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Influences of Drosophila circadian clock on sugar-mediated physiological changes

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Influences of *Drosophila* circadian clock on sugar-mediated physiological changes

By

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Abstract

It is widely known that high sugar consumption and poor sleep are detrimental to human health. Both are risk factors for obesity, which can lead to conditions such as heart disease. Despite this connection between sugar and sleep, little is known about how circadian clock dysfunction affects the physiological changes caused by increased sugar consumption. In this thesis, a mutant line of the model organism *Drosophila melanogaster* (Clk^{Jrk}, which contains a nonfunctional circadian rhythm gene known as Clk) that leads to circadian dysfunction, was exposed to a high sugar diet to observe how Clk affects sugar-related changes to food consumption, sleep, triglyceride levels, and starvation resistance. Despite previous research suggesting that the circadian rhythm affects feeding, Clk^{Jrk} flies did not substantially differ from wild-type flies in terms of food consumption. In addition, both Clk^{Jrk} and wild-type flies slept less on low-sugar food. However, unlike wild-type flies, Clk^{Jrk} flies did not experience increased starvation resistance on a high sugar diet, suggesting that a functioning Clk gene is essential for flies fed a high sugar diet to survive when starved. Lowered triglyceride levels in Clk^{Jrk} flies may explain the reduced starvation resistance of Clk^{Jrk} flies fed high sugar diets. The findings from this research provide a greater understanding of how sleep and sugar intertwine to affect health and disease. Future studies should explore the endocrine components of this relationship in *Drosophila*, particularly the *Drosophila* Insulin-Like Peptides (DILPs), which regulate nutrient storage and release.

Keywords. Sleep, feeding, *Drosophila melanogaster*, sugar, physiology, circadian rhythm

Introduction

Excess sugar consumption is rampant in the western world, contributing to a variety of health problems including diabetes and obesity (Freeman et al., 2018; Johnson et al., 2013). Obesity is particularly dangerous given that it can lead to other conditions, including heart disease (Virani et al., 2021). In the United States, the obesity prevalence was 42.4% during 2017-2018 (Hales et al., 2017). Globally, the World Health Organization (WHO) estimates that 39% of adults were overweight and 13% were obese in 2016 (WHO, 2021).

Interestingly, lack of sleep is a risk factor for obesity as well, and it can cause many of the same health conditions that high sugar consumption does. Further connecting these two risk factors is the knowledge that dysfunction of the circadian clock, an internal regulator of sleep and other cyclic behaviors, can also lead to obesity (Scheer et al., 2009; Shi et al., 2013). Thus, elucidating the impact of the circadian clock on physiological changes in response to a high-sugar diet holds direct relevance for human health.

Drosophila melanogaster, the fruit fly, is often used as a model organism for studying human physiology. This is because many of the molecular mechanisms underlying *Drosophila* and human physiology are conserved. Regarding sleep, both humans and flies respond to an internal circadian clock that controls the timing of sleep. This clock involves a negative feedback loop. In *Drosophila,* this loop consists of the products of genes *Period (Per)* and *Timeless (Tim)* inhibiting their own transcription. *Per* and *Tim* levels rise during the day and peak in the early evening, and as their protein products enter the nucleus, they inhibit their transcriptional activators *Clock (Clk)* and *Cycle (Cyc)*. In humans, the homologues of *Per* and *Tim* are known as *Per2* and *Ck1δ*, and the gene BMAL1 has the same function as *Clk* and *Cyc* (Dubowy & Sehgal,

2017). Regarding feeding and obesity, a high sugar diet leads to obesity and a diabetic-like state in *Drosophila*, just as it does in humans. The *Drosophila* Insulin-Like Peptides (DILPs) are homologues of human insulin and regulate nutrient storage and release (Hemphill et al., 2018; Musselman & Kuhnlein, 2018).

The Clk^{Jrk} line of *Drosophila* melanogaster, created by Allada et al. (1998), has a non-functioning Clk gene due to a premature stop codon in Clk mRNA. This leads to inhibited transcriptional activation of *Per* and *Tim* in *Drosophila*, and by extension an arrhythmic circadian clock. Whereas wild-type flies typically are most active in the early morning and late evening, with sleep occurring at night and during mid-day, Clk^{Jrk} flies have a more constant activity level throughout the day, do not have typical sleep patterns, and overall sleep less than wild-type flies. Because circadian rhythms also regulate the timing of feeding, the Clk^{Jrk} line of *Drosophila* is an ideal tool for studying the effects of sleep on sugar-induced obesity (Fulgham et al., 2021).

The goal of this senior thesis is to conduct an investigation into how the *Drosophila* circadian clock influences sugar-related obesity. To achieve this goal, the following four hypotheses were tested using Clk^{Jrk} flies and a wild-type line known as Iso31:

1. Clk^{Jrk} flies eat more food than wild-type flies, and this difference is amplified on a high-sugar diet;
2. Clk^{Jrk} flies sleep less than wild-type flies, and this difference is amplified on a high-sugar diet;
3. Clk^{Jrk} flies contain higher free triglycerides than wild-type flies, and this difference is amplified on a high-sugar diet; and

4. Clk^{Jrk} flies are more resistant to starvation stress, and this difference is amplified on a high-sugar diet.

Given that the *Drosophila* circadian clock is like the human circadian clock (Turek et al., 2013), the knowledge gained from this research could be used to develop treatments for sugar-related obesity that consider the importance of sleep. To test hypothesis 1, food consumption assays were conducted using the ConEx dye method to observe changes in feeding that may correlate with metabolic changes. High sugar (20% sucrose concentration) and low-sugar (5% sucrose concentration, the standard for *Drosophila* per May et al. 2019) diets were used to enable the study of sugar-related obesity. The ConEx method is ideal since it uses a dye that is mixed into fly food without changing the texture or taste of the food (Shell et al., 2018). To test hypothesis 2, sleep habits were monitored and hyposomnia was confirmed in the Clk^{Jrk} mutants using the *Drosophila* Activity Monitoring 2 (DAM2) system (Pfeiffenberger et al., 2010).

