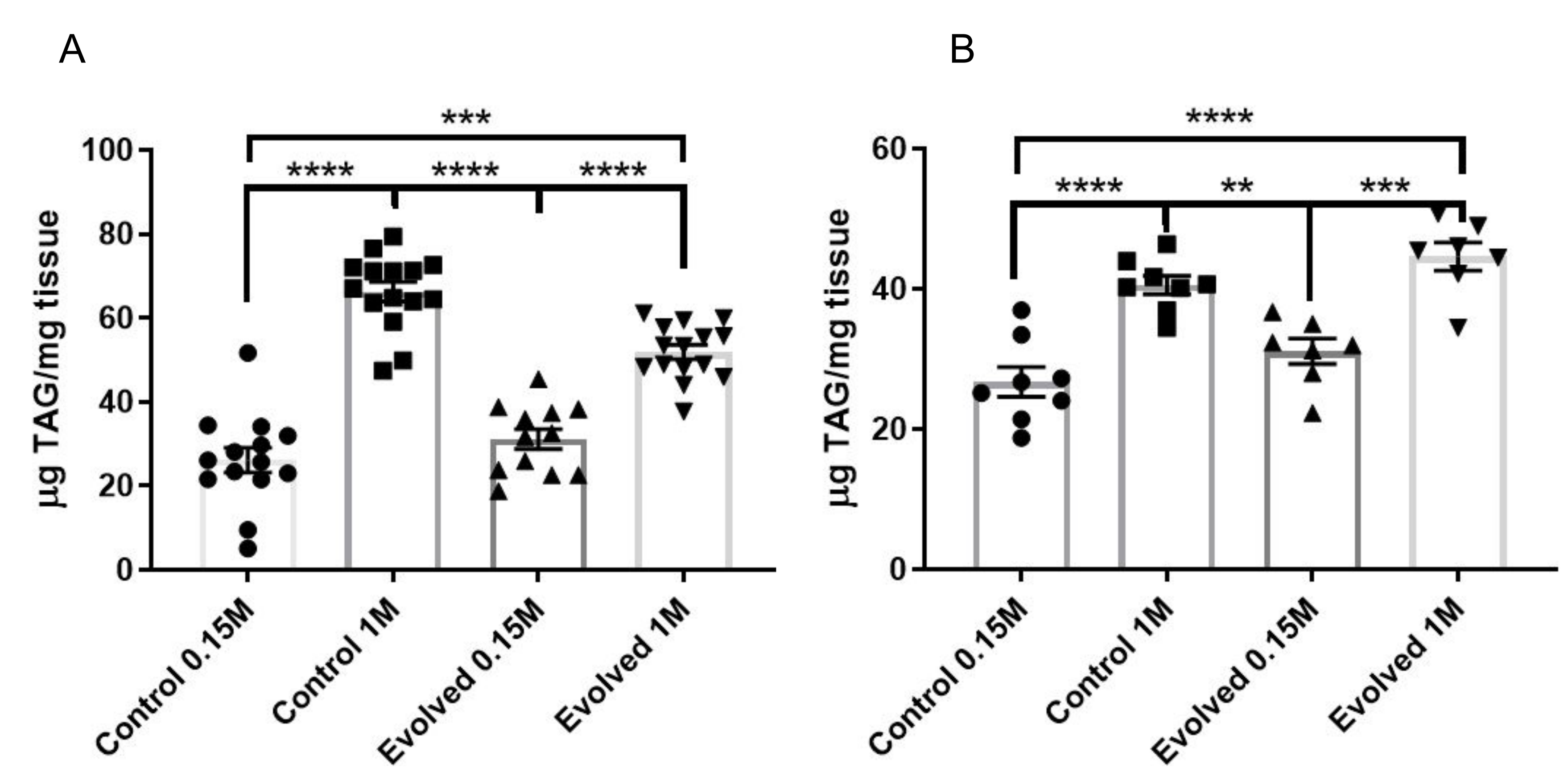

Figure 3. HS-fed flies exhibited increased TAG content. Flies reared for 1 week on 1M (HS) diets demonstrated significant ($p<0.0001$) increase in TAG in both populations compared to those on 0.15M (control) diets for a week. There was no significant difference between flies reared on 1M (HS) for a week despite maintenance on different diets (HS and control) prior to the assay. Females showed increased TAG/tissue than males for 1M (HS) diets. Significance was determined by ANOVA and Tukey's multiple comparison test. ** $p<0.01$ **** $p<0.0001$.

