The effects of fertilization and sex peptide on the survival of mated female *Drosophila melanogaster* exposed to high fat diet

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Abstract

We use *Drosophila melanogaster* to study the adverse effects that obesogenic diets have on physiological systems and many prior experiments explore the effects to high fat diets (HFDs). We see a significant decrease in lifespan when mated females are exposed to HFD, which is manifested in mass death within the first couple of days of exposure. These adverse effects are not seen in virgin female flies or on other obesogenic diets, including diets that are disproportionately high in sucrose relative to yeast. The HFD used by us utilizes coconut oil as a source of saturated fat. The cause of mass death isn't known, but we believe that it may be rooted in the behavioral changes due to mating, such as greater contact with the media to lay fertilized eggs. It may also be due to physiological changes resulting from the transfer of seminal proteins to female flies. An initial test involved male flies with reduced Sex Peptide (*SP*) production due to RNAi produced by the GAL4/UAS system. Males of BDSC strain *1947* have the insertion *P{GAL4-prd.F}RG1*, which causes expression of GAL4 in the accessory glands. These males were crossed with females of BDSC strain *25998* that have the insertion *P{TRiP.JF02022}attP2* that produces dsRNA for RNAi of *SP*. Males with RNAi against *SP,* along with isogenic controls, were then mated with virgin w^{1118} females. The resulting mated females were then put on a HFD (20% w/v coconut oil). Reduction in *SP* in the males involved in the mating showed very little impact on the abruptly shortened lifespan in mated females on a HFD. Further experiments that we are conducting will examine the effect of mating between females and sterile males using the tud^1 allele of the tudor (*tud*) gene. Sons of homozygous tud^1 females don't form a germline and thus are sterile, but still make all accessory proteins, and exhibit normal mating patterns. In addition, examining egg-laying on a high fat diet after mating will allow us to gain additional insight on how the observed mass death occurs.

Objectives

- Evaluate the effects of Sex Peptide (SP) and fertilization on lifespan during exposure to HFD
- Gain further insight into the mass death observed in mated females exposed to HFDs as seen in earlier Talbert lab studies. Notably, these same effects are not seen with virgin females.

Methods

Media: Flies were reared on a standard diet consisting of 5.2% cornmeal, 5.0% yeast extract, 1.0% agar, 3.0% sucrose,1.5% tegosept (20% w/v in 70% ethanol), 0.3% propionic acid and 0.3% tetracycline (10mg/mL in 70% ethanol). The normal diet (NM) consisted of 5.0% yeast extract, 1.5% agar, 5% sucrose, 1.5% tegosept and 0.3% propionic acid v/v. The high fat diet (HFD) consisted of the same amount of ingredients as the normal diet, except the there as an addition of coconut oil at 20% w/v. The percentages are the concentration units w/v unless otherwise noted.

Flies: All fly stocks were received from the Bloomington Drosophila Stock Center (BDSC). Stock 1947 GAL4 males were crossed with Stock 25998 SP RNAi females (from the Harvard Transgenic RNAi Project) to produce male offspring with active suppression of SP in the accessory glands. In order to create control male offspring, GAL4 males were crossed with Stock 36303 females that were isogenic to Stock 25998 but that lack the active UAS-RNAi sequence.

Stock 77892 males harbor the SP^0 deficiency and were crossed with Stock 8097 females that harbor a deficiency for multiple genes, including SP. Deficiency homozygous male offspring were collected. Control offspring were created by crossing males of 77892 with w^{1118} (Stock 5905).

For both manipulations above, SP-reduced male flies and control male flies were crossed with w^{1118} females to generate experimental mated females and control mated females for HFD exposure.

In addition we used Stock 1786 to produce flies that do not create sperm. This was done by mating female 1786 that where homozygous for the tud^1 allele with *Canton-S* males. The male offspring of this cross are sterile. Sterile males were mated with virgin female *Canton-S* flies, and these mated females were then used in the HFD lifespan assay. Normal *Canton-S* male flies were crossed with *Canton-S* females to generate mated female controls.

For all of the treatments above, virgin female experimental flies were housed at an equal population density. Flies were stored for maturity 2-3 days and then mated for 48 hours in a 3:1 female to male ratio. Males were removed and the mated females were subjected to the HFD lifespan assays.

Lifespan: The mated females were placed on HFD under uniform humidity at 23 0C on a 12-hour light/dark cycle. The flies were transferred to new media for the first three days then every 3 days and mortality recorded daily for each condition until all flies faced mortality (N=5 per condition, 20 per replicate, 100 flies per condition).