As part of hypothesis 3, lipids were quantified in Clk^{Jrk} and wild-type flies using the Infinity triglycerides assay. Given the obesity phenotype seen in circadian gene mutants (Shi et al., 2013), I expected to find differences in the amount of free triglycerides in these mutants.

To test hypothesis 4, a starvation assay was conducted on both genotypes. The DAM2 system was also used for this purpose. Starvation resistance was expected to be mediated by differing triglyceride levels in Clk^{Jrk} versus Iso31 flies.

Materials and Methods

General Fly Husbandry

The two strains used in this experiment were Clk^{Jrk} (Bloomington *Drosophila* Stock Center (BDSC) #24515), created by Allada et al. (1998) and Iso31 (BDSC #5905), a wild-type

control made co-isogenic with Clk^{Jrk} by backcrossing 7 times. Both were reared on a Nutri-Fly® diet (Flystuff, Genesee Scientific, San Diego, CA). This diet is a pre-mixed version of the standard cornmeal diet used by Bloomington *Drosophila* Stock Center, a major facility for the maintenance and distribution of *Drosophila* strains. Nutri-Fly food was supplemented with 1% propionic acid and 0.3% nipagin to prevent bacterial and fungal growth, respectively. Flies were seeded using fresh vials of the same diet and a 12L:12D cycle at 25℃. 7-10 pairs of flies were placed in each vial and allowed to mate for 48 hours before they were cleared and disposed of. Flies emerged between days 13-15, after which they were transferred to fresh Nutri-Fly® vials to mature and mate for 24 hours. After 24 hours, healthy-appearing males were selected and transferred to either 20S5Y ("high sugar", 20% sucrose, 5% yeast) or 5SY ("low sugar", 5% sucrose, 5% yeast) (see "*Drosophila* Food") diets for 5 days prior to feeding assay and 7 days prior to sleep, starvation, protein, and triglyceride assays. 2-3 days through the high and low sugar treatments, flies were transferred to fresh food of the same diet.

***Drosophila* Food**

The three diets used in this study were the pre-mixed Nutri-Fly® Bloomington Formulation for seeding, and the high and low sugar diets (20S5Y and 5SY) for experimentation. Details are given in Tables 1 and 2.

Table 1

Nutri-Fly® Ingredients

Ingredient	**Brand Name**	**Catalogue No.**
Nutri-Fly® Bloomington Formulation	Flystuff	66-121

Propionic Acid	Avantor J.T.Baker	02-003-884
Tegosept	Apex Bioresearch Products	20-259
Ethanol	Milipore	818760

Table 2

Sucrose-Yeast (SY) Ingredients

Ingredient	**Brand Name**	**Catalogue No.**
Nutri-Fly® *Drosophila* Agar, Gelidium	Flystuff	66-103
Sucrose	Cargill	62-112
Brewer's Yeast	MP Biomedicals	903312
Propionic Acid	Avantor J.T.Baker	02-003-884
Tegosept	Apex Bioresearch Products	20-259
Ethanol	Milipore	818760

Sleep Measurement

Sleep in male flies was measured using the *Drosophila* Activity Monitor 2 (DAM2, Figure 1) (Trikinetics). In *Drosophila* melanogaster, sleep is defined as a period of immobility longer than 5 minutes in which the arousal threshold is increased (Beckwith & French, 2019; Hendricks et al., 2000). Sleep data was taken for three days using 4 genotype/diet combinations

(Clk^{Jrk} on high sugar, Clk^{Jrk} on low sugar, Iso31 on high sugar, and Iso31 on low sugar). Sample sizes were 64,61,63, and 64 flies, respectively. Flies were supplied with the same diet they received during the 7-day period on either high or low sugar food. The first day was used to calibrate the system. Data from the sleep assay was analyzed using the protocol by Pfeiffenberger et al. (2010). Wake bins were defined as the total number of bins/day in which the fly was awake, total activity was the total number of infrared beam crossings (corresponding to movement) over a certain phase, bout length was the time in minutes in which a fly was awake ("wake bout", flanked by two periods of sleep) or asleep ("sleep bout", flanked by two wake periods), and % sleep described the percent of the time that a fly was asleep. Sleep eduction files were obtained, which show hour-by-hour graphs of average activity for flies. After the three-day assay, flies were transferred to tubes containing agar in place of food in order to run the starvation assay.

Figure 1

The DAM2 board and its tubes

Note. The DAM board (top image) emits infrared beams continuously and collects beam crossing readings over a specific time interval (every 60 s for this experiment) to determine if a fly is moving. If a fly crosses a beam, it is recorded as moving and awake. Flies are recorded as sleeping if there is no beam crossing, except when the fly never returns to an active state, in which case it is assumed to have died. The board contains 32 holes for glass tubes (bottom image) to be placed into. These tubes hold one fly each as well as either food (for the sleep assay) or agar (for the starvation assay). Top image reprinted from Trikinetics (2018).