Results

Glucose Analysis

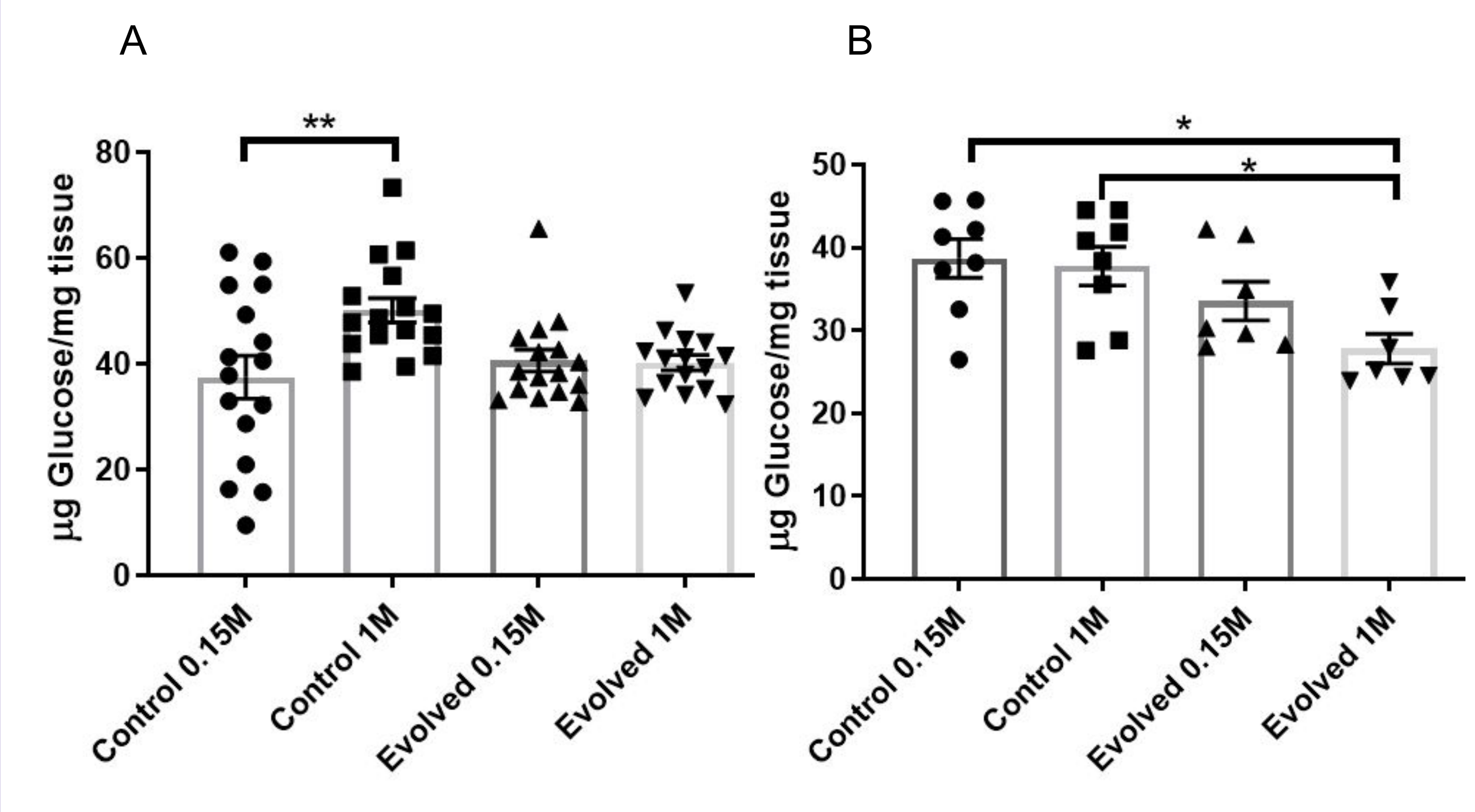

Figure 4. HS evolved flies exhibited reduced glucose content when fed the diabetogenic HS diet, compared to non adapted flies . (A) Females reared on 0.15M (control) diets then placed on 1M (HS) diets exhibited an increase in glucose concentration compared to those maintained on a 0.15M (control) diet for a week ($p<0.01$). They appear to also exhibit increased glucose concentrations on 1M and increased diet sensitivity, compared to the HS-evolved population. There was no difference in the average glucose concentration between diets for the evolved flies. (B) Males reared on 0.15M (control) diets depict a significantly higher ($p<0.05$) glucose concentration after 1 week of HS feeding, compared to those adapted to the 1M (HS) diet. Significance was determined by ANOVA and Tukey's multiple comparison test. ** $p<0.01$ **** $p<0.0001$.

Conclusions and Future Work

Our data suggest that there are already differences between control and evolved populations for several quantitative traits that are associated with HS feeding. Therefore, we will:

- Continue selection for ~15 more generations.
- Quantify additional phenotypic characteristics over time and across populations
  - High-sugar feeding survival
  - TAG content
  - Circulating glucose content
  - Insulin sensitivity
- Use DNA-seq and RNA-seq to identify genes associated with improved fitness in evolved populations
- Elucidate mechanisms by which alleles confer protective qualities

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