Statistical Analysis: All data were analyzed using the Student's T test to determine differences between groups.

Results

Flies showed no significant difference in lifespan when mated with males that produced reduced levels of SP in accessory glands.

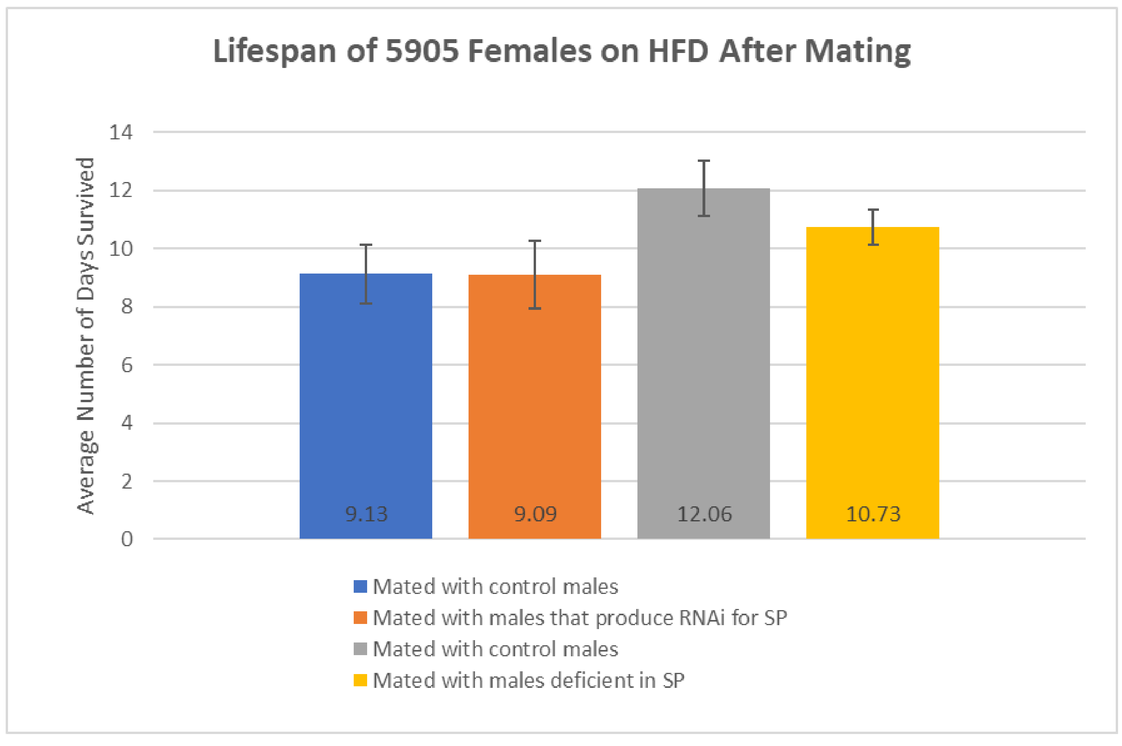

Figure 1: Effect on lifespan due to reduced amounts or absence of SP during HFD exposure. Lifespan was similar between females mated with males that exhibited normal amounts of SP and those that had reduced SP in accessory glands via *in vivo* RNAi. Lifespan was also similar between females mated with males that were homozygous for SP deficiency and those that were normal. Differences between groups were analyzed via Student's T-test. N=5 experiments per condition, 20 flies per experiment

Results (Continued)

Flies showed no significant changes in lifespan after mating with spermless males.