Starvation

The agar tubes, containing agar in a concentration of 1% w/v in water, each held one fly that had completed the sleep assay earlier. Flies were returned to their original spots in the DAM2 board, and flipping to agar tubes was done at ZT0 (when the lights in the incubator designated for the DAM2 boards turned on, or 9AM local time, when flies are most active). Flipping was done within one hour, and because fly activity peaks at this time, impact of flipping on endogenous sleep behavior should have been minimal. Any deceased flies were discarded during flipping. The same method used for the sleep assay was followed, except the assay was run until all flies had died and activity data was viewed in intervals of 1 hr.

Food Consumption

A modified version of the ConEx method (Figure 2, Shell et al., 2018) was used to measure food consumption for two days. MOCAP (Park Hills, MO) FC series flanged plastic caps (part # FCS.813) were double-wrapped around their centers with washi tape, then filled with high or low sugar food dyed with 1% w/v FD&C Blue No. 1 (Sigma-Aldrich, St. Louis, MO). 2-3 mm of space was left between the food and the top of the cap. Caps were placed in vials (Genesee Scientific, catalogue no. 32-116BR) with 2-3 flies each. Five vials were used per genotype/diet combination. After 48 hours of feeding, flies were removed and placed in pre-weighed vials for body weight measurement. 3 mL of deionized water was added to the empty vials. Following 5 seconds of vortexing, 200 µL of the liquid was pipetted in triplicates into a 96-well microplate. Deionized water, also in triplicate, was used as a blank. Plates were read at 630 nm using an Epoch microplate spectrophotometer (Agilent Technologies, Santa Clara, CA), and absorbance was normalized to fly number. Higher absorbance indicated greater food consumption. Assays were done on the same flies at 1 and 2 weeks of age. In between assays,

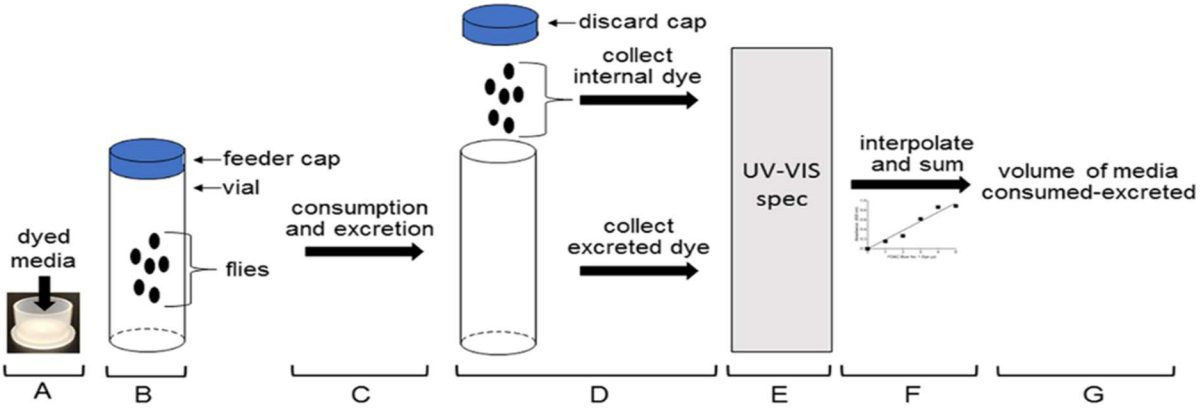
flies were fed on the same diet (undyed) diet they had received during the first assay, and flies were flipped to fresh food every 2-3 days.

Body Weight Measurement

The flies in pre-weighed vials were weighed on a VWR scale (Model 164AC, Radnor, PA). The weight of the pre-weighed vial was subtracted from the weight of the vial with flies to obtain the weight of the flies, which was divided by fly number to obtain the average weight per fly.

Figure 2

The ConEx Method



Note. Reprinted from Shell et al. (2018)

Protein and Triglyceride Measurement

Protein and triglyceride quantification was adapted from Hildebrandt et al. (2011) and Werthebach et al. (2019). To prepare samples for protein and triglyceride assays, eight flies were transferred to a 1.7 mL microcentrifuge tube and homogenized in 500 µL of 0.05% Tween20 with a pellet pestle. The homogenate was heat inactivated for 5 minutes at 70ºC, centrifuged at 5000 rpm for 5 minutes, then centrifuged at 14800 rpm for 15 minutes. The supernatant was

transferred to a new tube of the same size, then centrifuged at 14800 rpm for 15 minutes. 150 µL of the sample was transferred to another 1.7 mL microcentrifuge tube and another 100 µL was added to a 15 mL conical vial. The microcentrifuge tube was used for the triglyceride assay and the conical vial was used for the protein assay.

The Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Middletown, VA) was used for protein quantification. 1.5 mL of the reagent was added to the conical vial, then the vial was shaken and incubated at room temperature for 5 minutes. 200 µL of the liquid was pipetted in triplicates into a 96-well microplate. The reagent, also in triplicate, was used as a blank. Plates were read at 660 nm using the microplate spectrophotometer.

For triglyceride quantification, 600 µL of the Infinity triglycerides reagent (Thermo Fisher Scientific, Middletown, VA) was added to the microcentrifuge tube. The vial was shaken and incubated at 37ºC for 30 minutes. 200 µL of the liquid was pipetted in triplicates into a 96-well microplate. The reagent, also in triplicate, was used as a blank. Plates were read at 510 nm. Triglyceride readings were normalized in three ways: by fly number, by average weight per fly, and by protein readings.

Statistical Analysis

For statistical analysis, Microsoft Excel with statistical package added was used except for lifespan and mortality analysis. For those analyses, the web-based OASIS 2 tool (https://sbi.postech.ac.kr/oasis2/) (Han et al., 2016) was used. From this tool, the Kaplan-Meier estimator was used to determine the probability of mortality at a given time, and the log-rank test was used for survival curve comparison. Mean/median lifespan was also determined, and lifespan graphs were generated from data inputted to OASIS 2. ConEx data was analyzed with

Student's t-test. Sleep bout lengths were compared with the Mann-Whitney U test (both the Student's t-test and Mann-Whitney U test were used with significance at p values ≤ 0.05).

Results

Sleep Measurement

Flies that had been treated with high or low sugar food for 7 days were used in the DAM2 sleep assay. The DAM2 system generates a variety of sleep analyses, but for simplicity this thesis focuses on the data regarding total sleep, number of sleep bouts (defined as a continuous period of sleep), and average sleep bout length. Amount of total sleep encompasses the number of sleep bouts and their lengths for a given duration of time.

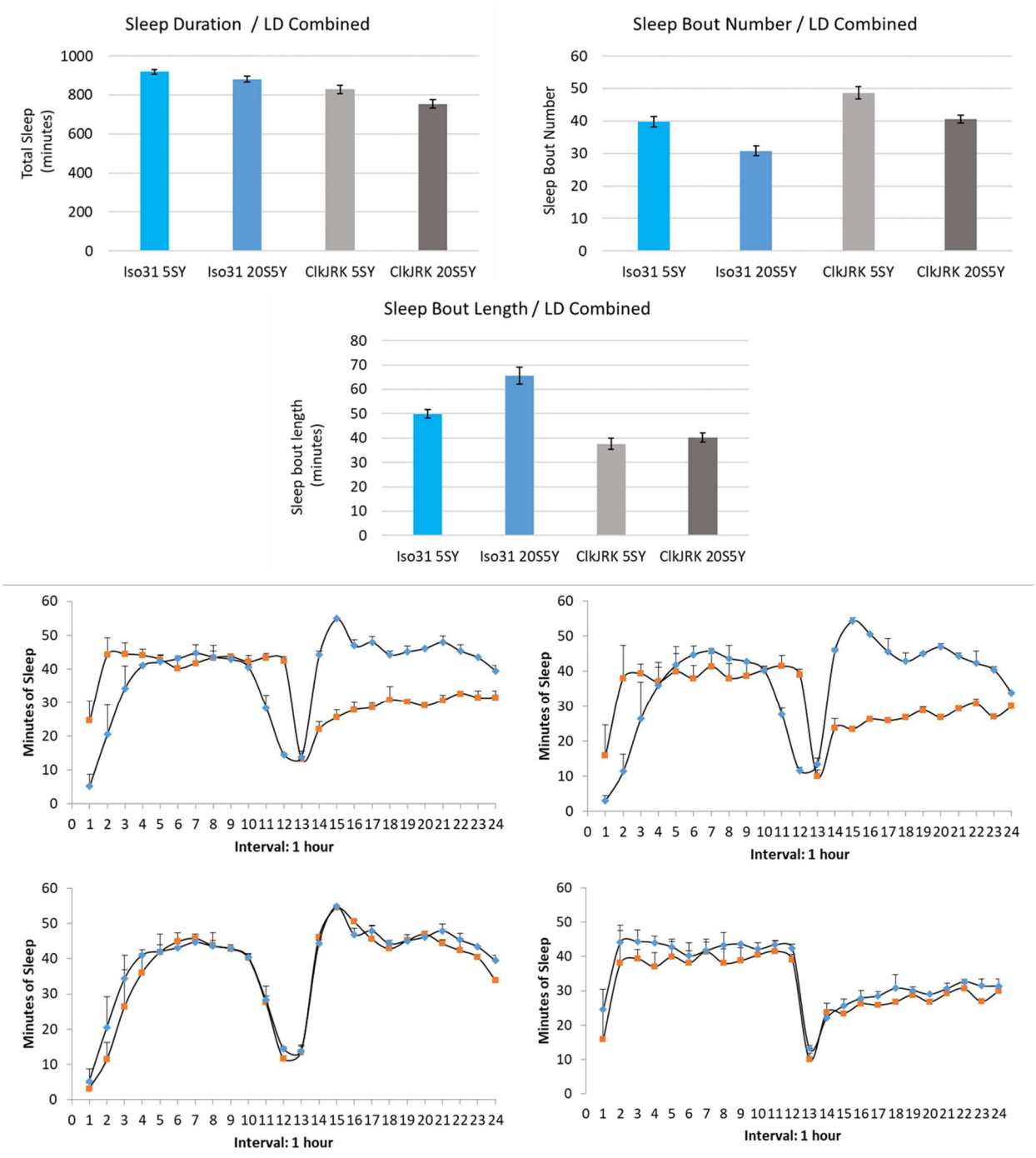
When comparing flies fed on low sugar versus high sugar food, both genotypes slept longer ($p = 0.0239$ for Iso31 and 0.00757 for Clk^{Jrk}) and had more bouts of sleep ($p = 3.23 \times 10^{-5}$ for Iso31 and $p = 3.61 \times 10^{-4}$ for Clk^{Jrk}) on low sugar food (Figure 3). However, only the wild-type Iso31 flies significantly differed in average bout length, with low sugar flies experiencing shorter bout lengths than high sugar flies (146.75% difference high sugar/low sugar, $p = 4.83 \times 10^{-5}$). In Clk^{Jrk} flies, the difference between average bout lengths on the two diets was not significant (120.88% difference high sugar/low sugar, $p = 0.182$) This suggests that a low sugar diet leads to sleep fragmentation in wild-type flies.