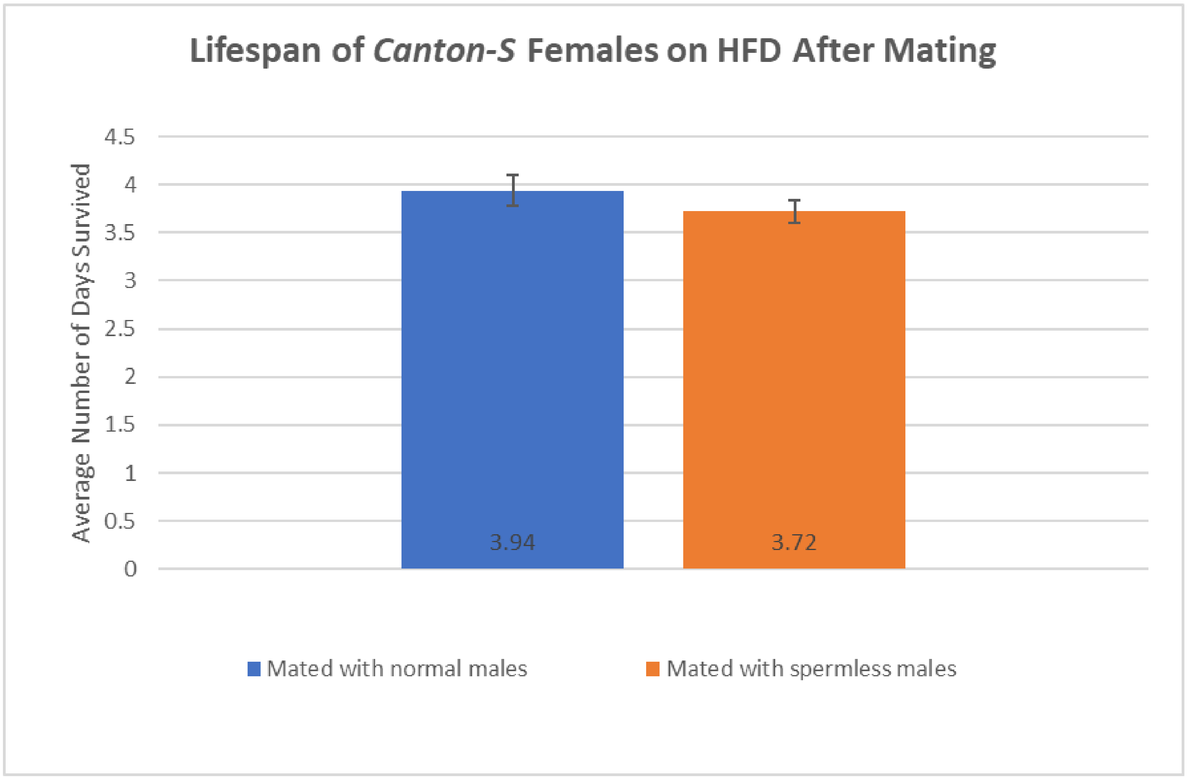

Figure 2: Effect on lifespan due to mating with sterile males during HFD exposure. Lifespan was similar between females mated with males that produced sperm and those that were sterile. Differences between groups were analyzed via Student's T-test. N=5 experiments per condition, 20 flies per experiment

Conclusion

- Female flies showed no significant change in lifespan on the HFD when mated with male flies that were producing less SP due to RNAi in accessory glands.
- Female flies also showed no significant change in lifespan on the HFD after being mated with male flies that were deficient in SP due to a homozygous deletion.
- The presence or absence of SP doesn't seem to have a significant effect on the short lifespan of mated females on the HFD.
- Female flies showed no change in lifespan on HFD when mated with male flies that were spermless.
- The presence or absence of sperm doesn't seem to have a significant effect on the short lifespan of mated females on the HFD.

Future Studies

- Examine egg laying behaviors to gain further insight on post-mating behaviors and how they may be leading to lower lifespan while on the HFD.

References

1. Chapman, T., Bangham, J., Vinti, G., Seifried, B., Lung, O., Wolfner, M. F., Smith, H. K., & Partridge, L. (2003). The sex peptide of Drosophila melanogaster: Female post-mating responses analyzed by using RNA interference. Proceedings of the National Academy of Sciences, 100(17), 9923–9928. https://doi.org/10.1073/pnas.1631635100
2. Hemphill, W., Rivera, O., & Talbert, M. (2017). RNA-Sequencing of Drosophila melanogaster Head Tissue on High-Sugar and High-Fat Diets. G3 Genes|Genomes|Genetics, 8(1), 279–290. https://doi.org/10.1534/g3.117.300397
3. Sharma, V., Pandey, A. K., Kumar, A., Misra, S., Gupta, H. P. K., Gupta, S., Singh, A., Buehner, N. A., & Ravi Ram, K. (2017). Functional male accessory glands and fertility in Drosophila require novel ecdysone receptor. PLOS Genetics, 13(5), e1006788. https://doi.org/10.1371/journal.pgen.1006788
4. Talbert, ME. Personal Communication. 2019-2020.
5. Tram, U., & Wolfner, M. F. (1998). Seminal fluid regulation of female sexual attractiveness in Drosophila melanogaster. Proceedings of the National Academy of Sciences, 95(7), 4051–4054. https://doi.org/10.1073/pnas.95.7.4051

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