When comparing flies Clk^{Jrk} and Iso31 flies fed on the same diet, Iso31 flies slept longer ($p = 2.97 \times 10^{-4}$ on low sugar, $p = 1.16 \times 10^{-6}$ on high sugar) and had longer average bout lengths than Clk^{Jrk} flies ($p = 9.85 \times 10^{-6}$ on low sugar, $p = 2.07 \times 10^{-9}$ on high sugar), indicating that the Clk^{Jrk} line is hyposomniac. However, Iso31 flies experienced fewer sleep bouts ($p = 2.50 \times 10^{-4}$ on low sugar, $p = 1.01 \times 10^{-6}$ on high sugar, Figure 3) while having increased sleep bout length,

suggesting the sleep pattern of the Clk^{Jrk} line is more fragmented. Sleep bout length was increased by 185.45% on a high sugar diet (percent difference Iso31/Clk^{Jrk}, $p = 2.07 \times 10^{-9}$) and by 153.50% on a low sugar diet ($p = 9.85 \times 10^{-6}$) in these wild-type flies. These results indicate that Clk^{Jrk} mutant flies have lost the ability to reprogram (i.e., increased sleep length by high sugar) their sleep pattern by sugar.

Figure 3

The effect of a high sugar diet on sleep



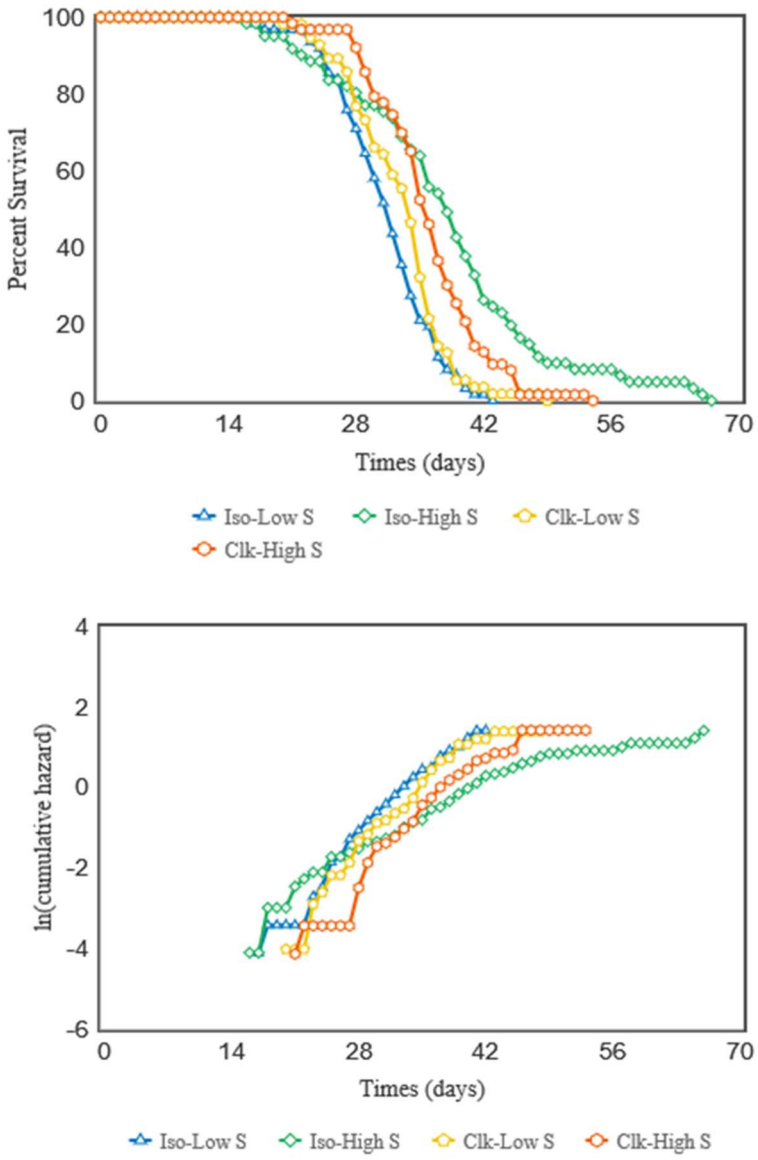
Note. The four bottom graphs show Iso31 5SY and Clk^{Jrk} 5SY (top left), Iso31 20S5Y and Clk^{Jrk} 20S5Y (top right), Iso31 5SY and Iso31 20S5Y (bottom left), and Clk^{Jrk} 5SY and Clk^{Jrk} 20S5Y (bottom right). Bars indicate standard error.

Starvation

The starvation assay was conducted with the same flies used in the sleep assay. Results showed that wild-type Iso31 flies on a high sugar diet were more resistant (121.04% increase in mean survival) to starvation than wild-type flies on a low sugar diet (Figure 4, $p = 5.30 \times 10^{-8}$). On the low sugar diet, starvation resistance was not significantly different ($p = 0.415$) between wild-type and Clk^{Jrk} mutant flies. However, on the high sugar diet, Clk^{Jrk} flies were more sensitive to starvation stress and died earlier ($p = 0.0547$, marginally significant). Iso31 flies on a high sugar diet had a 105.41% increase in mean survival compared to Clk^{Jrk} flies on the same diet. Like Iso31 flies, Clk^{Jrk} flies were more resistant to starvation on a high sugar diet than when on a low sugar diet ($p = 0.0028$), but as indicated by the p value, this difference in starvation resistance was not as strong as that seen in wild-type flies. This is further evidence that the physiology of Clk^{Jrk} flies does not respond to increased sugar consumption as the physiology of Iso31 flies does.

Figure 4

Differing Starvation Resistance in Clk^{Jrk} and Iso31 Flies



Note. Percent survival (top) indicates the percentage of the original sample size that was alive on a given day. Cumulative hazard (bottom, presented in natural log form) shows the cumulative odds of death within a given period of days.

Food Consumption

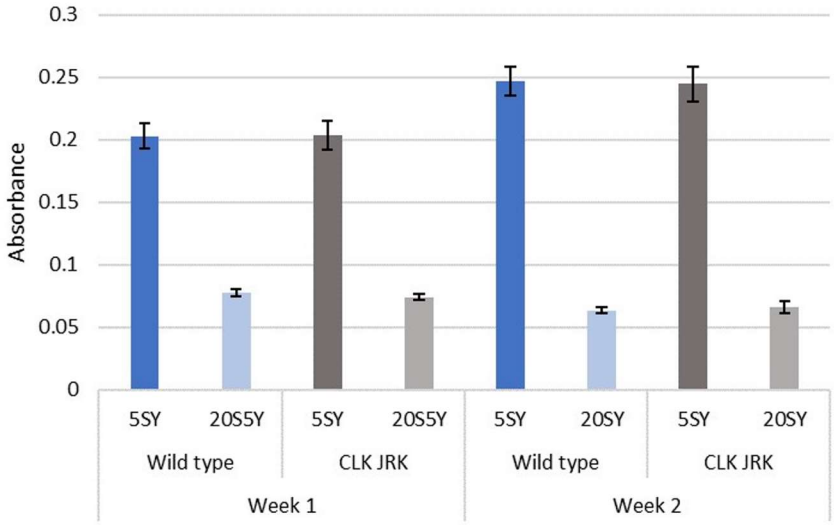
To determine if Iso31 and Clk^{Jrk} flies ate different amounts of food on low sugar versus high sugar food, the ConEx dye method was used. Two assays were conducted: the first on one-week old flies, and the second on those same flies when they were two weeks ok.

Surprisingly, genotype did not appear to have a significant effect on food consumption during week 1 ($p = 0.986$ for low sugar, $p = 0.335$ for high sugar) or week 2 ($p = 0.899$ for low sugar, $p = 0.645$ for high sugar). However, diet strongly affected food consumption (Figure 5). When genotype was kept constant, flies on the low sugar diet consumed more food than flies on the high sugar diet ($p= 4.46 \times 10^{-7}$ for Clk^{Jrk} week 1, $p = 1.34 \times 10^{-7}$ for Iso31 week 1, $p = 5.06 \times 10^{-8}$ for Clk^{Jrk} week 2, and $p = 1.15 \times 10^{-8}$ for Iso31 week 2). This "compensatory feeding" likely occurs because flies must eat more of the low sugar diet to consume the same amount of sugar as they would on the high sugar diet.

Age appeared to have an effect on food consumption. Flies of both genotypes on low sugar diets significantly differed in food consumption from week 1 to week 2. Specifically, feeding increased in the second week (Clk^{Jrk} $p = 0.0177$, Iso31 $p = 0.00482$). This increase suggests the presence of age-related changes in feeding. In addition, food consumption of wild-type flies on a high sugar diet significantly decreased from week 1 to week 2 ($p = 5.05 \times 10^{-4}$). The fact that Clk^{Jrk} flies did not experience this decrease suggests that they do not experience the same age-related feeding changes on high sugar food that wild-type flies do.

Figure 5

The effects of age and genotype on the consumption of high and low sugar food



Note. Bars indicate standard error.

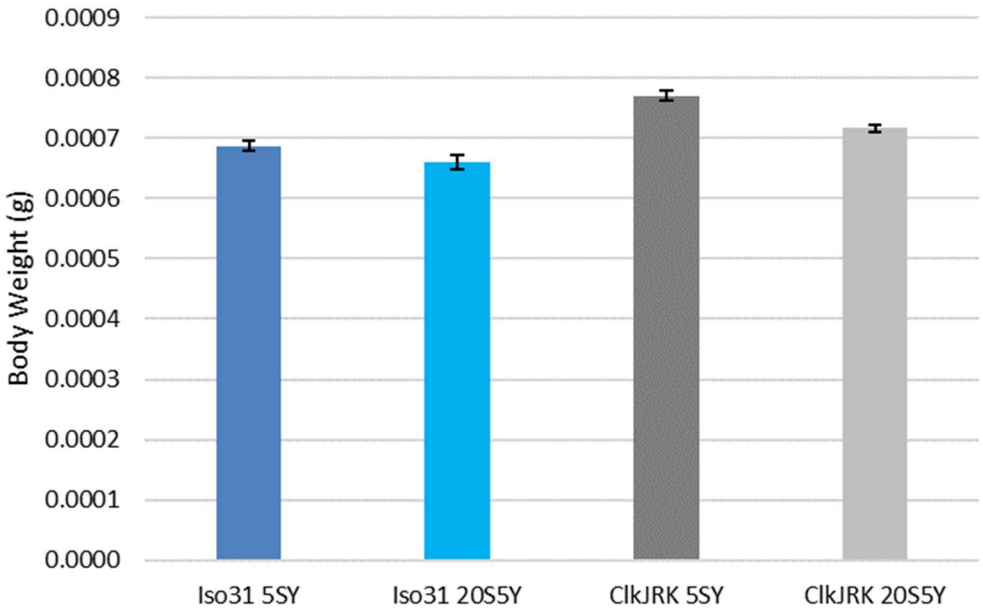
Body Weight

Body weight measurements were taken immediately after the ConEx assays concluded. However, flies were only weighed following the first week assay.

Regarding the effect of diet on body weight, Clk^{Jrk} flies that consumed high sugar food weighed less than Clk^{Jrk} flies that consumed low sugar food ($p = 3.11 \times 10^{-5}$) This effect was also seen in Iso31 flies ($p = 0.0430$). The more robust effect of the first trial was that flies that differed in genotype but not diet showed a difference in weight. Within both the low and high sugar groups, Clk^{Jrk} flies weighed more than Iso31 flies ($p = 6.82 \times 10^{-7}$ for low sugar, $p = 3.23 \times 10^{-4}$ for high sugar, Figure 6).

Figure 6

The effects of diet and genotype on body weight



Note. Bars indicate standard error.

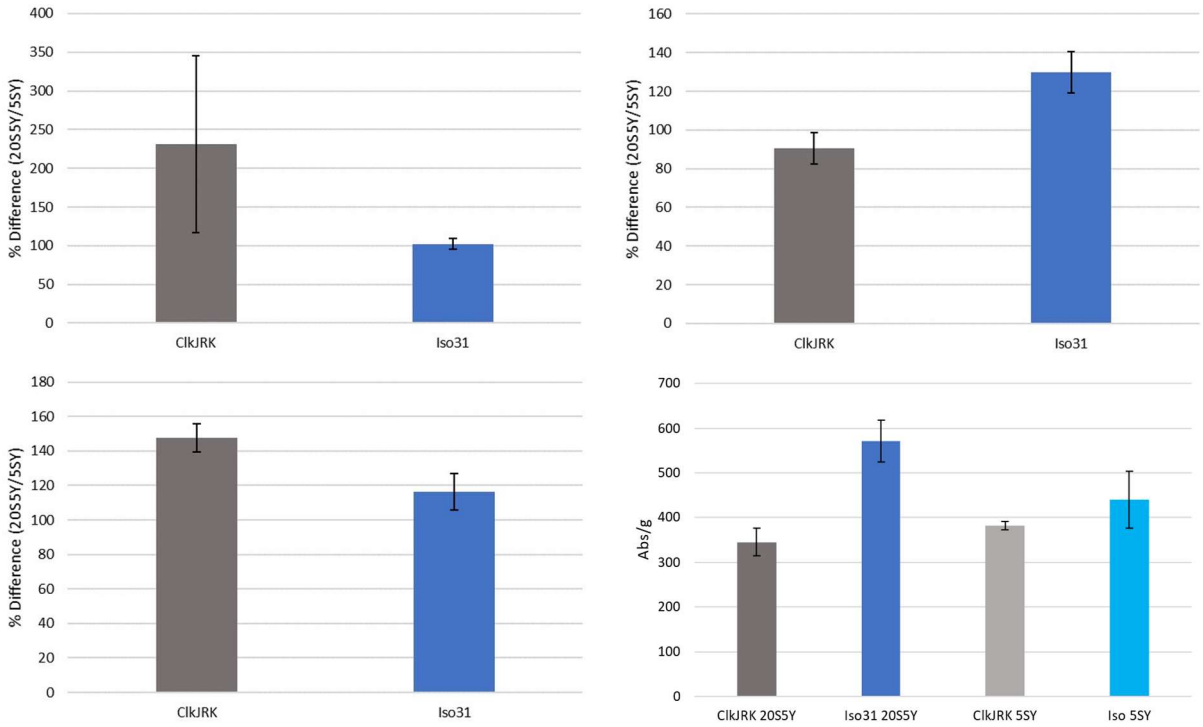
Protein and Triglyceride

To test the hypothesis that the Clk gene is important for protein and lipid metabolism on low and high sugar diets, total protein levels and triglyceride contents were measured using flies that had been treated with low or high sugar food for one week. Triglyceride levels were normalized by protein levels (which should have been relatively constant within genotypes since both diets contained the same amount of protein) (Xu et al., 2008). However, total protein levels may be also affected by circadian clock disruption in Clk^{Jrk} flies, so triglyceride levels were also normalized by body weight and fly number. Percent differences between the high sugar and low sugar treatment groups of each genotype were calculated.

No differences were found by genotype when triglycerides were normalized to protein levels, but when normalized to fly body weight, Clk^{Jrk} flies had a lower percent difference than Iso31 flies ($p = 0.0216$). As there was no significant difference in body weight compared by diet, this observation suggests that total protein levels are affected by either circadian disruption and/or sugar concentration. Although there was a trend that total protein levels are decreased in Clk mutant flies especially on a high sugar diet, statistical analysis did not support this possibility (data not shown). Also when normalized to body weight, Clk^{Jrk} flies on a high sugar diet had a lower triglyceride absorbance reading on high sugar food than Iso31 flies did ($p = 0.00804$, Figure 7). Due to differing results from each normalization method, it is difficult to determine if this finding is generalizable, so further investigation is needed.

Figure 7

Triglyceride readings from Clk^{Jrk} and Iso31 flies on low and high sugar diets



Note. Triglyceride readings are normalized to protein levels (top left), body weight (top right), and fly number (bottom left) and presented as the percent difference between the absorbance obtained from the assay on high sugar flies and the absorbance obtained from the assay on low sugar flies of the same genotype. The bottom right image displays absorbance values from each genotype/treatment group normalized to body weight, in order to show the difference between Clk^{Jrk} and Iso31 flies on high sugar diets. Bars indicate standard error.

Discussion

The *Drosophila* circadian clock affects a variety of behaviors, including sleep and food consumption (Beckwith & French, 2019). As an added layer to this phenomenon, food consumption can modify sleep in *Drosophila* (Catterson et al., 2010), and circadian clock disruption can promote obesity (Shi et al., 2013). In this thesis, the connections between excess sugar consumption (and the obesity that it supposedly causes), circadian clock disruption, and sleep were explored further.

In the sleep assay, flies slept longer on low sugar food. This implies that high sugar food promotes reduced sleep, which is in line with previous findings by Catterson et al. (2010) that increasing sucrose content decreases total sleep in a dosage-dependent trend. In the sleep assays discussed in this thesis, wild-type flies on high sugar diets experienced longer bouts of sleep on high sugar food compared to low sugar food. In other words, their sleep was more consolidated. This may imply that a low sugar diet induces more fragmented sleep, which is consistent with the observation by Lindford et al. (2012). Importantly, because Clk^{Jrk} flies did not experience this same difference in bout length on the two diets, they may have lost the ability to adapt their sleeping patterns to changes in diet. This is an example of how these mutant flies respond

differently to high sugar food, and further investigations into neuronal mechanisms should be undertaken.

The starvation results suggest that a functioning circadian clock is required for increased starvation resistance on a high sugar diet, and may indicate that a non-functioning clock leads to an abnormal physiological response on this diet, such as a difference in fat storage or usage. Clk^{Jrk} flies did not experience the starvation resistance on a high sugar diet that Iso31 flies did. This may be explained by the body weight-normalized triglyceride readings obtained in this paper. Clk^{Jrk} flies on a high sugar diet had lower triglyceride levels than wild-type flies on the same diet. This suggests that there is not a difference in fat metabolism, rather a difference in fat storage, but future metabolic assays should be conducted to confirm this.

Interestingly, genotype did not affect food consumption even though it did affect starvation resistance. Therefore, it is likely that starvation and sleep differences between Iso31 and Clk^{Jrk} flies do not depend on the amount of food consumed, instead on the amount of sugar consumed. This could hold implications for nutrition in humans, since high sugar consumption leads to reduced sleep in humans as well as *Drosophila* (Freeman et al., 2018), and could shift physicians' advice from advising patients to eat less to allowing them to continue eating their normal amount of food but choosing dishes that are low in sugar or fat. The main trend observed in the food consumption assay was a strong increase in food consumption when flies were given a low sugar diet versus a high sugar diet. The presence of this compensatory feeding phenomenon in both wild-type and Clk^{Jrk} flies suggests that a functioning Clk gene is not needed for flies to adapt their feeding to different sugar levels in food.

One caveat regarding the results of this study is that high sugar food was expected to induce obesity in flies, but this was not the case. Surprisingly, body weight decreased in flies fed

a high sugar diet. This could be due to the reduced food consumption of flies on this diet. However, a possible obese phenotype was induced in Clk^{Jrk} flies regardless of diet. The apparent lack of obesity on a high sugar diet may be unique to the fly species, but this remains to be seen. More careful analysis needs to be conducted to fully assess whether obesity was induced, employing other markers such as phosphorylated Akt levels and insulin resistance, since true obesity cannot be determined from weight alone. Because the possible obesity seen here was genotype-dependent and not diet-dependent, the results of this study should be looked at from a sugar consumption standpoint rather than an obesity standpoint. If obesity can be induced in other model organisms with a high sugar diet (or if obesity can be induced in *Drosophila* using a different high sugar feeding method), these results may be more applicable to obesity.

Conclusions

This research shows that Clk^{Jrk} flies respond to a high sugar diet differently than their wild-type counterparts in some ways (namely sleep and starvation), but do not differ in other ways (namely food consumption). Based on a literature survey, this is a novel observation and may provide insights into the roles of circadian clocks in obesity and/or sugar-related behavioral and physiological changes. In the future, metabolic assays should be conducted to confirm the evidence that Clk^{Jrk} flies store less triglycerides but do not metabolize them differently than wild-type flies. It would also be interesting to explore overall body composition of Clk^{Jrk} flies, to see why weigh more than wild-type flies but have lower triglycerides. Overall, this research contributes to knowledge of fly physiology and its connections to sleep, and may be applicable to human physiology as well.

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